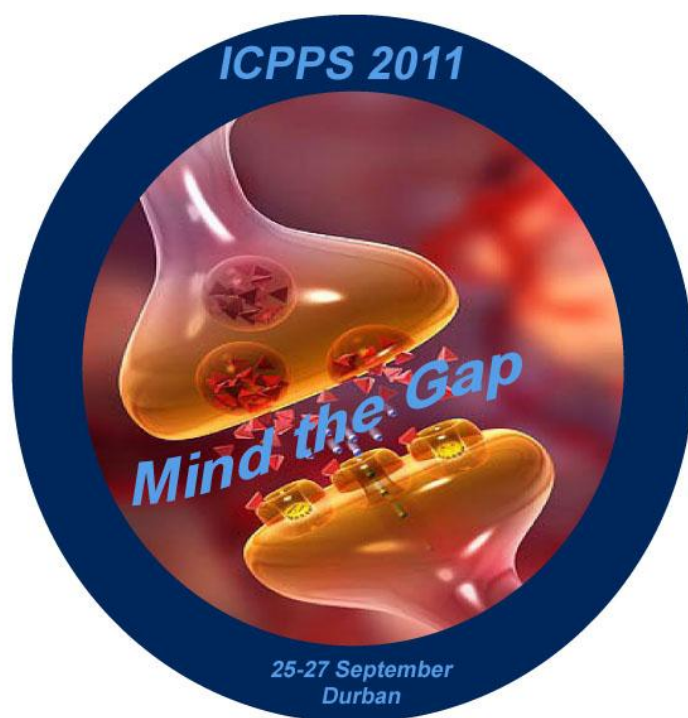


**THE 6TH INTERNATIONAL CONFERENCE ON
PHARMACEUTICAL AND PHARMACOLOGICAL SCIENCES**



PROGRAMME AND ABSTRACTS

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1. Welcome Letter: Congress Organising Committee

On behalf of the Conference Organising Committee, we would like to welcome you to 6th International Conference for Pharmaceutical and Pharmacological Sciences (ICPPS) to be held in Durban, South Africa from the 25th to the 27th of September 2011 at the Coastlands Hotel and Conference Centre in Umhlanga. We are excited to host this conference and to welcome you to the city where the fun never sets.

The theme of the conference is "Mind the Gap". The conference uniquely brings together in one place advancements in pharmaceutical and pharmacological sciences, health education, and policy and management issues and highlights the gaps that we need to be mindful of. A number of exciting papers will be presented at this year's conference spanning various disciplines. There are two special focus sessions and four plenaries. The presentations are likely to appeal to a wide range of people. We hope that presentations at this conference might lead to collaborations that would eventually lead to development of models, medicines, policy or measurement of impact. After all, at some point research needs to get out of a laboratory and into a community. Sharing of ideas is the fastest way that this can happen.

There are far too many people involved in making this conference possible and successful for me to mention here. There are three special mentions that I would like to make however. I'd like to thank the Scientific Advisory Committee, led by Dr Johannes Bodenstein for their hard work in evaluating all the abstracts. In addition Dr Frasia Oosthuizen and Dr U (Sara) Govinden worked tirelessly to get registration and finance sorted out. We especially would like to thank our sponsors for being willing to support this event and to ensure that sharing of research and ideas is possible.

Finally we would like to thank all of the delegates for attending this conference. We hope that you have a wonderful time, and that you will remember this conference long after it is over.

Warmest regards,

Prof Fatima Suleman

Conference Convener

2. Welcome Letter: APSSA



On behalf of The Academy of Pharmaceutical Sciences, I would like to invite and welcome you to the 6th ICPPS. This initiative of a combined conference of Pharmaceutical and Pharmacological Sciences in South Africa was established in 1996 and has become a highly stimulating scientific and social event, organised every 2-3 years by a different University.

The Academy of Pharmaceutical Sciences was established in 1979 and caters for the needs of its members involved in any sphere of pharmaceutical education, research and development. The mission of The Academy is to advance pharmaceutical sciences in South Africa, promote research and education, provide expert opinion and recognise excellence by providing a forum for free interchange and dissemination of scientific knowledge and skills by means of meetings, congresses, and publications. This is obtained by encouraging excellence in research and education and active participation in proceedings and activities of the scientific and pharmaceutical communities on national and international levels. Initiation of joint ventures like this ICPPS is an activity deemed expedient to realise these objectives.

This conference is a key event for the pharmaceutical scientist in South Africa and offers an attractive multidisciplinary scope of attendance and presentations. As always, it promises to be an event where scientific and social interactions could lead to lifelong collaboration and friendship. We look forward to your active participation.

Prof Sarel Malan

Chairman: Academy of Pharmaceutical Sciences of the PSSA

3. Welcome Letter: SASBCP



On behalf of the South African Society for Basic and Clinical Pharmacology (SASBCP) I wish to welcome all delegates to the 6th ICPPS 2011, hosted by the University of KwaZulu-Natal in the city of Durban. The ICPPS series has been established in 1996 as a bi- to quadrennial joint meeting between the Academy of Pharmaceutical Sciences of the PSSA (APSSA) and the SASBCP, and which has proven to be very rewarding. This year we are privileged to interact again with members of the Southern African Neurosciences Society, who share many common interests with our members.

Prof Suleman and her group at UKZN have worked very hard to put this excellent programme together. They have chosen a multidimensional theme "Mind the Gap", capturing many of the challenges we face in medicinal sciences in South Africa and on the African continent as a whole. As a scientific community we are responsible not only to foster the highest standards in science, but also to serve within the context of our local and wider communities. This is indeed what I believe this meeting will achieve as our members participate and engage at the highest level.

We are indeed privileged to welcome several international speakers this year, who will be making significant contributions by sharing their expertise and science. I am sure that there will be many discussions flowing from your participation and our engagement. May you also find the country and its people warm-hearted and your stay an enjoyable, unique experience.

Several sponsors and exhibitors have also come on board this year, for which we are sincerely grateful. By your valuable participation, you have contributed to capacity building in medicinal sciences in South Africa. I am sure that our members will engage with you and obtain important information from you to further our research, training and education programmes.

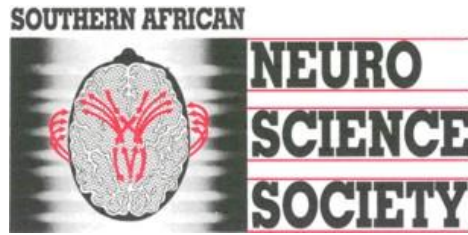
The SASBCP has become a very dynamic society, actively participating in the leadership of international committees, a 'Pharmacology for Africa' initiative (PharfA), local capacity building and as an organisation that offers new opportunities to its members for national and international collaboration and networking. We look forward to hosting the 17th World Congress of Basic and Clinical Pharmacology (WCP2014) in Cape Town, which will actively promote and build bridges in basic, clinical and translational aspects of the discipline.

As Society we remain committed to serve you as member, also by collaborating with the Organisers of the 6th ICPPS 2011, to create platforms for scientific excellence, networking and memorable experiences.

Prof Tiaan Brink

President: South African Society for Basic and Clinical Pharmacology

4. Welcome Letter: SANS



On behalf of the Southern African Neuroscience Society (SANS) I extend a warm welcome to you and your participation in a program of interesting topics.

Besides the exciting plenary sessions, it is especially encouraging to see the relatively large number of young scientists presenting their work and they therefore deserve all of our support. This is an excellent opportunity to get to know what our young scientists are researching and presents an opportunity to exploit common interests and networking.

I trust that you will have an enjoyable, memorable and stimulating experience, and I look forward to meeting you during the three days of the conference.

Prof Lauriston Kellaway

Chair of SANS

5. Programme

Sunday 25 th September 2011			
Time	African Fire 1-4		Heritage
09h00-12h00	Workshop 1	Workshop 2	Build
12h00-13h00	Lunch (Heritage)		
African Fire 1-4			
13h00-14h45	<p style="text-align: center;">Opening Ceremony</p> <p>Chair: Prof. F Suleman 13h00-13h15 – Welcome by the Deputy Vice Chancellor, College of Health Sciences, UKZN – Prof T.S. Pillay</p> <p style="text-align: center;">Session A: Paediatric Medicines – Session Chair: Mr A Gray</p> <p>13h15-13h35 – <i>Essential Medicines List for Children – the necessity and the challenges</i> (Mr A. Gray) 13h35-13h55 – <i>Paediatrician's dilemma when choosing medicines for children</i> (Dr T. Mitha) 13h55-14h15 – <i>Toxicity issues in dosage regimens for children</i> (TBC) 14h15-14h35 – <i>Formulating dosage forms for children – the necessity and the challenges</i> (Prof R.B. Walker) 14h35-14h45: Questions</p>		
14h45-15h15	Tea (Heritage)		
African Fire 1-4			
15h15-15h45	<p style="text-align: center;">Session B: Plenary Session 1 – Chair: Prof W. Daniels</p> <p>Topic: <i>New therapeutic opportunities for the treatment of Parkinson's disease: Focus on adenosine A_{2A} receptor antagonists</i> Speaker: Prof Micaela Morelli, Dept. of Toxicology, University of Cagliari, Italy</p>		
<p style="text-align: center;">Session C: Oral Presentations – Chair: Prof T. Brink</p>			
15h45-16h00	Greeff, OBW: A new evaluation model as an aid in the early diagnosis of dementia		
16h00-16h15	Russell, VA: Decreased dopamine D1 receptor signalling in prefrontal cortex and striatum of the SHR model of ADHD		
16h15-16h30	Van Zyl, PJ: Behavioural characterization of the Wistar-Kyoto rat model of depression		
<p style="text-align: center;">16h30 – 18h30 APSSA AGM</p>			
<p style="text-align: center;">18h30 for 19h00 Cocktail Function – The Heritage (Sponsored by Aspen)</p>			

Registration

Monday 26 th September 2011			
Time	African Fire 1-4		Foyer
08h30-09h00	Session D: Plenary Session 2 – Chair: Prof S. Malan Topic: <i>Computer assisted Drug Design (CADD) and Bioinformatics: Tools for designing drugs and understanding their activity</i> Speaker: Mahmoud E. Soliman , Department of Chemistry, University of Bath		Registration
Session E: Young Scientists Presentation (Parallel Sessions)			
	APSSA: Chair: Prof T. Govender	SASBCP: Chair: Prof B. Rosenkranz	
09h00-09h15	Booyesen, HP Thiocaffeine derivatives as inhibitors of monoamine oxidase	Brand, SJ Role of the peroxisome proliferator activated receptor (PPAR)- γ pathway in mood regulation and antidepressant action	
09h15-09h30	Dawood, Y Elucidation of the effect of oxidation and crosslinking on the drug entrapment efficiency and <i>in vitro</i> drug release behaviour of a starch-based multiparticulate drug delivery system	Erasmus, M An investigation into the role of noradrenergic receptors in conditioned fear: relevance to posttraumatic stress disorder (PTSD)	
09h30-09h45	Fourie, P Neuroprotective effects of amantadine-flavonoid conjugates	Fasinu, PS <i>In vitro</i> investigation of the effects of commonly used South African medicinal herbs on CYP1A2 activity employing human liver microsomes	
09h45-10h00	Frank, D Design and characterization of intravenously administered nanolipobubbles for targeted ovarian cancer therapy	Govender, K A bioavailability study of lumefantrine in mice; evaluating the application of Pheroid™ formulation	
10h00-10h15	Hazle, D Evaluation of the physicochemical and physicomechanical properties of optimized ciprofloxacin- and diclofenac-loaded co-blended alginate fibers for oramucosal delivery	Mokoena, M Ozone-induced oxidative stress is associated with a depressogenic effect in stress-sensitive rats	
10h15-10h45	Tea (Heritage) and Poster Session One		

Time	African Fire 1-4		Foyer
	Session F: Young Scientists Presentation (Parallel Sessions)		
	APSSA: Chair: Dr G. Killian	SASBCP: Chair: Prof D. Oliver	
10h45-11h00	Jhundoo, HD Formulation development and characterization of Labrafil® M 2130 CS solid lipid microspheres for the oral delivery of ketoconazole	Moller, M Social isolation rearing in rats alters plasma tryptophan metabolism and is reversed by sub-chronic clozapine treatment	Registration
11h00-11h15	Magnus, L Development and assessment of extemporaneous famciclovir oral formulations for paediatric use	Ozokwere, J Comparative performance of Sprague-Dawley rats using DMSO and DMF as cryoprotectants	
11h15-11h30	Moraal, C The design, synthesis and evaluation of aminocaffeine derivatives as inhibitors of monoamine oxidase	Pillay, P The effects of <i>Scilla nervosa</i> (Burch.) Jessop (Hyacinthaceae) aqueous extract on cultured Hep G2 cells	
11h30-11h45	Patel, S Developing materials to meet the information needs of caregivers of paediatric patients	Pretorius, A A preliminary investigation of the potential anticancer properties of quinoline derivatives	
11h45-12h00	Roux, W The isolation of three compounds from <i>Cotyledon orbiculata</i> and their antioxidant activities	Rahiman-Karim, BF An assessment of pathogen occurrence and resistance in a trauma intensive care unit of a private hospital under an antibiotic stewardship programme	
12h00-13h00	Lunch (Heritage) and Poster Session One		

Session G: Young Scientists Presentation (Parallel Sessions)			Foyer
African Fire 1-4			
	APSSA: Chair: Mr R. Rossiter	SASBCP: Chair: Prof O. Meissner	Registration
13h00-13h15	Shaikh, RP Synthesis of multilayered mucoadhesive membranes for prolonged and site-specific oral drug delivery	Schroeder, IE Effects of pentachlorophenol and its metabolites on cell viability and CYP1A1 metabolism	
13h15-13h30	Wadee, A The development of an <i>in situ</i> forming implant for the treatment of solid tumors: Determination of the effect of methotrexate release on colon carcinoma cells and <i>in vivo</i> studies in the rat model	Steyn, SF Effect of early-life exposure to the serotonin-norepinephrine reuptake inhibitor, venlafaxine, on behaviour in adulthood in stress-sensitive rats	
13h30-13h45	Verwey, MT The synthesis and <i>in vitro</i> antiplasmodial activity of acridine-triazine hybrids	Van Rensburg, L The influence of a synthetic lung surfactant on the permeability of antimycobacterial drugs through porcine lung tissue	
13h45-14h00	Chiwakata, MT Fragment based-type approach on synthesis of HMT analogues as potential anti-cancer agents	Willis, K Quantitative analysis of the active compounds, hypericin and hyperforin, in commercial products of St. John's Wort (<i>Hypericum perforatum</i> L.) by HPLC-MS/MS	
14h00-14h15	Munedzimwe, T The semi-synthesis of sargahydroquinoic acid derivatives as potential anti-plasmodial and anticancer agents	Wolmarans, P (De Wet) Natural stereotypy in deer mice and its association with frontal cortical and striatal serotonin transporter (SERT) density: implications for a putative animal model of OCD	
14h15-14h30	van Heerden, L Synthesis and <i>in vitro</i> antimalarial activity of a series of bisquinoline and bispyrrolo[1,2a]quinoxaline compounds		
14h30-15h00	Tea (Heritage) and Poster Session Two		

Time	African Fire 1-4
	Session H: Oral Presentations – Chair: Prof SY Essack
15h00-15h15	Mugabo, P Nevirapine plasma concentrations in premature infants exposed to single dose nevirapine for prevention of mother to child transmission of HIV-1
15h15-15h30	Rosenkranz, B Efavirenz levels in HIV infected children
15h30-15h45	Van Tonder, J A microplate method for multiparametric hepatotoxicity screening
15h45-16h00	Van Zyl, J A synthetic peptide-containing surfactant: Secondary structure and efficacy as therapeutic agent for respiratory distress syndrome
16h00-16h15	Samant, BS Synthesis and structural activity relationship study of halogenated aromatic compounds against human African trypanosomiasis
16h30 – 18h30 SASBCP AGM	
FREE EVENING/DINNER ON OWN	

Tuesday 27 th September 2011		
Time	African Fire 1-4	Foyer
08h30-09h00	<p>Session I: Plenary Session 3 – Chair: Prof CM Dangor</p> <p>Topic: <i>Safety and effectiveness of 1 % tenofovir gel in preventing HIV infection: Results of the CAPRISA 004 trial and next steps</i></p> <p>Speaker: Dr Leila Mansoor, CAPRISA 008 Co-Principal Investigator, Nelson R. Mandela School of Medicine, University of KwaZulu-Natal</p>	Registration
Session J: Oral Presentations – Chair: Prof S Hamman		
09h00-09h15	<p>Du Toit, L</p> <p>Conceptualisation and experimental optimisation of an intelligent intraocular implant for exemplification of the bioresponsive potential</p>	
09h15-09h30	<p>Khamanga, SMM</p> <p>A novel approach based on <i>Eigenvalues of Hessian</i> matrix to solve optimization problems in analytical methods development</p>	
09h30-09h45	<p>Lombard, MC</p> <p>Artemisinin-quinoline hybrids and hybrid dimers: Synthesis, <i>in vitro</i> and <i>in vivo</i> antiplasmodial activity</p>	
09h45-10h00	<p>Mufamadi, MS</p> <p>An implantable nano-enabled biorobotic intracranial device for neurotherapeutic applications</p>	
10h00-10h30	Tea (Heritage) and Poster Session Two	

10h30-11h00	<p align="center">Session K: Plenary Session 4 – Chair: Dr M. Mbandla</p> <p>Topic: <i>The organization of brain circuits underlying motivated behaviour</i></p> <p>Speaker: Dr Barry Richmond, Laboratory of Neuropsychology, National Institute of Mental Health, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland</p>	Foyer
Session L: Oral Presentations – Chair: Prof L. Kellaway		
11h00-11h15	<p>Ndlovu, BC (SANS Young Scientist) The effects of oleanolic acid on dopamine cell lines following a toxic insult: Implications for Parkinson’s disease</p>	Registration
11h15-11h30	<p>Mohamed Moosa, Z (SANS Young Scientist) The effects of methylmercury exposure in a Parkinsonian model</p>	
11h30-11h45	<p>Strydom, B 8-Aryl- and alkyloxycaffeine analogues as inhibitors of monoamine oxidase</p>	
11h45-12h00	<p>Poka, MS Investigation of natural polymer systems to control nicotinic acid release</p>	
12h00-12h15	<p>Hayeshi, R Nanomedicine for improved efficacy of TB Drugs</p>	
12h15 – 13h15 Lunch (Heritage) and Poster Session Three		

Session M: Oral Presentations – Chair: Ms S. Boschmans		Registration	Foyer
13h15-13h30	Jobson, R Through the gaps: Paradigm hopping		
13h30-13h45	Kapp, E Interventions to maintain quality of pharmaceutical care within the public health care sector: The changing role of the pharmacist		
13h45-14h00	Meyer, R Restructuring of practical training at the NWU School of Pharmacy		
14h00-14h15	Moch, S Does exit-level assessment of Wits medical students test rational prescribing skills?		
14h15-14h30	Suleman, F AIDS Online International (AOI): an internationally synchronized online university course on HIV/AIDS education, prevention, and behavioural research – a pilot study for international collaboration		
14h30-15h00	Tea and Poster Session Three		
15h00-17h00	Session N: Special Focus Session on Human Resources for Health – Chair: Ms L. Osman		
	15h00-15h20: <i>A Recap of the rationalisation of Schools of Pharmacy – the principles</i> (Prof O. Greeff)		
	15h20-15h40: <i>Perspectives from a Global level – Pharmacy Education and the Global Workforce</i> (Prof B. Futter)		
	15h40-16h00: <i>Why are we here? (NHI/HR Strategy/Mid Level Workers/Specialities)</i> (Ms L. Osman)		
	16h00-16h20: <i>Producing “Jack of all trades”?</i> (Ms G. Enslin)		
	16h20-16h40: <i>The Australian experience of increasing HR production – the case study of pharmacists</i> (Prof D. Newby)		
	16h40-17h00: Questions/Feedback from Rapporteur (Mr A. Gray)		
19h00 for 19h30	Banquet/Gala Evening (Heritage) – Sponsored by Clicks		

6. Plenary Speakers



Micaela Morelli



Mahmoud E.S. Soliman



Leila E. Mansoor



Barry J. Richmond

7. Exhibitors



8. Invited Speakers



Andy Gray



Thahir A. Mitha



Rod B. Walker



Oppel B.W. Greeff



Billy Futter



Lorraine Osman

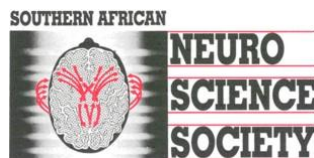


Gill Enslin

9. Sponsors



NATIONAL BIOPRODUCTS INSTITUTE



Proudly sponsored Micaela Morelli and Barry Richmond



Proudly sponsored David Newby

10. Biosketches

10.1 Plenary Speakers

10.1.1 Micaela Morelli

Micaela Morelli has a degree in Biological Sciences and is a Full Professor of Pharmacology.

She holds the following academic and professional positions:

- From 1995- belongs to CNR Institute of Neurogenetic and Neuropharmacology
- From 2001- belongs to Centre of Excellence on Neurobiology of Dependence
- 1997-2000- Chairman of the Department of Toxicology, University of Cagliari
- 2001-2006- elected member of the Italian Neuroscience Society Council
- From 2004- Council of the International Basal Ganglia Society (IBAGS)
- From 2005- Italian representative on the International Brain Research Organisation (IBRO)

She serves on the Editorial Board Committees of Neurotoxicity Research, Experimental Neurology, International Review in Neurobiology, Journal of Caffeine Research and Frontiers of Neuroanatomy.

For more than 20 years Professor Morelli has devoted her research interests to the study of drugs active in the central nervous system and particularly to drugs for the treatment of Parkinson's disease. She is an author of more than 130 publications in international journals with impact factor.

10.1.2 Mahmoud E.S. Soliman

Dr Mahmoud E.S. Soliman holds a Bachelor Degree in Pharmaceutical Sciences (Faculty of Pharmacy, ZU, Egypt, 1988); a Masters Degree in Pharmaceutical Organic Chemistry (Faculty of Pharmacy, ZU, Egypt, 2004) and a PhD in Computational Chemistry and Drug Design (University of Bath, UK, 2009).

His main interest is related to the design and study of biologically and therapeutically oriented targets by employing the applications of computational methods to study the problems of chemical and biological reactivity, with particular focus upon the transition state, environmental effects on mechanisms, the origins of catalysis, and the interpretation of kinetic isotope effects:

- Mechanistic pathways and transition states for reactions in enzymes and solutions
- Design of enzyme inhibitors and exploring the binding and catalytic theme of the designated targets
- Adopting computational approaches to understand protein structure and function

He has 7 peer-reviewed publications. Current research support and collaboration include an anti-HIV project with UKZN; an anti-influenza project with the University of Bath, UK and a project on computational support with UCT and Bath.

10.1.3 Leila E. Mansoor

Dr Leila E. Mansoor (BPharm, PhD) is a pharmacist by profession and obtained her BPharm degree in 2000 and a PhD in Pharmacy Practice in 2005. She joined CAPRISA in 2006 as a post-doctoral trainee. Dr Mansoor was responsible for overall co-ordination of the CAPRISA 004 tenofovir gel trial including: monitoring of study performance accrual, retention and quality assurance targets; ensuring regulatory and ethics committee compliance; maintenance of essential documents, communication with sites and coordinating the planning & implementing of the dissemination of the study results. Her research interests include HIV prevention clinical research, adherence support and measures in clinical trials, social science research focusing on patient's knowledge about, attitude to and perceptions of their medicine and disease state. Dr Mansoor's current role at CAPRISA is as the UKZN CAPRISA HIV/AIDS Clinical Trials Unit (CTU) Coordinator and is the co-Principal Investigator of CAPRISA 008, the tenofovir gel implementation trial.

10.1.4 Barry J. Richmond

Barry Richmond was trained as a physician (M.D. 1971) with Board certifications in Paediatrics (1976), and Neurology with Special Competence in Child Neurology (1979). He has been in the NIH intramural research program since the end of my Neurology residency training in 1976. He has been a Senior Investigator and Chief of the Section on Neural Coding and Computation since 1996. His research targets how higher brain functions, particularly perception, memory and motivation, arise from the cooperative activity of neurons. Two large themes are connected through an emphasis on visual performance. The first is to understand neural correlates of visual perception itself, with the ultimate goal of learning how the cooperative activity of single neurons gives rise to visual perception and interpretation of objects in the environment. The second is to understand how motivation arises, and how cues, especially visual cues, are learned and interpreted to set motivational levels. He has worked on neural coding, the neural basis of motivated behaviour (and what is now being called neuroeconomics), and recently application of genetic techniques to modify targeted cell types and receptors.

10.2 Invited Speakers

10.2.1 Andy Gray

Andy Gray B Pharm, MSc (Pharm), FPS is a pharmacist whose research interests include policy analysis (in particular, the development and implementation of National Drug Policies), rational medicines use and the application of Highly Active Antiretroviral Therapy in resource-constrained settings.

He is a Senior Lecturer in the Department of Therapeutics and Medicines Management, Nelson R Mandela School of Medicine, University of KwaZulu-Natal, Durban, South Africa. He is also consultant pharmacist for the Centre for the AIDS Programme of Research in South Africa. Mr Gray is a Fellow and Honorary Life Member of the Pharmaceutical Society of South Africa, a past President of the South African Association of Hospital and Institutional Pharmacists, a past President of the Hospital Pharmacy Section and currently Chairman of the Board of Pharmaceutical Practice of the International Pharmaceutical Federation (FIP). He has been a member of the Scheduling and Naming Expert Committee of the South African Medicines Control Council since 2000. He has been appointed as a Member of the World Health Organization's Expert Panel on Drug Policies and Management and has served as a member and co-chairperson of the Expert Committee on the Selection and Use of Essential Medicines, as chairperson of the sub-committee on Essential Medicines for Children and on the WHO Guidelines Review Committee. He is currently non-executive chairman of JEMBI Health Systems, a not-for-profit company focused on the furthering of computer/IT-based healthcare solutions for the developing world.

Widely published, he has served as a reviewer for a number of international and local journals, is associate editor of the South African Pharmaceutical Journal and serves on the international editorial advisory boards of International Journal of Clinical Pharmacy, Southern Med Review and the GaBI Journal. He has also been a member of the editorial committee of the South African Health Review.

Mr Gray has also reviewed funding applications for several agencies, including the Health Systems Trust, National Research Foundation, Medical Research Council and UNITAID. He has been actively involved in the development and assessment of medicines and other health-related law in South Africa.

10.2.2 Thahir Mitha

Dr Thahir Ayob Mitha (MBCChB, FCP (SA) Paeds) is currently in private practice as a Paediatrician after having been involved as Consultant/Lecturer in the Department of Paediatrics, King Edward Hospital/University of Natal from 1993-1994.

Dr Mitha has been Chairperson of the KZN Paediatric Group from 2007-2009, served as Secretary/Treasurer of the South African Paediatric Association between 2001-2009 and is a Committee Member of South African Paediatric Vaccine Advocacy Group (SADVAG).

10.2.3 Rod B. Walker

Professor Walker (B.Pharm., Ph.D., MPS (SA)) is currently the Dean and Head of the Faculty of Pharmacy at Rhodes University in South Africa. He completed his BPharm (1983) and PhD in Biopharmaceutics and Pharmacokinetics (1995) at Rhodes University and was the National Distinguished Teacher of the Year in Pharmacy (2000). Professor Walker has recently been appointed as a visiting Professor at School of Health and Life Sciences at Aston University in the United Kingdom.

Professor Walker is a registered Pharmacist in South Africa and has to date, published over 70 research papers, book chapters and abstracts and has delivered over 180 presentations at conferences. He has successfully supervised 12 MSc and 4 PhD students and is currently supervising or co-supervising 2 PhD and 7 MSc students.

He serves as a reviewer for scientific and professional journals and has been appointed to the Editorial Board of several journals including Drug Development and Industrial Pharmacy, Dissolution Technologies and Pharmaceutics amongst others and his research focuses on the development, manufacture and assessment of pharmaceutical dosage forms and regulatory science.

Professor Walker is one of the few Pharmaceutical Scientists who have been rated by the National Research Foundation in South Africa, as a C category researcher. He has attracted research funding from the University, National Research Foundation and several pharmaceutical companies and has worked as a Senior Scientist in a Pharmaceutical CRO in the USA where he was involved in the development of sustained release technologies.

Professor Walker was recently appointed by the Minister of Health to serve on the Medicines Control Council. Professor Walker is currently the Chairman of the Head of Schools Committee of the South African Pharmacy Council.

Professor Walker is an active member of several international professional societies including the American Association of Pharmaceutical Scientists, the Controlled Release Society in which he has served as a member and Co-Chair of the Board of Scientific Advisors and serves on several other Committees. He is a member of the South African Academy of Pharmaceutical Sciences and of which he was a member of the Executive Committee and the Pharmaceutical Society of South Africa. Professor Walker has also organised or co-organised four conferences in South Africa.

10.2.4 Opper B.W. Greeff

Prof Greeff holds a Bachelors degree in Medicine and Surgery MBChB (Pret); Post graduate qualifications in Primary Health Care, FCFP (SA); MPharmMed (Pret); Pharmaceutical Medicine FFPM (Royal Colleges of Physicians (UK)) and a MD (Psychiatry) (KZN).

From 1973 until 1977, Prof Greeff worked as a General Practitioner, was employed as Medical Advisor for Wellcome Southern Africa in 1978 and Medical Director with Roussel Laboratories from 1981 to 1990. During this period he was intimately involved in the commercialisation activities of both companies.

In 1990 he founded Clindepharm International, the first Contract Research Organisation in South Africa, which he sold to Quintiles Transnational Corporation in August 1997 and continued his employment at Quintiles.

In 2002 he was appointed CEO of Early Development & Laboratory Services worldwide for Quintiles in the USA and in the same year as President: Global Product Development Services (PDS), and was appointed on the Executive Committee of Quintiles.

Opper also chairs the Asia-Pacific Management Board and was appointed Chairman of the Quintiles-Fisher JV, Cenduit, an IRS enterprise in 2007.

In August 2008 Prof Greeff was appointed professor and head of the Department of Pharmacology, School of Medicine, Faculty of Health Sciences at the University of Pretoria, retaining his board positions, and relocating back to South-Africa.

10.2.5 Billy Futter

Emeritus Associate Professor Billy Futter M Comm PGDHE (Rhodes) retired from the Faculty of Pharmacy, Rhodes University after 25 years of teaching managerial, administrative and social sciences. He has been an external facilitator on a distance education programme at Fairleigh Dickerson University, USA since 2004. He has been involved in official capacities for inspecting pharmacy schools, developing unit standards for pharmacists, curriculum design. Keen on innovating in teaching, his contributions were acknowledged when he was selected as Pharmacist of the Year in 2004 by the Academy of Pharmaceutical Sciences.

He has published extensively in South African pharmacy professional journals on management issues and patient safety, presented numerous papers and posters at pharmacy conferences and published in international journals.

His current focus has been as strategic lead for WHO/UNESCO/FIP Pharmacy Education Taskforce (since 2006). This body aims to enable the sustainability of a pharmacy workforce that is relevant to local needs.

He is also an active sportsman regularly taking part in a wide range of sports including open water swimming, trail running and race walking. He has earned age group national colours for Biathlon and competed as a member of the South African national age group team in the Biathle and Triathlon World Championships.

10.2.6 Lorraine Osman

Lorraine Osman's work has taken her into most sectors of pharmacy, including academia, community pharmacy, drug information, private hospital pharmacy and the pharmaceutical industry. She spent fourteen years at the Wits School of Pharmacy, first at the Technikon and then at the University, where she was the head of the Pharmacy Practice division. Her current employer is the Pharmaceutical Society of South Africa, where she is the head of public affairs. Lorraine is the editor of the SA Pharmaceutical Journal, the South African Pharmacist's Assistant magazine, the PSSA electronic newsletter and the Pharmacy Law Compendium.

Lorraine is the Vice President of the South African Pharmacy Council. In that capacity, she has chaired the Human Resource Task Team and has been involved with the development of the scopes of practice and qualifications for pharmacy technicians and authorised pharmacist prescribers from their inception. She also serves on the Education Committee, the Committee for Preliminary Investigation and the Continuing Professional Development Committee.

10.2.7 Gill Enslin

Gill has been Head of Department: Pharmaceutical Sciences since 2002. She obtained her doctorate in 2006, the focus of which was the use of chitosan and other compounds as absorption enhancers for hydrophilic macromolecules. She has been a member of the Niche Area “Natural Products in Drug Development” since its inception, and has supervised a number of masters students and published and presented various papers on the topic at national and international conferences. Dr Enslin is also involved with the development of higher education qualifications and curricula for the Bachelor of Pharmacy and pharmacy support personnel.

11. Abstracts

11.1 Oral Presentations

11.1.1 Session A

Globally, improving child health is a priority. The World Health Organization has pointed out that “access to simple, affordable medicines could prevent or treat the conditions which cause more than 8.1 million deaths of children under five each year”. WHO’s “Make medicines child size” campaign has tackled a number of the barriers preventing achievement of this goal. This session will focus on the process of developing the WHO Model Essential Medicines List for Children. In 2011, WHO also developed a priority list of medicines for mothers and children. WHO has underlined that in order to improve access, priority medicines should be:

- manufactured according to quality standards;
- licensed for use by regulatory authorities;
- on National Essential Medicines lists;
- part of national standard treatment guidelines;
- procured from the supplier of a quality product;
- in the supply chain; and
- prescribed by health care professionals who know how to use them.

This session will also focus on the difficulties associated with paediatric dosing and the toxicities that may result from inappropriate use. It will highlight the need for new and appropriate dosage forms, focusing on local research into new formulations.

The World Health Assembly Resolution WHA60.20 (2007) “Better medicines for children” can be accessed at <http://www.who.int/childmedicines/publications/WHA6020.pdf>

11.1.2 Session B

11.1.2.1 Morelli, M

New Therapeutic Opportunities for the Treatment of Parkinson's Disease: Focus on Adenosine A_{2A} Receptor Antagonists

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The motor symptoms of Parkinson's disease (PD) are due primarily to the degeneration of the dopaminergic neurons of the nigrostriatal pathway. However, several other brain areas and neurotransmitters other than dopamine such as adenosine, because of the extensive interaction of its receptors with the dopaminergic system have been implicated in the pathophysiology of the disease. Treatment of PD is largely unsatisfactory due to the several side effects such as "on/off", "wearing-off" and dyskinesia, associated with dopaminergic therapy, therefore, recent assessments have called for a broadening of therapeutic options beyond the traditional dopaminergic drug arsenal. In my presentation I will review the interactions between dopamine and adenosine receptors that underpin the preclinical and clinical rationale for pursuing adenosine A_{2A} receptor antagonists as symptomatic and potentially neuroprotective treatment of PD. Adenosine A_{2A} receptors have a selective localization in richly dopamine-innervated areas, offering a unique opportunity to modulate basal ganglia function mediated by dopamine. Indeed, A_{2A} receptor antagonists have been shown to restore motor function, either alone or in combination with dopaminergic drugs, in experimental models of PD. Moreover, in clinical trials, adenosine A_{2A} receptor antagonists reduce "off" time in patients with PD receiving optimal dopaminergic therapy without the exacerbation of dyskinesia. Finally, adenosine A_{2A} receptor antagonists would appear to help prevent neurodegeneration in PD, raising the possibility of their use as neuroprotective agents.

11.1.3 Session C

11.1.3.1 Greeff, OBW

A New Evaluation Model as an Aid in the Early Diagnosis of Dementia

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Purpose:

Dementia is highly prevalent in people over the age of 65. Considering the demographic shift in the age structure of the world's population, it has become the most important disease of old age. The Comprehensive Dementia Evaluation Model (CDEM), a new evaluation model for the early diagnosis of dementia was developed which included the Folstein Mini-Mental State (FMMS), the Wechsler Memory Scale (WMS), the Trailmaking Test (TMT) and the evaluation of word fluency with the Controlled Oral Word Association Test (COWAT). Various other clinical measurements were performed to obtain conclusive diagnoses. The individual rating scales were quantified and weighted, and the model applied to apparently healthy elderly volunteers. Where uncertainty remained as to the diagnosis of dementia, a MRI was performed.

Methods:

Subjects: Forty-four patients who responded to a newspaper article were included in the study. Informed consent was obtained. None of the subjects were previously diagnosed with dementia. The patients were between the ages of 54 and 84.

DSM-III: An independent evaluator tested the subjects according to the DSM-II criteria in order to ascertain the sensitivity of the CDEM model to distinguish between healthy and slightly demented subjects.

CDEM: An independent evaluator tested the subjects according to the FMMS, WMS, TMT and COWAT tests. The average of each test was used in a predetermined ratio to ascertain the diseased state of each patient.

MRI: Discrepancies between the EEG, VEP and CAT scan of three subjects necessitated the use of MRI to confirm the diagnoses.

Results:

Clinical evaluation revealed no co-morbid diseases which were not properly managed. Based on the DSM-III evaluation, none of the patients were diagnosed as demented. The CDEM model successfully identified 5 out of 6 mildly demented patients, demonstrating that the model can differentiate between normal and pre-clinically demented subjects with a high accuracy rate. Data obtained from the MRI indicated that this is a useful tool in the evaluation and diagnosis of different forms of dementia. It was concluded that CDEM is a useful aid in the early diagnosis of SDAT and can be applied in the screening and evaluation of the efficacy of agents used in the treatment of dementia.

11.1.3.2 Russell, VA

Decreased Dopamine D1 Receptor Signalling in Prefrontal Cortex and Striatum of the SHR Model of ADHD

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Purpose:

The spontaneously hypertensive rat (SHR) displays the characteristic behavioural disturbances of attention-deficit/hyperactivity disorder (ADHD, hyperactivity, inattention and impulsivity). Evidence suggests that there may be an age-dependent alteration of the dopaminergic system, giving rise to increased dopamine transporters (DAT) and dopamine D1 receptors (DRD1) in adulthood. The aim of the present study was to measure dopamine-related protein levels in striatum and prefrontal cortex of SHR compared to control Wistar-Kyoto (WKY) rats.

Methods:

This study was approved by the University of Cape Town Faculty of Health Sciences Animal Ethics Committee (ref no. 009/065). Duplicate pooled (n = 5) samples of prefrontal cortex and striatum of prepubertal (35-day-old) SHR and WKY were sonicated in 1 M triethylammonium bicarbonate buffer and centrifuged at 19,000 rpm for 30 min at 4°C. Supernatants were subjected to iTRAQ labelling and matrix-assisted laser desorption/ionization tandem mass spectrometry (MALDI-MS/MS) by the Centre for Proteomic and Genomic Research (CPGR) at the University of Cape Town. The MS/MS spectra were analysed with ProteinPilot using the Ratus ratus database. Proteins detected with > 95% confidence were tested for significant differences ($p < 0.05$) between SHR and WKY.

Results:

Consistent differences between SHR and WKY were found in prefrontal cortex and striatum, suggesting global changes in cortico-striato-thalamo-cortical circuits. The greatest difference between SHR and WKY was found in the dopamine- and cAMP-regulated phosphoprotein, Mr 32 kDa (DARPP-32, also known as protein phosphatase 1 regulatory subunit 1B, PPP1R1B) which was 33-fold lower in SHR striatum than in WKY striatum and 16-fold lower in SHR prefrontal cortex compared to WKY prefrontal cortex.

Discussion:

DARPP-32 is an important integrator of information in dopaminergic neurons. It is regulated by dopamine, glutamate, GABA, and serotonin. The present results show that SHR have decreased DARPP-32 and therefore a diminished capacity to regulate protein phosphatase-1. Uninhibited, protein phosphatase-1 removes phosphate groups from CaMkinase II and glutamate receptors, inactivating them. This would impair glutamate signalling. Results are consistent with an attempt by SHR to upregulate glutamate transmission in striatum and prefrontal cortex.

11.1.3.3 Van Zyl, PJ

Behavioural Characterization of the Wistar-Kyoto Rat Model of Depression

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Purpose and Methods:

Major depression, a heterogeneous neuropsychiatric disorder, has received significant attention with regard to the monoamine theory of depression and forms the basis of our knowledge in clinical treatment of depression¹. However, in search of strategies to identify neurobiological changes and ultimately improve treatment outcomes, various animal models of depression have been studied. The Wistar-Kyoto (WKY) rat is hypersensitive to stressful situations and displays behavioural characteristics in patients with depression^{2,3}. It has also been found that behavioural differences are present between WKY rat strains, with the WKY from Charles River a more suitable model for depression^{4,5}. The aim of this study was to characterize the WKY rat as a model of depression by comparing depression-like behaviour in the forced swim test (FST), elevated plus maze (EPM) as well as open field test (OFT) between WKY rat strains and Wistar control strain.

Results:

The FST revealed significantly higher levels of immobility in both WKY rat strains relative to the Wistar strain. Significantly higher immobility was only found within onset of the FST in the Charles River WKY rat strain obtained from USA when compared with Harlan WKY obtained from UK. Both WKY strains displayed more passive behaviour in the OFT, without displaying any anxiety in the EPM compared to the Wistar strain. These findings are in accordance with previous results demonstrating behavioural and biological differences among different WKY rat strains and its respective controls. Currently the effect of the antidepressant desipramine is used to measure effects on ultrasonic vocalizations and FST behaviour in the WKY rat strain obtained from USA. This behavioural result will be used for follow-up biochemical analysis of protein changes at time of lifting of despair. The use of the animals was approved by the University of Cape Town Animal Ethics Committee (Ref no. 010/036).

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11.1.4 Session D

11.1.4.1 Soliman, MES

Computer Assisted Drug Design (CADD) and Bioinformatics: Tools for Designing Drugs and Understanding Their Activity

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Drug design is an integrated growing discipline. It involves the study of effects of biologically active compound on the basis of molecular interaction in terms of molecular structure or its physicochemical properties involved. The development of new methods in the field of molecular biology and computer science, has improved the tools for drug design significantly. More and more new drugs are developed with the help of computer technique. The field of bioinformatics has become a major part of the drug design that plays a key role for validation drug targets. Bioinformatics can help in understanding of complex biological processes and help improve in understanding of complex biological processes and help improve drug discovery.

CADD techniques have changed the way in which potential new drugs are discovered. In the past, a large number of synthetic chemical compounds or natural products for desirable effects were the only route. The increasing cost of screening compounds and the decreasing yield of new and unique lead compounds from the natural sources have made this route less favourable in recent years. Armed with the information that computational tools can provide, researchers in the pharmaceutical industry can precisely gain insight into the 3D structural and mechanistic features of a protein and hence create potential drug candidates.

Depending upon the information we have about the receptor and ligand, computer assisted drug design (CADD) could be divided into three major techniques, mechanism-based approach, direct drug design and indirect drug design.

Quantum mechanics/molecular mechanics (QM/MM) combined with molecular dynamic simulations (MD) have proved a robust tool for exploring the mechanistic features of glycosidase enzymes^{1,2}. A hybrid docking studies with MD simulations has been a robust technique to fully characterize the binding and interaction theme of synthesized potential HIV PR inhibitors³⁻⁵.

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11.1.5 Session E1 APSSA

11.1.5.1 Booysen, HP

Thiocaffeine Derivatives as Inhibitors of Monoamine Oxidase

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Purpose:

Parkinson's disease (PD) is the result of the degeneration of dopaminergic neurons in the Substantia Nigra pars compacta (SNpc) of the brain. This leads to striatal dopamine (DA) deficiency which is the cause of all the major symptoms of PD. The major enzyme responsible for the metabolism of DA in the brain is monoamine oxidase (MAO). There are two isoforms of this enzyme, MAO-A and MAO-B, which are attached to the outer membrane of mitochondria. Inhibitors of MAO are used to reduce the catabolism of DA and to conserve the depleted supply of DA in the Parkinsonian brain. Based on reports that C8-substituted caffeine analogues are potent reversible inhibitors of MAO, a series of 8-thiocaffeine derivatives were synthesized and evaluated as inhibitors of recombinant human MAO-A and MAO-B.

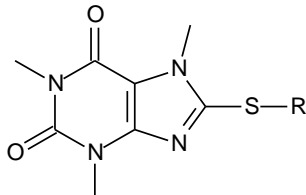
Methods:

The thiocaffeine derivatives were synthesized by reacting an appropriate thiol with 8-chlorocaffeine in the presence of a base. Ethanol served as solvent for these reactions. 8-Chlorocaffeine, in turn, was prepared by reacting caffeine with chlorine (Cl₂) in chloroform. The 8-thiocaffeine derivatives were subsequently evaluated as inhibitors of recombinant human MAO-A and -B using kynuramine as substrate. Since kynuramine is oxidized by the MAO's to 4-hydroxyquinoline, a fluorescent compound, the enzyme activities could be measured fluorometrically. The inhibition potencies were expressed as the IC₅₀ values. Time dependant studies were carried out and Lineweaver-Burke plots were constructed in order to determine if the thiocaffeines are reversible and competitive inhibitors of the MAO enzymes.

Results:

The results documents that the series of 8-thiocaffeine derivatives are MAO-B selective inhibitors. The most potent inhibitor was 8-(4-bromobenzyl)caffeine with an IC₅₀ value of 0.16 μM towards MAO-B and 2.61 μM towards MAO-A (Table 1). It was also shown that the thiocaffeine derivatives are reversible MAO-A and -B inhibitors and that they are competitive inhibitors.

Table 1. Selected 8-thiocaffeine derivatives and their IC₅₀ values for the inhibition of MAO-B.

	R	IC ₅₀ (μM)
	-(CH ₂) ₂ -C ₆ H ₅	0.223
	-CH ₂ -(4-Cl-C ₆ H ₄)	0.192
	-CH ₂ -(4-Br-C ₆ H ₄)	0.16
	-CH ₂ -(4-F-C ₆ H ₄)	0.34
	-(CH ₂) ₂ -O-C ₆ H ₅	0.332

11.1.5.2 Dawood, Y

Elucidation of the Effect of Oxidation and Crosslinking on the Drug Entrapment Efficiency and *In Vitro* Drug Release Behaviour of a Starch-Based Multiparticulate Drug Delivery System

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Purpose:

The purpose of the study was to develop a starch-based multiparticulate (SBM) drug delivery system through periodate mediated oxidation and subsequent covalent (epichlorohydrin: ECH) or ionic (sodium trimetaphosphate: STMP) crosslinking and to evaluate the effects of these modifications on the drug entrapment efficiency and drug release behaviour using model drug sulfasalazine (SFZ).

Methods:

Oxidation of starch multiparticulates: SBM samples (5g) were immersed in 50mL sodium(meta)periodate (SMP) solution (0.2M) and allowed to oxidize under constant stirring for 1.5 hours at a constant temperature of $25\pm 1^\circ\text{C}$. Thereafter the oxidized beads were removed, washed with 1000mL deionized water and allowed to dry at 50°C for 4 hours.

Crosslinking: Two sets of SBM samples (4g) were each immersed into 28.5mL of a gelling solution (20% sodium hydroxide (1M), 32% ethanol (99%^{v/v}) and 48% deionized water) and allowed to gel for 4 hours. Thereafter 1.5mL ECH (99%^{v/v}) or 1.5mL sodium STMP (60%^{w/v}) was added to each set respectively and allowed to crosslink under constant stirring for a further 2 hours at 25°C before being removed and dried at 50°C for 4 hours. The same procedure was conducted on oxidized SBM

Drug entrapment: Six formulations consisting of unmodified; oxidized; STMP-crosslinked, ECH-crosslinked, oxidized-ECH-crosslinked and oxidized-STMP-crosslinked SBM (1g) were each placed into 5mL SFZ solution (75mg/mL) and allowed to hydrate under constant stirring for 4 hours at 25°C before being removed and dried at 50°C for 4 hours. Samples from each batch (0.2g) were then homogenised in 10mL sodium hydroxide solution (0.1M) and SFZ content was analysed using UV spectroscopy.

Scanning electron microscopy: SEM analysis was performed on SBM to determine surface and core morphology.

Fourier Transformed Infra-Red Spectroscopy: FTIR was performed out to observe the effect of modification on SBM structure and mechanism of drug entrapment.

In vitro drug release: Drug release was performed using a USP dissolution apparatus II. Accurately weighed SFZ-loaded SBM (0.2g) were placed in 900mL PBS at $37\pm 0.5^\circ\text{C}$ and 50rpm. Samples (5mL) were withdrawn after 10 minutes and then hourly for 10 hours. SFZ content was analysed using UV spectroscopy and fractional SFZ release was determined.

Results and Conclusions:

Drug entrapment results demonstrate that modifications of SBM enhance their drug entrapment efficiency and further ECH-crosslinking of oxidized beads yielded the highest DEE of $40.90\pm 1.53\%$. FTIR results indicate that the SBM are modified during oxidation and crosslinking and show interactions with SFZ which does not occur with unmodified beads. SEM results show SFZ adsorbed onto the surface of the SBM which accounts for the burst release observed during in vitro drug release. SFZ was entrapped within the core of the SBM which facilitates the extended release observed. ECH-crosslinked-oxidized beads demonstrated the lowest burst release ($10.9\pm 0.67\%$) after 10 minutes and released only $39.16\pm 2.58\%$ of SFZ within 12 hours. Thus covalent-crosslinking of oxidized SBM proved to be the most promising method in formulating a drug delivery system.

11.1.5.3 Fourie, P

Neuroprotective Effects of Amantadine-Flavonoid Conjugates

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Purpose:

Neurodegenerative diseases like Parkinson's and Alzheimer's diseases affect millions of people around the world. Oxidative stress has been implicated in the pathogenesis of a number of neurodegenerative diseases, cancer and ischemia. The brain is particularly vulnerable to oxidative damage because of its high utilization of oxygen, high levels of polyunsaturated fatty acids, relatively high levels of redox transition metal ions and low levels of antioxidants. Oxidative stress occurs due to an imbalance in the pro-oxidant and antioxidant levels. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are highly reactive and react with biomolecules, including proteins, lipids, carbohydrates, DNA and RNA. It has been suggested that cognitive or memory defects are the consequences of oxidative stress and that the above mentioned imbalance potentiates neurodegeneration. Flavonoids possess a wide spectrum of biological activities including antioxidant activity. Another class of compounds, the polycyclic cage compounds (such as amantadine), which are NMDA-receptor antagonists, show that receptor antagonism can notably reduce neurodegeneration by blocking the Ca²⁺ influx which leads to cell death through apoptosis. For the purpose of this study amantadine-flavonoid conjugates were synthesised consisting of both an NMDA receptor inhibitor as well as an antioxidant moiety. The hypothesis being that neuronal degeneration will be restricted, *via* the high propensity of the compounds to scavenge reactive oxygen species as well as monitoring the proper functioning of Ca²⁺ homeostasis in the CNS.

Methods:

Amantadine-flavonoid derivatives were synthesised using standard laboratory procedures and structures were determined with standard methods such as NMR, IR and mass spectrometry. The synthesised compounds were tested in a selection of biological assays, to establish the relative antioxidant properties. The biological assays employed were the superoxide anion Nitro Blue Tetrazolium (NBT) assay and the lipid peroxidation Thiobarbituric Acid (TBA) assay. The NBT assay evaluated the ability of the synthesised compounds to scavenge free radicals and consequently inhibit the reduction of NBT to nitro blue diformazan, the free radical indicator. The ability of the synthesised compounds to inhibit hydrogen peroxide induced lipid peroxidation was assessed using the TBA assay.

Results:

Results obtained from both the NBT and TBA assay indicate weak antioxidant activity for the synthesised compounds when compared to the known antioxidant, Trolox[®].

11.1.5.4 Frank, D

Design and Characterization of Intravenously Administered Nanolipobubbles for Targeted Ovarian Cancer Therapy

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Purpose:

This study aims to develop a camptothecin (CPT)-loaded nanolipobubble (NLB) intravenous formulation for the targeted treatment of ovarian cancer. This research is based on the pre-formulation studies undertaken to develop nanoliposomes (NL's), as the precursor to NLB preparation, ranging in size between 100-150nm, to facilitate adequate extravasation through the leaky vasculature present in tumour tissue. Furthermore, drug release kinetics of the formulation was elucidated at physiological and tumour pH (pH 7.4 and 6.0, respectively), to determine the targeting potential of the formulation.

Methods:

Preparation of CPT-loaded and placebo NL's: Varying concentrations and ratios of distearolyphosphatidyl choline (0.1-0.3% w/v) and either distearolyphosphatidylethanolamine-m-PEG (DSPE-m-PEG) or cholesterol (CHO) (0.1-0.3% w/v) was dissolved in chloroform:methanol (9:1; 10mL). PBS (pH 7.4; 10mL) was subsequently added to the organic phase under ultra-sonication (60% amplitude for 90 seconds). Tween® 80, Span® 80 and polyvinylpyrrolidone (PVP) (0.1-0.3mL) were employed as surfactants. The resultant emulsion was subjected to evaporation under vacuum (65°C) for 3 hours, forming a NL suspension. In CPT-loaded formulations, CPT was incorporated during ultrasonication.

Determination of NL size and size distribution: The NL suspension was analysed for size and size distribution profiles over a 3 hour period, whilst being maintained at 37°C. Effect of CPT loading on size of the NL's was investigated.

Elucidation of drug release profiles: NL's were centrifuged to remove unbound drug, and the sediment was re-suspended in PBS (pH 7.4 and 6.0; 100mL) and maintained in a shaker bath at 37°C at 25 rpm. Samples were withdrawn at pre-determined intervals and drug release was evaluated by UV spectroscopy.

Evaluating interactions of formulation components: Formulations were subjected to Fourier Transform Infra-Red (FTIR) analysis to determine the interactions of lipids and surfactants, and the effects of these interactions on the drug release kinetics and stability of the formulation.

Determination of NLB lifetime: Sulphurhexafluoride gas was incorporated into the NL, forming NLB's. The NLB's were injected into a hydrogel and observed using a microultrasound imaging system.

Results:

Variation in the lipid component and surfactant proved to be a factor in size and stability of the NL's. In general, the use of CHO instead of DSPE-m-PEG resulted in a 20-40nm increase in NL size and an unfavourable size distribution profile (PDI>0.2). Although more stable, PVP-containing formulations also displayed a dramatic increase in size (>200nm). Drug release was fairly rapid (100% in 15 minutes), thus polymer coating is being investigated as a means of retarding drug release. FTIR analysis highlighted only a physical interaction between lipids and surfactants, which had no significant influence on stability or drug release kinetics. NLB lifetime studies are currently underway.

11.1.5.5 Hazle, D

Evaluation of the Physicochemical and Physicomechanical Properties of Optimized Ciprofloxacin- and Diclofenac-Loaded Co-Blended Alginate Fibers for Oramucosal Delivery

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Purpose:

Periodontal disease (PD), a prevalent condition worldwide, is characterized as a chronic bacterial infection with cascading inflammatory reactions that if left untreated may lead to the permanent tooth loss. The aim of the study was to design, formulate and evaluate (*in vitro*) a novel polymeric matrix system to deliver an antimicrobial and anti-inflammatory drug over 10 days for the treatment of PD.

Methods:

Alginate combined with glycerol was crosslinked with barium cations forming a monolithic fiber incorporating ciprofloxacin and diclofenac sodium as the model antimicrobial and anti-inflammatory agents respectively. A 3-Factor Box-Behnken Design was employed to statistically optimize the fibers according to their tensile properties and drug release. The optimized formulation (3.14%^{w/v} alginate, 22.54mL glycerol and 10.00%^{w/v} barium chloride) was evaluated according to drug entrapment, drug release and hydration behaviour at pH 4 and 6.8, vibrational transitions (FTIR), antimicrobial activity and tensile properties.

Results:

Drug release at pH 4 occurred as a result of drug diffusing through the polymeric matrix however at pH 6.8 the disruption of the fiber structure led to drug release as a consequence of the swelling and erosion of the matrix. Ciprofloxacin was sufficiently released from the drug-loaded fibers inhibiting growth of *Escherichia coli*, *Enterococcus faecalis* and *Streptococcus mutans* over 10 days. The physicomechanical and physicochemical properties were related to the degree of crosslinking, the effect of the plasticizer and the interaction of formulation components affecting the strength, flexibility and drug release from the matrix, which may be attributed to monomeric composition of polymer and the crosslinker ion present as well as the interactions with the plasticizer. The promising *in vitro* results advocate further analysis of the fibers.

11.1.6 Session E2 SASBCP

11.1.6.1 Brand, SJ

Role of the Peroxisome Proliferator Activated Receptor (PPAR)- γ Pathway in Mood Regulation and Antidepressant Action

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Introduction:

A high incidence in major depression world-wide, a serious psychiatric disorder, and the evident shortfalls in efficacy of current drug treatments have stimulated research to explore new avenues to identify novel drug targets. While new hypotheses have focused on impaired neurogenesis and neurotrophic signalling in depression, the importance of cardiovascular, inflammatory and metabolic disorders as risk factors for developing depression has opened up new avenues for investigation. The peroxisome proliferator-activated receptor (PPAR)- γ is a major signalling molecule in the action of insulin, of which its agonist rosiglitazone has recently been demonstrated to display antidepressant-like effects in rodents. Furthermore, clinical evidence has confirmed the beneficial effects of PPAR- γ agonists in depression, putatively due to their neuroprotective, neurotrophic, insulin sensitizing and anti-inflammatory actions. The purpose of this study was to investigate the possible antidepressant-like effects of the closely related PPAR- γ agonist, pioglitazone, in a genetic animal model of depression and to compare this to gliclazide, a non-insulin-sensitizing hypoglycaemic drug. Furthermore, we investigated whether pioglitazone or gliclazide can augment the antidepressant-like actions of imipramine, a prototype antidepressant.

Methods:

Animals: Male FSL rats (weighing 220-250g) were used. *Drug Treatment:* Rats (n=12/group) received either the vehicle, pioglitazone (30; 70; 120 mg/kg p.o.), imipramine (20 mg/kg p.o.) or gliclazide (10 mg/kg p.o.), pioglitazone (70 mg/kg p.o.) + imipramine (20 mg/kg p.o.) co-administration or gliclazide (10 mg/kg p.o.) + imipramine co-administration. All drugs were administered once daily for seven days in a 4% isopropyl-methylcellulose suspension via oral gavage (2.5ml.kg⁻¹). *Behavioural testing:* Animals were assessed in the forced swim test (FST) in order to determine antidepressant-like effects, together with assessment of locomotor activity. Cognitive performance was accessed using the novel object recognition task (ORT).

All experiments were performed in accordance with the ethics application NWU-00099-10-S5 as approved by the research ethics committee of the North-West University, Potchefstroom.

Results:

As expected, imipramine produced robust antidepressant-like effects in FSL rats, as observed in the FST and ORT. Pioglitazone, however, failed to demonstrate any notable antidepressant-like response in the FST at any of the doses tested, nor did it alter cognitive performance in the ORT or augment imipramine's effects in either the FST or ORT.

Conclusion:

Contrary to earlier studies performed in healthy animals using rosiglitazone, pioglitazone failed to demonstrate a notable antidepressant response in FSL rats. The lack of effects may either be due to pharmacokinetic issues associated with the oral route or pioglitazone's low potency on the PPAR-receptor (compared to that of rosiglitazone). Using the subcutaneous route as an alternative will be explored. Further studies using rosiglitazone as alternative to pioglitazone may be necessary.

11.1.6.2 Erasmus, M

An Investigation into the Role of Noradrenergic Receptors in Conditioned Fear: Relevance to Posttraumatic Stress Disorder (PTSD)

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Purpose:

Post-traumatic stress disorder (PTSD) is characterized by enhanced recall and reduced extinction of fear memory following a prior traumatic event. Current treatment options remain inadequate in treating the disorder and a deeper knowledge of the neurobiological pathways underlying the processing of fear memory is required. Noradrenalin plays a prominent role in this process, and clinical studies have suggested that immediate post-trauma administration of the non-selective β -blocker, propranolol, may be effective in preventing the later onset of PTSD. However, more research is required to delineate the separate roles for α_1 , α_2 , β_1 and β_2 receptors in fear memory acquisition, consolidation and extinction. The aim of this study is to set up a behavioural method for analysing fear memory processing and to validate the role of noradrenergic receptors in this response.

Methods:

Male Wistar Rats (180-230 g) were exposed to a fear conditioning passive avoidance paradigm based on the premise that centrally acting (eg. propranolol) but not non-centrally acting (eg. nadolol) β -blockers prevent the development of PTSD. An acute treatment three-tiered dose response analysis of propranolol (5, 10, 20 mg/kg sc) and nadolol (2, 10, 20 mg/kg sc) was undertaken to establish predictive and construct validity. Thereafter centrally active α_1 (prazosin: 0,1;1,5mg/kg sc), α_2 (yohimbine: 1;5;10 mg/kg sc), β_1 (betaxolol: 1;5;10 mg/kg sc) and β_2 (ICI 118551: 0,4;1,0; 4,0 mg/kg sc) selective antagonists were studied. The test involved 2 day conflict training where latency to move from a lighted to a darkened chamber, and the subsequent experiencing of a foot shock in the darkened chamber, was assessed. Drug or vehicle (1 ml/kg sc) was administered after training on day 1 as a single dose with avoidance behaviour assessed 24 hrs later. All experiments were approved under ethics approval number NWU-00007-11-S5 by the Research Ethics Committee of the North West University Potchefstroom.

Results:

Post-trauma administration of propranolol, 10mg/kg, was maximally effective in reducing fear memory, while nadolol was without effect, thus confirming the critical role of central β -adrenoceptors in the consolidation of fear memory. Further elaboration of these findings using adrenoceptor-selective modulators found that the β_1 selective antagonist, betaxalol dose-dependently attenuated fear conditioning, with a maximal effect at 10mg/kg. Similarly, the β_2 selective antagonist, ICI 118551, also attenuated fear conditioning but only at doses of 1mg/kg or lower, with higher doses having no effect. The α_1 antagonist, prazosin, was effective in attenuating passive avoidance behaviour at all doses tested, while the α_2 antagonist yohimbine was ineffective. In conclusion, both β_1 and β_2 receptors play a dominant role in mediating passive avoidance responding. These data underscore the value of propranolol, a non-selective β -blocker, in preventing the development of PTSD. While the inhibition of α_2 receptors has minimal therapeutic benefit in this model, selective block of α_1 receptors does offer clinical utility in the treatment of conditioned fear responses, and thus supports clinical evidence of its use in PTSD.

11.1.6.3 Fasinu, PS

***In vitro* Investigation of the Effects of Commonly Used South African Medicinal Herbs on CYP1A2 activity Employing Human Liver Microsomes**

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Purpose:

Herb-drug interaction (HDI) is a major clinical concern especially with concomitant consumption of medicinal herbs and prescription medicines. *In vitro* liver-based technologies present scientifically relevant medium for investigating the potential for HDI. CYP1A2 is one of the major metabolic enzymes accounting for 11% of all CYP-mediated drug metabolism. The purpose of the current study therefore, was to investigate the effects of 15 commonly used herbal medications in South Africa on the metabolic activity of CYP1A2 employing human liver microsomes (HLM) and phenacetin as a standard probe substrate.

Methods:

Graded concentrations of phenacetin (0.25 – 50µM) were incubated with 0.25mg/mL HLM in Eppendorf tubes for 15 min in a shaking water bath (100rpm; 37°C). An NADPH-regenerating system prepared with glucose-6-phosphate dehydrogenase and reduced NADP⁺ in a cofactor solution was used to initiate the metabolic reaction. The reaction was halted by the addition of ice-cold acetonitrile (-20°C) containing 2µg/mL thiacetazone as an internal standard. The resulting mixture was centrifuged (5000g, 10 min) to precipitate the microsomal proteins. Supernatants were analysed by HPLC/MS. The methodology was optimized by profiling the rate of metabolism against the substrate concentrations to determine the enzyme kinetic parameters (K_m and V_{max}). Further incubations were performed at the determined K_m in the presence of the herbal extracts, prepared in percentage dilutions, according to the recipe of the traditional healers. Control incubations with α -naphthoflavone, a CYP1A2-specific inhibitor, were performed concurrently. The influence of the aqueous herbal extracts on CYP1A2 activity was determined and compared. All determinations were performed in triplicates.

Results and Discussion:

The results showed that 7 out of the 15 herbal extracts investigated demonstrated significant inhibition of phenacetin metabolism to varying degrees (30-86%). These included *Hypoxis hemerocallidea*, *Spirostachys Africana*, *Bowiea volubilis*, *Chenopodium album*, *Kedrostis Africana*, *Lauridia tetragonia* and *Pachycarpus concolor*.

Conclusions:

Given the role of CYP1A2 in the metabolism of a wide range of therapeutically important drugs, the results showed a potential for herb-drug interaction especially if such drugs are administered concomitantly with the herbal medications investigated.

Ethics:

This research was approved by the University of Stellenbosch Human Research Ethics Committee with Ethics Reference number N10/09/307

References:

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Acknowledgement:

Hope Cape Town, for financial support.

11.1.6.4 Govender, K

A Bioavailability Study of Lumefantrine in Mice; Evaluating the Application of Pheroid™ Formulation

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Purpose:

Each year 350-500 million cases of malaria occur world-wide and over 1 million people die, most of them young children in sub-Saharan Africa. The clinical efficacy of most antimalarials may be undermined due to poor oral bioavailability. This dilemma may be addressed by applying novel methods of drug formulation and delivery systems to current antimalarial agents. Lumefantrine (lume) is a lipophilic antimalarial with variable oral bioavailability reportedly due to the 'food effect'. This in turn results in compromised treatment efficacy. Pheroid™ technology is a fatty acid based delivery system that may be applied in drug formulation. The aim of this study was to determine if an improvement in lume solubility using the Pheroid™ technology would improve its bioavailability and eliminate the reported 'food effect'.

Methods:

Study design: The experiment was designed with the intention of applying population PK modelling to the data. The reference group was administered lume dissolved in a standardized oral solution of DMSO:Water (1:9). The test group was administered the Pheroid™ formulated lume. Each group included 20 mice dosed via oral gavage at 10mg/kg. Whole blood samples (20ul) were collected via tail bleeding at 0.5, 1, 1.5, 3, 5, 7, 10, 12 and 24 hour time points, alternating blood collection between two subgroups of 10 mice. The blood samples were collected in Eppendorf tubes and stored immediately at -80°C until sample analysis. The experiment was performed twice, firstly in the fed state during which food was available to mice throughout the experiment, and secondly in the starved state during which the mice were starved for 12 hours prior to drug administration and food returned 1 hour after drug administration. Ethical clearance no: 010/027.

Instrumentation & Data Analysis: An Agilent 1200 series HPLC system and Applied Biosystems API 3200 triple quadrupole mass spectrometer was used to analyse samples. Whole blood samples were extracted using a protein precipitation method and chromatography was performed using a PFP column.

Results and Conclusions:

In the reference group the highest calculated blood concentration of lume was 90ng/ml at 5 hours post dose and 2126ng/ml at 1.5 hours post dose, under fed and starved conditions respectively. In the test group the highest calculated blood concentration of lume was 1060ng/ml at 10 hours post dose and 1048ng/ml at 5 hours post dose, under fed and starved conditions respectively. In the reference group results were observed which differed to published reports, with lume concentrations in the starved group more than 20 times the concentration under fed conditions. In the test group there is a 1.1% difference between the fed and starved states, indicating improved consistency with the use of Pheroid™ formulated lume. It may be concluded that when lume is administered in a soluble form, the associated 'food effect' and variable oral bioavailability may be overcome.

11.1.7 Session F1 APSSA

11.1.7.1 Jhundoo, HD

Formulation Development and Characterization of Labrafil[®] M 2130 CS Solid Lipid Microspheres for the Oral Delivery of Ketoconazole

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Purpose:

Lipid-based formulations are promising drug delivery systems that may be used to enhance the oral bioavailability of hydrophobic molecules when conventional formulations fail to achieve a similar purpose. The objective of this research was to develop, optimize and characterize aqueous dispersions of solid lipid microspheres (SLM) for the hydrophobic drug, ketoconazole (KTZ) in an attempt to improve its oral bioavailability.

Methods:

KTZ-loaded SLM aqueous dispersions were manufactured using a simple micro-emulsion technique. Four batches *viz.*, batches SLM 01-SLM 04 were manufactured using Labrafil[®] M 2130 CS as the solid lipid matrix and a surfactant system consisting of Pluronic[®] F68, Tween 80 and sodium cholate. The crystalline and polymorphic nature of Labrafil[®] M 2130 CS and a binary mixture of Labrafil[®] M 2130 CS and KTZ before and after exposure to a temperature of 60°C for one hour was investigated using DSC and FT-IR prior to formulation development and optimization studies. SLM dispersions were characterized in terms of particle size, shape and surface morphology using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The zeta potential (ZP) of SLM was evaluated in HPLC water with a conductivity of 18 µS/cm using a Zetasizer Nano. Drug loading capacity (DLC) and encapsulation efficiency (EE) of SLM for KTZ were determined by ultrafiltration and HPLC. The stability of SLM dispersions was investigated on days 0, 7, 30 and 90 following storage at 25°C/60% RH and 40°C/75% RH.

Results:

DSC data of Labrafil[®] M 2130 CS showed the presence of a single thermal event prior to and after heating, suggesting that exposure of the lipid to relatively high temperatures does not change its polymorphic form. These data were supported by FTIR, which revealed lack of significant change in chemical bond shift, broadening of peaks or occurrence of unique bands in the IR spectrum. However, both DSC and FTIR data showed a slight decrease in the crystallinity of the lipid following exposure to heat. DSC data of a binary mixture of Labrafil[®] M 2130 CS and KTZ prior to and following exposure to heat revealed the presence of separate peaks typical of Labrafil[®] M 2130 CS and KTZ. In addition, there was a slight decrease in the peak maximum of Labrafil[®] M 2130 CS following the addition of KTZ to the lipid. FT-IR data of the binary mixture were similar to those obtained for KTZ and Labrafil[®] M 2130 CS separately. SEM and TEM data showed the presence of discrete spherical microspheres with a smooth surface with size ranging between 12.7-18.6 µm on the day of manufacture. However, aggregation of particles occurred for batches SLM-01 and SLM-02 following one day and one week of storage at 40°C/75% RH and 25°C/60% RH, respectively. Particles in Batches SLM-03 and SLM-04 were relatively stable at 25°C/60% RH as aggregation could only be observed after at least one-month of storage and the ZP of the particles in these batches remained relatively constant. The DLC and EE of SLM for KTZ were between 0.3-0.9% and 24-58% respectively on the day of manufacture and remained relatively constant following storage for three months.

Conclusions:

Discrete and spherical KTZ-loaded SLM may be produced by a simple micro-emulsion technique using Labrafil[®] M 2130 CS. Two batches of the four batches manufactured *viz.*, SLM-03 and SLM-04 were relatively stable following storage at 25°C/60% RH for at least one month, albeit were highly unstable when stored at 40°C/75% RH.

Acknowledgements:

The authors acknowledge financial support from the Joint Research Committee of Rhodes University (RBW) and the Andrew Mellon Foundation (HDJ).

11.1.7.2 Magnus, L

Development and Assessment of Extemporaneous Famciclovir Oral Formulations for Paediatric Use

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Introduction:

Active Pharmaceutical Ingredients (API) such as the antiviral agent, famciclovir (FCV), that are required for paediatric treatment are not commercially available in age-appropriate dosage forms. Common practice is therefore the preparation of an oral liquid dosage form using commercially available tablets, capsules or powdered API and dispersing or dissolving it in a vehicle that a patient can easily swallow. Commonly used vehicles include methylcellulose, syrup simplex or a combination thereof or where possible, commercially available suspending agents such as Ora-Sweet[®] can be used. The manipulation of adult dosage forms however by crushing, reconstitution or division can lead to inaccurate dosing, degradation and loss of the API. Furthermore several factors are overlooked when manufacturing extemporaneous formulations that include the physical and chemical properties of the API and excipients, compatibility between the API and excipients in addition to potential stability and bioavailability issues. The aim therefore, is to develop and assess the stability of extemporaneously prepared FCV suspensions for paediatric use.

Method:

The stability of FCV 25 mg/ml suspensions was assessed in syrup, hydroxypropyl methylcellulose, Ora-Sweet[®] and a buffered aqueous solution (pH 6) after storage at 25 °C/60% RH and 40 °C/75% RH over a 6 week period. Formulations were manufactured using methylparaben and propylparaben alone or in combination and sodium metabisulphite, ascorbic acid or citric acid were used as antioxidants. The formulations were manufactured using FCV powder or FCV from commercially available crushed tablets. The resultant formulations were assessed for API content using a validated stability-indicating HPLC assay, viscosity and appearance and the data was analysed using statistical methods. The degradation rates were calculated for each formulation and a degradation rate profile plotted. Investigations into the compatibility of FCV and various excipients were undertaken using infra-red spectroscopy (IR) and differential scanning calorimetry (DSC).

Results and discussion:

FCV was found to undergo major degradation in the presence of sucrose, as seen in the formulations in which syrup and Ora-Sweet[®] were used as the vehicle. FCV was found to be more stable when the API powder, dispersed in HPMC or the buffered aqueous solution (pH 6) was used as the vehicle. The stability of FCV was found to be dependent on the type of excipients used and investigations into the compatibility of FCV with the various excipients used in the formulations identified potential interactions between the API and some formulation components. The viscosity of the formulations varied, with some showing a decrease in viscosity over the 6 week period. Colour changes were observed for the formulations in which syrup was used as the vehicle.

Conclusion:

FCV is more stable in HPMC based vehicles when used as API powder. Careful consideration of excipients is necessary for the formulation of stable oral dosage forms for paediatric use. In particular caution is required when extemporaneous manufacturing approaches are used to produce age-specific dosage forms.

Acknowledgements:

The authors acknowledge financial support from the Joint Research Committee of Rhodes University and the NRF (RBW), the Henderson and DAAD scholarship programmes (LM).

11.1.7.3 Moraal, C

The Design, Synthesis and Evaluation of Aminocaffeine Derivatives as Inhibitors of Monoamine Oxidase

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Purpose:

Monoamine oxidase (MAO) is responsible for dopamine catabolism in the brain and is therefore especially important in the treatment of Parkinson's disease (PD). MAO-B inhibitors provide symptomatic relief by reducing dopamine catabolism in the PD brain. (E)-8-(3-Chlorostyryl)caffeine (CSC) is a reversible inhibitor of MAO-B and also an antagonist of the adenosine A_{2A} receptor. Since adenosine A_{2A} receptor antagonists are also used in the treatment of PD, compounds such as CSC, which blocks both the A_{2A} receptor and inhibits MAO-B, may have enhanced therapeutic potential in the treatment of PD. In this study a series of 8-aminocaffeine derivatives were synthesized and tested as inhibitors of MAO-B. These aminocaffeines are structural analogues of CSC, and may possess similar biological properties with respect to the inhibition of MAO-B and the antagonism of A_{2A} receptors.

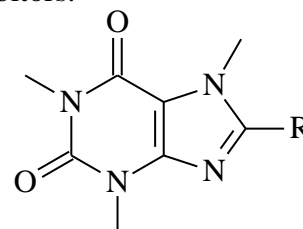
Methods:

8-Chlorocaffeine was condensed with the appropriate amine at high temperatures to produce 8-aminocaffeine derivatives. The inhibitory activities of the compounds were towards recombinant human MAO-A and -B, determined and expressed as the corresponding IC₅₀ values.

Results:

The results showed that human MAO-B was most potently inhibited by 8-[methyl(4-phenylbutyl)amino]caffeine with an IC₅₀ value of 2.97 μM. Human MAO-A was most potently inhibited by 8-[2-(3-chlorophenyl)ethylamino]caffeine with an IC₅₀ value of 5.78 μM. It was found that methylation of the amine group increased inhibition activity and selectivity towards MAO-B. For example 8-[4-(phenylbutyl)amino]caffeine inhibited MAO-B with an IC₅₀ value of 7.56 μM while its methylated derivative, 8-[methyl(4-phenylbutyl)amino]caffeine, has an increased inhibition with an IC₅₀ value of 2.97 μM. The selectivity for MAO-B inhibition also increased compared to MAO-A when the amine was methylated. For example 8-[4-(phenylbutyl)amino]caffeine inhibited MAO-A with an IC₅₀ value of 25.51 μM (selectivity index, SI = 3.38), while the methylated amine, 8-methyl(4-phenylbutyl)aminocaffeine, inhibited MAO-A with a value of 37.7 μM (SI = 12.69). From these results it may be concluded that methylated 8-aminocaffeine derivatives are promising lead compounds for the development of selective MAO-B inhibitors.

-R	IC ₅₀ (μM) MAO-A	IC ₅₀ (μM) MAO-B
-NHC ₅ H ₈ C ₆ H ₅	25.51 ± 10.28	7.56 ± 0.54
-NCH ₃ C ₄ H ₈ C ₆ H ₅	37.70 ± 6.4	2.97 ± 0.54
-NHC ₂ H ₄ C ₆ H ₄ Cl	5.78 ± 0.41	4.91 ± 3.45



11.1.7.4 Patel, S

Developing materials to meet the information needs of caregivers of paediatric patients

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Purpose:

Medication errors are common, harming at least 1.5 million Americans and costing the USA \$3.5 billion per year. Focused patient counselling on appropriate medication use may help to reduce medication administration errors. In South Africa, two of the main barriers to pharmacist-patient counselling are language and low literacy. Although incorrect administration of medicines to children is commonly reported in the literature and by local health providers, no tools or aids exist to facilitate the communication process with caregivers having limited health literacy. The objective of this study was to develop a simple poster incorporating visual aids to communicate paediatric medicine administration information targeted at low-literate caregivers; to evaluate its effectiveness in communicating the information and on knowledge acquisition; and to investigate the association of demographic variables with knowledge.

Method:

Pictograms were designed illustrating methods of administering oral liquid dosage forms and eye, ear and nose drops to children. A black and white A3-size poster incorporating pictograms and a minimum of simple text was developed and was translated into Shona. Interviews were conducted with 32 caregivers via an interpreter at a public healthcare facility in Harare. Ethical approval was obtained from the Faculty of Pharmacy's Ethics Committee and permission to conduct the interviews was obtained from Belvedere Maternity Hospital, Harare. Inclusion criteria included: role as caregiver, over the age of 21 years, first language Shona speaker. Demographic data were recorded and baseline knowledge relating to medicine administration was evaluated using a 12-item test. After studying the poster, caregiver knowledge was re-evaluated. Significance of the change in knowledge scores from baseline to post-baseline was evaluated using the Student's T-test and the association of selected demographic variables such as age, sex and education with knowledge was analysed using ANOVA at a significance level of 0.05.

Results:

Despite the majority of caregivers (84%) having more than 10 years of schooling, baseline knowledge was poor (45.6%) indicating a high potential for medication errors. After reading the poster, knowledge increased significantly to 69.3%. Education, age and sex were not significantly associated with knowledge. A simple poster containing pictograms accompanied by a minimum of text was shown to significantly increase caregiver knowledge of paediatric medication administration. The combination of pictorial illustrations and simple, easy to read text served as a useful aid in communicating health information. However, when used alone, the poster proved inadequate in fully informing readers on methods of administering medication. Verbal counselling, using this poster as a tool for caregiver education, is therefore preferable and constitutes best practice. Based on the findings of this study, a checklist was developed to assist healthcare providers design simple illustrated health information.

11.1.7.5 Roux, W

The isolation of three compounds from *Cotyledon orbiculata* and their antioxidant activities

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Purpose:

Epilepsy is one of the world's most prevalent central nervous system disorders and affects more than 70 in 1000 children in South Africa. Most of these cases are people in rural areas of South Africa where communities rely on the use of traditional medicine. *Cotyledon orbiculata* is widely used in traditional medicine to treat epilepsy and other central nervous system disorders. Oxidative stress and dysfunction of the mitochondria are emerging as important factors in the pathogenesis of epilepsy. Oxidative stress is not only the result of seizures, but also contributes to epileptogenesis. The need to screen these plants for antioxidant activity and toxicity is very important to understand underlying mechanisms of action, efficacy of treatment and safety of traditional medicine.

Methods:

Isolation of compounds: Plant material of *Cotyledon orbiculata* was extracted using a soxhlet extractor and methanol as solvent. These extracts were filtered and concentrated using a Buchi rotavapor. The concentrated extracts were then UV irradiated to reduce the amount of chlorophyll in the mixture. The resulting liquid was analysed using HPLC (high pressure liquid chromatography) and three major peaks were selected for isolation by using an Agilent 1200 series fraction collector.

Biological assays: Three assays were performed to assess the antioxidant activity and toxicity of the isolated compounds.

Thiobarbituric acid assay: This assay quantifies the extent of the inhibition of lipid peroxidation in rat brain homogenates by the isolated compounds. The concentration of the isolated compounds used in the assay ranged from 0.3125 mg/ml to 2.5 mg/ml.

Nitroblue tetrazolium assay: This assay quantifies the ability of extracts to scavenge superoxide radicals in a biological sample (rat brain homogenate). The NBT assay was performed using the same concentrations range for the isolated compounds as in the TBA assay.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay: The MTT assay indicates the toxicity of isolated compounds towards mammalian cells. Neuroblastoma cells were used to test the compounds in a concentration range of 0.08 mg/ml to 10 mg/ml.

Results:

All compounds were able to attenuate lipidperoxidation as seen from the results obtained from the TBA assay. Compound 1 and 2 had the highest activity, with compound three having the weakest activity. Compound 2 had the highest activity at a concentration of 1.25 mg/ml.

All compounds were able to scavenge superoxide radicals. Compound 1 showed the best activity. Compound 1 showed activity across the whole concentration range, where compound 2 and 3 were only active in some concentrations.

Compound 2 at a concentration of 10 mg/ml had toxic effects on the neuroblastoma cells used. The other 2 compounds were non-toxic.

11.1.8 Session F2 SASBCP

11.1.8.1 Mokoena, M

Ozone-Induced Oxidative Stress is Associated with a Depressogenic Effect in Stress-Sensitive Rats

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Purpose:

Depression has been associated with oxidative stress, while a role of environmental toxins in general has also been linked to an increased incidence of anxiety-related disorders. Ozone in particular, is an urban environmental toxin and chronic exposure to ozone per inhalation is associated with systemic and central oxidative stress. There is therefore a need to clarify the role of environmental toxin – induced oxidative stress on the development of mood disorders and the effects on antidepressant action. In this study we investigated the effects of ozone inhalation as a chronic stressor on depression-related behaviour and markers of neurobiological stress and antidepressant response in stress sensitive rats.

Methods:

Flinders Sensitive Line (FSL – stress-sensitive) and Flinders Resistant Line (FRL) male rats were provided by the Animal Centre of the North-West University (Ethics approval number: NWU-00008-07- A8).

Ozone Exposure and Drug Treatment:

The rats inhaled either 0 or 0.3 parts per million (ppm) ozone 4 hours daily for 15 days and simultaneously treated with saline or hydroxypropyl methylcellulose control, desipramine (15 mg/kg) or melatonin (50 mg/kg) 2 hours before dark cycle begins.

Novel Object Recognition Test (NORT):

To measure visual learning and memory, the rats were habituated in the NOR box for 10 minutes 2 days prior to the testing day. On the day of testing the rats were exposed to 2 experimental trials. In the 1st trial each rat was exposed to 2 identical objects for a period of 5 min. Following 90 minutes inter-trial interval, one familiar object was replaced with a novel object in the 2nd trial and the rat left to explore for 5 minutes. Total object exploration time was scored for both trials.

Elevated Plus Maze (EPM):

The elevated plus maze was used to assess anxiety responses of the rats. Each rat was placed at the centre of a cross-shaped elevated maze with two open arms without walls and two enclosed by high walls. The behaviour was recorded for 5 minutes. The behaviours recorded were the time spent and entries made in the open and closed arms.

Forced Swim Test (FST):

To assess depressive-like behaviour and antidepressant action, the rats were each placed in a cylindrical tank containing water and video recorded for 7 minutes. Immobility, climbing and swimming behaviour was scored to isolate depressive-like behaviour, adrenergic and serotonergic mechanisms respectively.

Results:

Ozone impaired memory in the novel object recognition test, induced anxiogenic activity in the elevated plus maze and depressive-like effects in the forced swim test. Additionally, ozone attenuated the antioxidative activity of melatonin and the antidepressant-like effects of desipramine. The data therefore suggest that chronic ozone inhalation may be depressogenic in stress sensitive rats. It furthermore emphasizes a causal role of environmental toxins in the epidemiology of affective disorders such as major depression.

11.1.8.2 Moller, M

Social Isolation Rearing in Rats Alters Plasma Tryptophan Metabolism and is Reversed by Sub-chronic Clozapine Treatment

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Purpose:

Schizophrenia is associated with increased oxidative stress, although the source of this redox disequilibrium is unknown. Altered tryptophan metabolism has been described in the disorder, possibly linked to inflammation and glutamate-directed excitotoxicity. We have demonstrated that social isolation rearing (SIR) in rats, a putative neurodevelopmental animal model of schizophrenia, is associated with cognitive and other behavioural changes akin to schizophrenia, as well as altered frontal cortical D₁ and NMDA receptor binding and increased oxidative stress. These bio-behavioural changes are reversed by antipsychotic treatment. Tryptophan is catabolized via the kynurenine pathway to quinolinic acid (QA), a NMDA receptor agonist with excitotoxic actions; kynurenic acid (KYNA), a NMDA receptor antagonist, purported to have neuroprotective actions; and 3-hydroxy-anthranilic acid (3OHAA), a pro-apoptotic messenger. Together they contribute to the neuroprotective-neurodegenerative balance in the brain, which in turn will impact on cellular redox. To explain increased oxidative stress in SIR rats and in schizophrenia, we studied whether tryptophan metabolism and neuroprotective-neurodegenerative balance are altered in post-natal SIR rats. Moreover, we also investigated whether any observed imbalance in tryptophan metabolism could be reversed with clozapine.

Methods:

Male Sprague-Dawley (SD) rats (10 rats/group) were used, (Ethics approval number NWU-0035-08-S5). In a non-treatment arm, two groups of rats were randomly separated at weaning and exposed to either 8 weeks SIR or 8 weeks social rearing. In the treatment arm, four groups of rats exposed to either SIR or social rearing received either saline or clozapine (5mg/kg i.p) for the last 11 days of rearing. Animals were sacrificed and plasma tryptophan, kynurenine, KYNA, anthranilic acid, 3OHAA and QA were analysed using a solid phase extraction (SPE) liquid-chromatography electrospray ionization tandem mass spectrometry method, developed and validated in our laboratory for the simultaneous detection of tryptophan and the above-mentioned metabolites. The neuroprotective ratio was expressed as: Neuroprotective ratio = 1000 x plasma KYNA (μM) / plasma kynurenine (μM).

Results:

Plasma tryptophan, kynurenine, anthranilic acid, 3OHAA and QA were significantly elevated in treatment naive and saline treated SIR rats versus their socially housed controls, with a significant decrease in KYNA as well as a significant decrease in the neuroprotective ratio. Clozapine treatment significantly reversed these alterations in SIR animals, with limited effects in the socially reared controls. SIR in rats thus significantly disrupts tryptophan metabolism via the kynurenine pathway with increased risk for neurodegenerative changes in the brain, possibly explaining the behavioural and neurochemical alterations observed in SIR animals and possibly in schizophrenia.

11.1.8.3 Ozokwere, J

Comparative Performance of Sprague-Dawley Rat Hearts Using DMSO and DMF as Cryoprotectants

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Purpose:

Heart transplantation is one of the most effective treatment options for congestive heart failure. Current organ storage methods can preserve the human heart for only about four to six hours. The organ donor pool could be dramatically increased if the preservation time could be lengthened and hearts stored for weeks or even month's prior to transplantation. This study describes the performance characteristics of explanted Sprague-Dawley rat hearts before and after cryopreservation using 10 % dimethylsulphoxide (DMSO) and 30 % dimethylformamide (DMF) in Tyrode solution.

Methods:

A modified Morgan perfusion model was used for this study. Male Sprague-Dawley (ethical approval AREC/2009/09/002) hearts were harvested and arrested in a cold (< 10°C) Tyrode solution (pH 7.4) for 5 minutes. The hearts were mounted on the aorta and vena cava to allow reperfusion in a doubled walled water jacket at 37°C for baseline performance studies. The hearts (n=3) were cooled to 4, -20, -80 and -176°C (liquid nitrogen), and stored for 6 hours. This study was extended to 48 hours and 7 days at -176°C (n=6). Cardiac output (aortic and coronary) and an electrocardiogram were obtained during baseline studies, followed by cryopreservation and after thawing at times T0, 10, 20, 40, 60, 120 min, 6, 8, 12 and 24 hours. Reperfused hearts were monitored for as long as possible. Ethical approval (AREC/2009/09/002) for the use of laboratory animals was obtained from the Tshwane University of Technology, Ethics Committee and the Animal Ethics committee before experimental work commenced.

Results:

The average heart rate of the Sprague-Dawley rats reduced from 396 beats / minutes to 184 beats / minutes after anaesthesia. The average survival time of the hearts under the experimental conditions were 7 hours 32 minutes with an average aortic output at 8 hours of 0.62 ml and 0.52 ml at 12 hours for DMF and 0.61 ml for 8 hours and 0.35 ml for DMSO at average survival time of 9 hours 44 minutes. A 100 % recovery after cryopreservation with DMSO and DMF was achieved after storage for 6 hours, 48 hours and 7 days in liquid nitrogen. DMSO and DMF were equally effective cryoprotectants in this study.

11.1.8.4 Pillay, P

The Effects of *Scilla nervosa* (Burch.) Jessop (Hyacinthaceae) aqueous extract on cultured Hep G2 cells

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Purpose:

Scilla nervosa, a medicinal plant indigenous to Southern Africa, is traditionally used to treat a diverse range of illnesses. The Zulu people use aqueous decoctions of the bulbs as analgesics in the treatment of rheumatic fever (Bangani et al., 1999). Bulbs have been identified to contain homoisoflavanones and stilbenes (Du Toit et al., 2010). It has been recently demonstrated in our laboratory that extracts prepared from the bulbs possess potent anti-inflammatory properties (Du Toit et al., 2011) and these findings therefore rationalise the traditional use of the plant as an anti-inflammatory agent. However, little information is known about the plant's toxicity and only one historical report suggested 0.5-1 kg of the fresh plant in the flowering stages was toxic to sheep (Van der Walt and Steyn, 1946). Therefore, current research is required into the potential toxicity of especially the bulbs on the liver as major detoxifying organ.

In this study we investigated the effects of an aqueous extract of the bulbs in cultured Hep G2 human liver cells, a model system for liver metabolism and toxicity of xenobiotics.

Methods:

Cytotoxicity—Hep G2 cells were cultured to confluency, incubated with the extract (varying concentrations; 24 h) and viability was measured using the MTT assay. The IC₅₀ value was determined and used as a reference concentration in the subsequent assays. **DNA Damage**—Single cell gel electrophoresis with fluorescent DNA staining was employed to determine single strand DNA breaks. **Oxidative Damage**—Lipid peroxidation was measured by quantifying the levels of malondialdehyde in a colorimetric assay. **Apoptosis**—The apoptotic potential was determined using fluorescence-activated cell sorting (FACS) and luminometry with commercial Annexin V FITC Caspase-Glo 8/9 assays, respectively. **Mitochondrial Membrane Potential**—FACS and the commercial JC-1 Mitoscreen assay was used.

Results and Discussion:

The cell viability of Hep G2 cells showed a dose-dependent decrease and the IC₅₀ for the extract was determined as 0.03 mg/ml. DNA fragmentation, as evidenced by an increase in tail length, was more pronounced in the treated cells. Lipid peroxidation increased 1.9-fold. The percentage of apoptotic cells was higher than the controls, and the intracellular activities of caspase 8 (1.3-fold) and ATP (1.2-fold) increased, while caspase 9 decreased (1.1-fold). There was no change in mitochondrial depolarisation. Results suggest that liver cells are sensitive to an aqueous extract of the bulbs of *S. nervosa*. Future research could be directed towards determining the effects *in vivo*. Our results and future studies would support the policies of the government and United Nations which aim to promote the development, use and regulation of traditional medicine.

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Acknowledgements:

This study was supported by an NRF Masters Freestanding Blockgrant Scholarship.

11.1.8.5 Pretorius, A

A Preliminary Investigation of the Potential Anticancer Properties of Quinoline Derivatives

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Purpose:

Derivatives of the quinoline moiety have been shown to exert a range of biological activities, including anti-neoplastic activity. It has also been reported that quinoline derivatives modulate multidrug resistance in cancer through the inhibition of P-glycoprotein (P-gp), a transmembrane xenobiotic transporter. The clinical application of quinoline derivatives in the treatment of malignancies has been limited due to non-selectivity. Two novel hydroxyquinoline derivatives, HQ5 and HQ10, were synthesised as potential anticancer agents. Preliminary screening indicated the selective toxicity of these compounds to cancer cell lines. HQ5 and HQ10 were investigated for potential anticancer properties as pertains to their ability to circumvent multidrug resistance and possible mechanism of action.

Methods:

Rhodamine 123 efflux assay: The ability of HQ5 and HQ10 to inhibit the function of P-gp was investigated with a flow cytometric Rhodamine 123 efflux assay. Verapamil, a known P-gp inhibitor, was used as positive control. Three P-gp expressing cell lines, COLO 320DM, HepG2 and SH-SY5Y cells, were loaded with rhodamine 123. Thereafter the cells were exposed to HQ5, HQ10 and verapamil at three different concentrations and analysed flow cytometrically for fluorescent intensity.

Cell cycle analysis: Cell cycle analysis studies were performed using the MCF-7 breast cancer cell line after 24, 48 and 72 hours of exposure to HQ5 and HQ10. Samples were stained with PI and RNase and analysed using a flow cytometric method.

Apoptosis/necrosis: An MCF-7 breast cancer cell model was used to determine the effect of the test compounds on the induction of apoptosis or necrosis after five and seven days of exposure to HQ5 and HQ10. A standard flow cytometric method using Annexin V-FITC and Propidium Iodide (PI) was used.

Results:

Data indicated that HQ5 and HQ10 did not abrogate the function of P-gp in COLO 320DM, HepG2 or SH-SY5Y cell lines. In contrast, an enhancement of P-gp activity was observed in all the cell lines used. After investigating the effect of the hydroxyquinolines on the cell cycle progression of MCF-7 cells, it was observed that HQ5 and HQ10 arrested the cell cycle at the G₁ checkpoint. As predicted for exposure to a G₁ blocker, both of the experimental compounds induced apoptosis in MCF-7 breast cancer cells in a dose and time dependent manner.

11.1.9 Session G1 APSSA

11.1.9.1 Shaikh, RP

Synthesis of Multilayered Mucoadhesive Membranes for Prolonged and Site-Specific Oral Drug Delivery

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Purpose:

The purpose of this study was to formulate and evaluate a novel polymeric drug delivery system comprising an upper gastric and intestinal targeted multilayered membrane system, for site-specific oral delivery of model drugs Rifampicin and Isoniazid respectively.

Methods:

Mucoadhesive Membrane Fabrication: Solutions of chitosan (1.0-2.0% w/v) for the gastric targeted component and pectin (1.3-2.7% w/v) for the intestinal targeted component, added to a solution containing polyvinylpyrrolidone (4.0-8.0% w/v) and Poly(vinyl alcohol) (PVA) (2.00-4.75% w/v) before film-casting and dried at room temperature for membrane formation.

Preparation of Electrospun Drug-loaded nanofibrous membranes: Solutions (10% w/v PVA with 2% w/v INH and 8% w/v PVA with 2% w/v RIF) were electrospun on a horizontal electrospinning rig with an optimised collector to capillary tip distance of 24cm or 28cm for INH and RIF nanofibrous membranes respectively with an applied voltage of 20kV.

Modification by Vapour-Induced Crosslinking: Crosslinking was performed in the presence of GA vapours in a closed environment. INH- and RIF-loaded PVA nanofibrous membranes were suspended over 15-60mL of the GA solution and allowed to crosslink for 6-24 hours.

Drug Entrapment Efficiency (DEE) Determination: Accurately weighed samples of drug-loaded nanofibrous membranes were dissolved in 100mL of PBS (INH: pH 6.8; RIF: pH 1.2). The drug content was analysed by UV spectrophotometry and computed from a standard linear curve of the drug in PBS.

In-Vitro Drug Release Study: *In vitro* drug release studies were performed using a USP 33 apparatus II rotating paddle method, set at 50rpm, in which a single nanofibrous membrane of known mass was placed in 900mL of PBS (INH: pH 6.8; RIF: pH 1.2) at 37°C. Sampling occurred at hourly intervals for 12 hours and with analysis by UV spectroscopy.

pH-responsive Mucoadhesivity Analysis: Mucoadhesive membranes were exposed to test parameters as described in drug release studies and tested for mucoadhesion at 2 hour intervals over 12 hours using a textural analyser.

Scanning Electron Microscopy Studies: Surface morphology of the nanofibrous membranes was analysed by SEM to qualitatively elucidate nanofiber diameter and network density.

Elucidation of Tensile properties: Physicomechanical properties of the crosslinked and non-crosslinked nanofibrous membranes were determined using a nanoTensile[®] tester.

Ethics approval for *in vivo* studies obtained from Wits Animal Ethics Screening Committee, AESC No: 2009/01/05.

Results and Conclusions:

DEE tests demonstrated a high entrapment of 98.77±1.384% and 95.07±1.988% of INH and RIF respectively. Drug release results showed that the crosslinking time and quantity of GA had a profound effect on prolonging the release characteristics of the nanofibrous membranes. SEM analysis revealed uniform, cylindrical fibers with diameters of 372.5±28.61nm and 631±57.78nm for INH and RIF respectively. Crosslinking produced denser packing of nanofibers within the membrane which facilitated prolonged drug release characteristics. Crosslinking notably decreased the elasticity and increased the ultimate strength and yield stress of the nanofibrous membrane. Mucoadhesion, determined by measuring the work of adhesion from a typical force: distance obtained from textural analysis was 7.5e⁻³±2.96e⁻³J and 2.03e⁻²±5.43e⁻³J for optimised gastric targeted membranes and intestinal targeted membranes respectively. Membranes maintained their mucoadhesive properties over 12 hours only showing a decrease in mucoadhesion of 20-40% after 8 hours.

11.1.9.2 Wadee, A

The Development of an *In Situ* Forming Implant for the Treatment of Solid Tumors: Determination of the Effect of Methotrexate Release on Colon Carcinoma Cells and *In Vivo* Studies in the Rat Model

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Purpose:

An implant which forms at the site of a tumor and releases drug *in situ* provides many benefits. High local concentrations of drug at the site can be achieved as well as a reduction in systemic side-effects. Poly(methylvinyl ether) is a biocompatible, thermoresponsive polymer which converts from a solution to a solid with a change in temperature. As a result the polymer in solution form can be injected at the site of the tumor and will form a solid implant *in situ* due to its response to the increase in temperature. This study sought to determine whether the release of the entrapped chemotherapeutic, methotrexate (MTX) occurred over a month and whether the amount of drug being delivered was sufficient to cause cancer cell death. A pilot study in the non-tumor bearing rat model was also conducted.

Methods:

Preparation of implant formulations: Optimized formulations of poly(methylvinyl ether) (20% w/v, Calcium Chloride: 0.125M) were prepared and loaded with MTX at a concentration of 5mg/mL. Formulations prepared for animals were prepared from autoclaved polymer and drug was added to the formulation under aseptic technique.

Determination of the release of MTX from the implant: Drug release studies were performed and samples were drawn every 3 days for 30 days. Drug content was assayed using UV spectroscopy. Drug elutes collected were also utilized to determine whether MTX was being released in sufficient quantities to inhibit the growth of cells.

Utilization of flow cytometry and an MTT assay for the determination of cell viability: Colon carcinoma cells (HT29) which were used as the model for solid tumors, were grown in Dulbecco's Modified Essential Medium (supplemented with 10% FBS and 1% Pen-Strep) to 75% confluency. Elutes collected from the drug release studies on days 1, 7, 15 and 30 were then added to cells and incubated over 24 hours. Flow cytometry and MTT assays were then conducted on the treated cells to determine cell viability.

Determination of the release of MTX in the rat model: Sprague-Dawley rats (200-250g) were divided into two groups of 25 rats each. Animals were followed over a period of 10 days. Control animals received two intraperitoneal injections (on days 1 and 5) delivering a total dose of MTX 2mg/rat/10 days, whereas the study group received a once-off implant delivering a dose of 2mg/rat/10 days. Rats were monitored for toxicity throughout the study. Ethics approval for *in vivo* studies obtained from WITS Animal Ethics Screening Committee, AESC Number: 2009/37/05.

Results and Conclusions:

Release of MTX exceeded 30 days with mean fractional drug release after 30 days of 25% of the entrapped drug. The MTT assay showed that a significant percentage of cells were non-viable after treatment with elutes from the implant. This was confirmed by flow cytometry. Rats in the control group did not develop any significant toxicity as a result of the intraperitoneal injection possibly due to the rapid clearance of the MTX. Rats that received the implant began to develop significant toxicity to MTX after 6 days. This indicated that MTX was being released in sufficient quantities and in a sustained manner and shows promise for utilization of this formulation as an *in situ* forming implantable system delivering chemotherapeutics to the site of solid tumors.

11.1.9.3 Verwey, MT

The Synthesis and *In Vitro* Antiplasmodial Activity of Acridine-Triazine Hybrids

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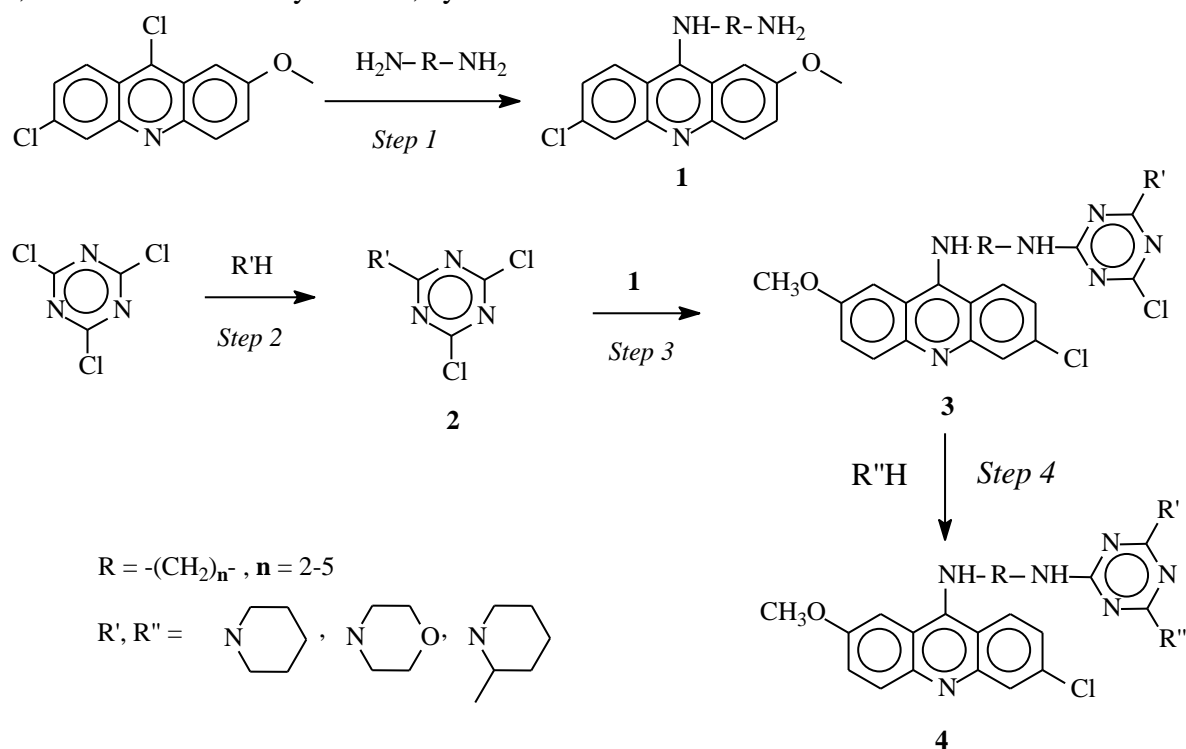
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Purpose:

Malaria is endemic in 92 countries and kills over an estimated 2 million people each year. This is due the widespread resistance the malaria parasite *P. falciparum* has developed against the classic anti-malarial drugs such as chloroquine, quinine, artemisinin etc., which calls for the search for new drugs an emergency. The aim of this study is to synthesize a range of new hybrid compounds of acridine and triazine and to evaluate their *in vitro* activity against various strains of *P. falciparum*.

Methods:

The synthesis of acridine-triazine hybrid consists of 3-4 nucleophilic substitutions involving 6,9-dichloro-2-methoxyacridine, cyanuric chloride with different diamines.

**Scheme:** Synthetic routes for acridine-triazine hybrids**Results:**

The intermediate compounds 1 and 2 were synthesized in 65-85 % yields, and their structures were confirmed by NMR and MS.

11.1.9.4 Chiwakata, MT

Fragment Based-Type Approach on Synthesis of HMT Analogues as Potential Anti-Cancer Agents

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Purpose:

Halogenated monoterpenes (HMTs) isolated from marine algae have been demonstrated to show cytotoxic selectivity towards cancerous cells (oesophageal and breast cancer). Selectivity is one of the ideal properties required in newer agents to lower side effect profiles. An understanding of the chemistry behind biological activity of HMTs has become important. A fragment based-type approach allows fragmentation of drug leads into smaller molecules and enables mapping out of pharmacophoric elements. Hence this study was aimed at synthesizing a series of HMT analogues focusing on carboxylic acid and ester derivatives.

Methods:

A series of benzaldehyde derivatives with selected substituents at the *ortho* position to the aldehyde functional group was selected. Cinnamate derivatives were prepared via condensation reactions between appropriate benzaldehyde derivatives and ethyl acetate in the presence of sodium and ethanol, and by Wittig reactions using 1-ethoxycarbonyl ethylidene-triphenylphosphorane. Conversion of aromatic aldehydes to dichlorides was carried out in the presence of thionyl chloride and dimethylformamide. Carboxylic acids were obtained by hydrolysis of corresponding ester derivatives. Products synthesized were characterized by nuclear magnetic resonance (NMR).

Results:

The required compounds were synthesized in yields varying from 60 to 70%. The spectroscopic data were consistent with the structures of the synthesized compounds.

Conclusion:

Condensation reactions (Claisen type and Wittig reactions) can be used to convert aromatic aldehydes into α , β unsaturated esters. Dichloro moieties can be prepared from α , β unsaturated aldehydes by use of a chlorinating agent; whereas corresponding carboxylic acids are easily obtained by hydrolysis of their parent esters.

Acknowledgements:

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11.1.9.5 Munedzimwe, T

The Semi-Synthesis of Sargahydroquinolic Acid Derivatives as Potential Anti-Plasmodial and Anticancer Agents

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Purpose:

We have recently reported the presence of a number of antiplasmodial metabolites from the South African brown alga *Sargassum heterophyllum*. Of particular interest is sargahydroquinolic acid (SHQA) and sargaquinolic acid (SQA), which has been found to exhibit moderate antiplasmodial (IC₅₀: 12.0 µM) and cytotoxic activity (IC₅₀:68.4 µM)

The mechanisms by which SHQA and structurally related metabolites bring about this activity as well as the pharmacophoric groups responsible for its antiplasmodial and anticancer activity remain unknown. In an effort to identify the functional groups responsible for the biological activity of SHQA, a series of SHQA derivatives were synthesised.

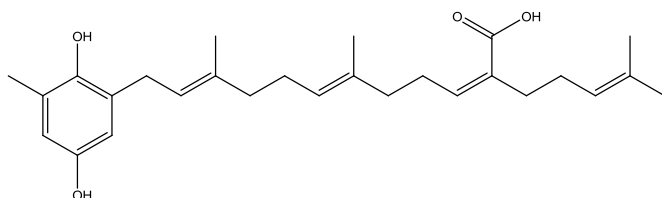


Figure 1. Chemical structure of Sargahydroquinolic acid (SHQA).

Methods:

SHQA was isolated from *S. heterophyllum* as published previously. Sargaquinol was obtained via LiAlH₄ reduction of SHQA while Dess-Martin periodinane oxidation of sargaquinol afforded sargaquinol. Oxidation of SHQA to sargaquinolic acid (SQA) proceeded smoothly using either Ag₂O or MnO₂. The acetylated derivative of SHQA was obtained via treatment of the latter with acetic anhydride/pyridine while methylation using CH₃I or dimethylsulphate gave the hydroquinone and quinone di-methylethers respectively. Sargachromenol was extracted directly from the natural source. The structures of all synthesised compounds were confirmed by standard spectroscopic techniques including one and two dimensional NMR techniques and comparison to published data where it existed.

Conclusion:

All compounds were synthesized in reasonable yield and gave satisfactory NMR spectra.

11.1.9.6 Van Heerden, L

Synthesis and *In Vitro* Antimalarial Activity of a Series of Bisquinoline and Bispyrrolo[1,2a]Quinoxaline CompoundsL. van Heerden*, D.D. N'Da*, J.C. Breytenbach* and J.W. Breytenbach[†]*Pharmaceutical Chemistry, School of Pharmacy, [†]Statistical Consultation Services, North-West University, Potchefstroom 2520

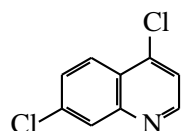
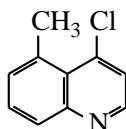
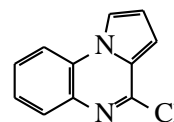
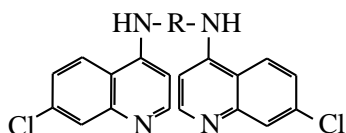
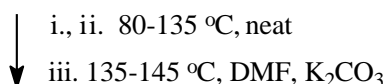
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Introduction:

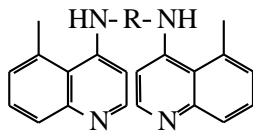
Malaria was estimated to claim the lives of 863 000 people in 2008.^[1] The widespread resistance to chloroquine is mainly associated with reduced concentrations of the drug in the parasites digestive food vacuole. It is possible that the biscompounds can circumvent this mechanism due to their decrease in conformational mobility which makes them less efficiently extruded by proteinaceous transporters and efflux proteins.

Aim of study:

The purpose of this study is to synthesize and evaluate the *in vitro* antimalarial activity of a series of biscompounds.

4,7-dichloroquinoline
i.4-chloro-5-methylquinoline
ii.6-chloropyrrolo[1,2-a]quinoxaline
iii.

Series 1: (1)-(6)



Series 2: (1)-(6)



Series 3: (1)-(6)

Methodology:

The series of biscompounds were synthesized through nucleophilic aromatic substitution between chloroquinoline^[2] or chloroquinoxaline^[3] and diamines.

(1) - (4) : R = -(CH₂)_x-N(R')-(CH₂)_z- ; (5), (6) : R = -(CH₂)_x-NH-(CH₂)_y-NH-(CH₂)_z-
Compounds:

(1) R' = H, x = 2, z = 2, (2) R' = H, x = 2, z = 3, (3) R' = H, x = 3, z = 3,

(4) R' = CH₃, x = 3, z = 3, (5) R' = H, x = 2, y = 2, z = 2, (6) R' = H, x = 3, y = 2, z = 3

Results:

Thirteen compounds have been synthesized successfully and their structures verified by ¹³C NMR, ¹H NMR and MS. Research in progress is to complete series 3 and determine the *in vitro* antimalarial activity of all compounds.

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[3] J. Gullion *et al.* J. Med. Chem. 47, 1997-2009 (2004).

11.1.10 Session G2 SASBCP

11.1.10.1 Rahiman-Karim, BF

An Assessment of Pathogen Occurrence and Resistance in a Trauma Intensive Care Unit of a Private Hospital Under an Antibiotic Stewardship Programme

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Introduction:

Due to the increasing use of antibiotics in the intensive care units and the increased resistance of micro-organisms to these antibiotics, it has become the duty of all healthcare professionals to preserve the antibiotics that we currently have.

Aim:

This research aims to assess pathogen occurrence and resistance under an antibiotic stewardship programme in an intensive care unit in a private hospital.

Method:

An antibiotic stewardship programme was instituted in a private hospital in Johannesburg. This program aimed to monitor and promote rational use of antibiotics through purposive control of appropriateness of the drug, dose and delivery systems. All antibiotic prescriptions during a six-month period were scrutinised, and the existing programme of sampling for infection and testing for resistance was maintained. The program was instituted as an addition to the fully comprehensive infection control program existent in the ward. All prescriptions, including drug, dose, dosage interval and delivery were noted on a Microsoft Excel spreadsheet. Specimens from standard ward procedures were cultured in the laboratory and results were also analysed by incorporation into the spreadsheet. Simple descriptive statistics were generated for antibiotic use, occurrence of infections and resistant bacteria. Existent statistics for the same months in the previous year were analysed for comparison.

Results:

Over the six-month period the number of MDR/PAN *Acinetobacter* infections decreased with the introduction of daily clinical antibiotic ward rounds from 25 in 2009 to 9 in 2010, this was in spite of an increase in the number of carbapenems used. Carbapenem usage increased by 5.1% in 2010 whilst colimycin usage decreased by 14.2%. The number of *Klebsiella* infections showed a 5.9% decrease in incidence and *Clostridium difficile* infections showed a significant 57.2% reduction. These indicate the necessity of using antibiotics rationally and preserving antibiotics that we currently have by the implementation of an antibiotic stewardship programme in conjunction with a comprehensive infection control programme.

11.1.10.2 Schroeder, IE

Effect of Pentachlorophenol and its Metabolites on Cell Viability and CYP1A1 Metabolism

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Purpose:

A number of *in vitro* assays exist to detect the mechanisms of cellular hepatotoxicity. However, these assays determine single end-points of toxicity. This study assessed a cost effective quantitative test battery of *in vitro* assays using a single 96-well microplate.

Methods:

The organochlorine pesticide pentachlorophenol (PCP) and two of its major metabolites tetrachlorobenzoquinone (TCBQ) and tetrachlorohydroquinone (TCHQ) were used as known hepatotoxins. The HepG2, a hepatocarcinoma cell line, was used to assess cytotoxicity using the neutral red uptake assay. CYP1A1 activity was determined using ethoxy-resorufin-*O*-deethylation as surrogate. Reactive oxygen species (ROS) generation was investigated by measuring dichlorofluorescein diacetate cleavage by H₂O₂. Effects on mitochondrial membrane potential was determined using JC-1 staining. Apoptosis were investigated by assessing caspase-3 activity whilst necrosis was investigated by determining plasma membrane integrity using propidium iodide staining.

Results:

The IC₅₀'s of PCP, TCBQ and TCHQ were 70.09, 140.8 and 132.1 µM respectively. PCP at 5, 10 and 50 µM concentrations induced CYP 1A1. TCHQ also induced CYP1A1, to a lesser extent at 50 and 100 µM concentrations whilst TCBQ showed no significant induction of this enzyme. TCBQ produced a large increase in (ROS) generation at all test concentrations (5 - 150 µM). ROS generation caused by TCHQ was evident at 100 µM and 150 µM concentrations, however PCP appeared to have no significant effect on ROS generation. An increase in mitochondrial membrane potential was observed for TCBQ and TCHQ at concentrations > 10 µM, however PCP did not seem to have an observable effect. PCP and TCHQ did not significantly increase caspase-3 activity whilst TCBQ showed a small increase in caspase-3 activity at 5 µM. None of the test compounds appeared to affect the membrane integrity of the cells.

Conclusion:

PCP toxicity differed from that of its metabolites in that it did not induce mitochondrial effect or ROS generation, like its two major metabolites. In conclusion this battery of tests can be used to provide valuable insight into mechanism of toxicity studies.

11.1.10.3 Steyn, SF

Effect of Early-Life Exposure to the Serotonin-Norepinephrine Reuptake Inhibitor, Venlafaxine, on Behaviour in Adulthood in Stress-Sensitive Rats

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Introduction:

Depression during childhood and adolescence had been considered an uncommon condition, whereas more recent epidemiological studies suggest an alarming trend for a persistent escalation in the prevalence of depression in these age groups. Even though there is limited knowledge about the safety and long-term effects of treatment with antidepressants early in life on neurodevelopment and susceptibility to psychiatric disorders later in life, the number of prescriptions of these drugs for children and adolescents has increased significantly. The objective of the current study was to investigate the effects of early-life (pre-natal and post-natal) chronic treatment with the dual action serotonin-norepinephrine reuptake inhibitor, venlafaxine, in stress-sensitive rats on late-life measures of cognition, anxiety-like and depressive-like behaviour. In addition, the study also investigated which age was more associated with optimal behavioural changes later in life following the chronic administration of venlafaxine, viz. pre-natal versus early post-natal phase, or both.

Methods:

Stress-sensitive Flinder's Sensitive Line (FSL) rats and their controls, Flinder's Resistant Line (FRL) rats, were employed for the current study. Pregnant dams were injected subcutaneously for 14 days with 10 mg/kg venlafaxine or saline from pre-natal days 15 to 1. New-born pups were then injected subcutaneously with 3 mg/kg venlafaxine or saline for 14 days from postnatal days 3 to 17. Doses were determined from previous studies reported in the literature. Four rat treatment groups (n = 8/group) of both FSL and FRL rats received injections during pre-natal + post-natal ages as follows: saline + saline, venlafaxine + saline, saline + venlafaxine and venlafaxine + venlafaxine. Following the drug treatments, all rat groups were subjected to a battery of behavioural tests, including the object recognition test (ORT), locomotor activity test (LOCO - Digiscan®), elevated plus maze (EPM) and forced-swim test (FST) on either postnatal day 21, 35 or 60 (separate treatment groups for each age group). All animal procedures were approved by the Ethics Committee of the North-West University (approval number: NWU-00045-10-S5), and are in accordance with the guidelines of the National Institutes of Health guide for the care and use of laboratory animals.

Results:

Preliminary data suggest that none of the early-life treatment regimens influence behaviour or cognition in control FRL rats, as observed in the ORT, EPM or FST on post-natal days 21, 35 or 60. As expected, in stress-sensitive FSL rats following pre- and post-natal administration with saline control, depressive-like behaviour in the FST was significantly enhanced relative to corresponding FRL rat groups as observed at post-natal days 35 and 60, but not 21. Importantly, depressive-like behaviour as observed in FSL rats at post-natal day 60 was reversed following pre- and/or post-natal treatment with venlafaxine, relative to the corresponding FRL rat groups. Such reversal of depressive-like behaviour in FSL rats were not observed at post-natal days 21 or 35, suggesting a delayed response. Conversely, preliminary data from the ORT, LOCO or EPM did not reveal any significant differences between the various FSL treatment groups, including at post-natal day 60.

Conclusions:

The current data therefore imply that early-life administration of venlafaxine to stress-sensitive (but not control) rats induce a delayed reversal of depressive-like behaviour, manifesting at post-natal days 35 and 60, but not earlier. Preliminary data do not support similar changes in anxiety-like behaviour or cognition.

11.1.10.4 Van Rensburg, L

The Influence of a Synthetic Lung Surfactant on the Permeability of Antimycobacterial Drugs Through Porcine Lung Tissue

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Purpose:

The pulmonary route is very attractive for drug delivery as it offers an alternative mode of delivery to intravenous and oral systems. For this reason mixing of a pharmaceutically active agent with a pulmonary surfactant may provide an attractive method of improving drug delivery through the natural barriers of the lung. In particular, the spreading behaviour of surfactant, combined with the ability to disperse drugs that are insoluble in aqueous solutions might improve their bioperformance by altering their solubility, thermodynamic activity, diffusion rate and consequently the drug penetration/permeation profile, that is, their transport across the membrane. The objective of this study was to investigate the role of a synthetic surfactant, Synsurf[®] in the permeability of the antimycobacterial drugs isoniazid (INH) and pyrazinamide (PZA) through porcine lung tissue.

Methods:

Diffusion kinetics of drugs: Frozen porcine bronchial tissue was thawed in PBS buffer (pH 7.4) to room temperature. Thereafter, it was carefully cut into approximately 4 mm² sections and placed into a flow-through perfusion apparatus. The Ethics committee of Stellenbosch University granted ethical clearance: Ethics Reference Project No 95/019; Approval date 16/2/2011. Permeability experiments were conducted over 24 hours and samples (collected every 2 hours) were assayed by HPLC detection. Statistical analyses were carried out using an F-test to establish steady state diffusion kinetics. Mean steady state flux values were compared statistically using a t-test at a significance level of 5%.

Surface tension measurements and critical micellar concentration of Synsurf[®]: Analyses were done using a Digidrop operating system. A range of concentrations were prepared of Synsurf[®]. Five droplets of each concentration were assessed and the mean used to find the surface tension of the various concentrations.

Results:

The calculated critical micelle concentration (cmc) of Synsurf[®] was found to be ~200 µg/ml. *In vitro*, a significant enhancement of INH flux across porcine bronchial tissue (19%) was found when the drug was mixed with Synsurf[®]. In contrast to this, PZA flux values across bronchial tissue were retarded with Synsurf[®] at high concentrations of surfactant.

Conclusion:

In conclusion, the synthetic lung surfactant Synsurf[®] alters the permeability characteristics of the pulmonary epithelium to antimycobacterial drugs. This finding warrants further investigation on the clinical relevance of Synsurf[®] as a drug delivery agent. Studies evaluating the surface properties of drug Synsurf[®] drug combinations as well as the chemotherapeutic properties of such combinations are in progress.

11.1.10.5 Willis, K

Quantitative Analysis of the Active Compounds, Hypericin and Hyperforin, in Commercial Products of St. John's Wort (*Hypericum Perforatum* L.) by HPLC-MS/MS

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Purpose:

Herbal supplements are used in the treatment of a variety of illnesses. Despite the numerous advances and advantages of conventional medicines, the popularity of herbal supplements as an alternative has grown, due in part to the availability of herbal medicines without prescription, the belief that because they are natural they are safer and the dissatisfaction with side effects of conventional medicines. As the growth in the use of herbal supplements continues, concerns over their quality, safety and efficacy has been raised. Unlike conventional medicines, the majority of herbal medicines are not subject to strict regulations during manufacturing, therefore variation in the amounts of active compound content have been observed. Consistency in composition of herbal medicines is key to the safety and efficacy of these products.

St. John's Wort is a popular herbal medicine used for the treatment of mild to moderate depression. The active compounds, thought to be responsible for its antidepressant effects, are hypericin and hyperforin. In this study hypericin and hyperforin concentrations of multiple batches of four commercial St. John's Wort products were investigated.

Methods:

Sample extraction: 100 mg of finely crushed St. John's Wort product from each brand was mixed with methanol and sonicated for 20 minutes at ambient temperature. The solutions were centrifuged at 1000g for 5 minutes and the supernatant collected. This extraction process was repeated six times, after which the supernatants were combined and filtered (0.2 µm particle size). The extraction procedure was performed under strict exclusion of light.

Liquid chromatography: HPLC analysis of the extracts was carried out on a fused-core Ascentis Express C18 column (Supelco Analytical) using gradient elution.

Mass spectrometry: Mass spectra were obtained using a 4000 QTrap LC/MS/MS system (Applied Biosystems), equipped with an electrospray ionisation source operated in negative ionisation mode. Selected MRM transitions were used to quantify for hypericin, hyperforin and ketoprofen (internal standard).

Results:

Tablet/capsule weight from the same batches differed by up to 24%. The large differences in weight suggest that there is variability within the amount of active compounds per tablets/capsules.

Moreover, the actual amount of hypericin present differed from the amount claimed to be present by the manufacturers in the products where this was given: a maximum of 66% of the claimed amount was found.

Furthermore, the amount of hypericin and hyperforin present in tablets/capsules differed between multiple batches of the same brand by up to 35% and 89% respectively with the difference between brands being as much as 94% and 99% respectively.

Conclusion:

Large variations in brand product consistency of St. John's Wort were found and the active compound variation between brands was significant.

11.1.10.6 Wolmarans, P (De Wet)

Natural Stereotypy in Deer Mice and its Association with Frontal Cortical and Striatal Serotonin Transporter (SERT) Density: Implications for a Putative Animal Model of OCD

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Purpose:

Deer mice (*Peromyscus maniculatus bairdii*) present with different levels of abnormal stereotypical behaviour that allows the animals to be classified into high (HSB), low (LSB), and non- (NSB) stereotypy cohorts. Earlier studies in our laboratory have established the strong face and predictive validity of the model, together with some evidence of construct validity. The treatment specific response to selective serotonin reuptake inhibitor (SSRI) and the persistent, seemingly purposeless nature of these behaviours closely emulate that of OCD. However, construct validity needs to be further explored. Given the selective response of OCD to SSRIs, serotonin represents a major role player in the neurobiology of OCD. This study aimed to strengthen the construct validity of the model by investigating frontal cortical and striatal serotonin transporter (SERT) binding, the neurobiological target for SSRIs, in treatment naive animals expressing different degrees of stereotypy.

Methods:

Behavioural assessment: All animals were screened for repetitive stereotypy on a weekly basis for 9 weeks during the dark cycle using the Accuscan[®] Animal Activity Monitoring System. Data were collected after weeks 5 and 9, with week 5 data used to divide the animals into two cohorts, viz. HSB and combined NSB plus LSB. *Neurochemical analysis:* Following final behavioural analysis the animals were sacrificed by decapitation and the relevant brain areas dissected, snap frozen and stored at -80°C until later SERT binding analysis. SERT binding was performed in frontal cortical and striatal tissue using [3H]-paroxetine as radioactive ligand and fluoxetine to determine non-specific binding. Ethical Clearance Number: NWU-00066-10-S5.

Results:

No significant difference in cortical SERT densities were observed in treatment naive HSB versus LSB/NSB mice. However, striatal SERT densities were found to be significantly lower in the HSB mice compared to the LSB/NSB cohort. In addition we found that HSB animals presented with significantly higher cortical SERT densities as compared to that in the striatum. Interestingly, a significant opposite relationship was evident in the LSB/NSB group. High stereotypy in deer mice is therefore related to significantly altered serotonergic signalling in the striatum, thus introducing a new level of construct validity for this putative animal model of OCD.

11.1.11 Session H

11.1.11.1 Mugabo, P

Nevirapine Plasma Concentrations in Premature Infants Exposed to Single Dose Nevirapine for Prevention of Mother to Child Transmission of HIV-1

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Background:

There are no pharmacokinetic data for premature infants receiving single dose nevirapine (sdNVP) for prevention of mother to child HIV transmission (MTCHT).

Methods:

Infants below 37 weeks gestation were prospectively enrolled. Mothers received sdNVP during labour. Infants received sdNVP 6 mg for birth weight (BW) above 2 kg and 2 mg/kg if BW below 2 kg. They also received zidovudine for 7 days, 12 mg twice daily if BW above 2kg and 4 mg/kg twice daily if BW below 2kg. Blood was collected on day 1, 2, 4, 6, 8, 14 and 21 days after birth. NVP concentrations were determined by liquid chromatography mass spectrometry. The study was approved the Ethics committees of the Universities of the Western Cape (Reference Nb 04/7/10) and Stellenbosch (Reference Nb 04/10/183).

Results:

Of 100 infants enrolled, evaluable data were obtained from 81 infants, 58 born to mothers receiving sdNVP during labour (Group I) and 23 to mothers who missed NVP (Group II). 29.6% infants were small for gestational age (SGA). Median (range) C_{max} , T_{max} , AUC and $T_{1/2}$ were 1438 (350-3832) ng/ml, 25h50 (9h40-83h45), 174134 (22308-546408) ng*h/ml and 59.0 (15.4-532.6) h for Group 1 and 1535 (635-4218)ng/ml, 17h35 (7h40-29h), 168576 (20268-476712) ng*h /ml and 69.0 (22.12-172.3) h for Group II. For Group II, the median (range) V_d and Cl were 1702.6 (623.7-6189.8) ml and 34.9 (6.2-163.8) ml/h for Group II. AUC was higher ($p=0.006$) and CL ($p<0.0001$) lower ($p<0.0001$) in SGA infants. Plasma concentrations exceeding 100ng/ml were achieved over 8 days in 78% from Group I and 70.0% from Group II. MTCHT rate was 4.8%.

Conclusion:

sdNVP dosage of 6 mg for BW above 2 kg and 2 mg/kg if BW below 2 kg is appropriate in premature newborns. Even if maternal sdNVP is omitted, sdNVP maintains infant plasma concentrations above 100ng/ml through the 1st week of life.

11.1.11.2 Rosenkranz, B

Efavirenz Levels in HIV Infected Children

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Background:

ARV treatment of HIV infected children in South Africa remains limited. There is only insufficient information about the pharmacokinetics of ARVs in the paediatric population. The aim of the present study was to quantify exposure to the NNRTI efavirenz (EFV) in children treated with HAART and to identify factors associated with inadequate drug exposure (1,2).

Methods:

Paediatric patients treated at Tygerberg Children Hospital for at least 4 weeks with an efavirenz containing ARV regimen were included in the study approved by the Ethics Committee of Stellenbosch University. A blood sample was taken; serum was prepared and stored at -80°C until analysis of EFV concentrations at the TDM laboratory at the Infectiology Clinics, Wuerzburg University using an established HPLC method (3). Results are reported as mean±SD and range. The project has been approved by the Health Research Ethics Committee of the Faculty of Health Sciences (no. N09/05/151).

Results:

The analysis was performed on 26 blood samples of 24 patients (14 male, 10 female; age 9.3±3.1, range 3.7-15.9 years, BMI 15.4±3.0 (7.0-21.9) kg/m², WHO stage 4: N=12, stage 3: N=11, stage 1: N=1). The EFV dose was 319±89 (200-600) mg/day corresponding to 18.3±16.9 (10.8-20.0) mg/kg/day. EFV serum concentrations showed great variability (4,049±6,862, range 156-36,340 ng/ml) (therapeutic range: 1,000-4,000 ng/ml). 12% of the samples were below and 15% above the therapeutic range. Of 7 patients hospitalised for treatment of opportunistic infections, 3 were below and 2 above the therapeutic range. 2 patients received TB treatment including rifampicin; one was below, the other above the therapeutic range. Of 5 patients on TB treatment without rifampicin, 4 were within the therapeutic range and one below. Of 6 underweight patients, 3 were within the therapeutic range, 2 below and one above. Samples from all 8 patients without any co-morbidities were within the therapeutic range.

Conclusion:

Opportunistic infections, underweight and a rifampicin containing TB regimen were identified as potential risk factors for EFV concentrations below or above the therapeutic range in this paediatric patient population. TDM monitoring may support the choice of the most appropriate dose of EFV in such patients (4).

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11.1.11.3 Van Tonder, J

A Microplate Method for Multiparametric Hepatotoxicity Screening

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Purpose:

Hepatotoxicity is one of the major causes of drug attrition. This is partially due to the lack of current methodologies to detect or accurately predict hepatotoxicity during early drug development. Research has shown that *in vitro* cytotoxicity testing is just as accurate as *in vivo* animal studies in predicting acute, lethal human doses of compounds. Considering the cost difference between *in vitro* and *in vivo* testing it is clear that room exists for *in vitro* methods to be used to more accurately predict toxicity. The present study aimed at miniaturising a battery of six different *in vitro* toxicity assays onto a single microplate to assess the toxic effects of the well-known organochlorine pesticide DDT and its metabolites DDE and DDD in an established liver-derived cell line, HepG2.

Methods:

Parameters that were examined include: cell viability (Neutral red uptake), phase I metabolism (ethoxyresorufin-*O*-deethylase activity), oxidative stress (2',7'-dichlorodihydrofluorescein diacetate cleavage), mitochondrial toxicity (JC-1 staining) and mode of cell death: apoptosis (caspase-3 activity) / necrosis (Propidium iodide staining).

The toxin exposure for each assay was dependent on the time it would take for that specific toxic response to occur. Therefore, exposure time prior to conducting the different assays ranged from 1 h - 24 h. Viability, phase I metabolism and necrotic cell death was measured after 24 h exposure, whereas, apoptotic cell death, oxidative stress and mitochondrial effects were measured following 6 h, 3 h, and 1 h exposure, respectively. Each plate was set up in such a way that it included relevant blanks, negative and positive controls as well as 5 concentrations of the toxin, ranging from 5 μ M to 150 μ M. A well-established positive control was included for each assay to determine whether the assay performance was achieved.

Results:

All three tested toxins produced similar results. Viability decreased in a dose-dependent manner yielding IC₅₀ values of 54 μ M, 64 μ M and 44 μ M for DDT, DDE and DDD, respectively. Evaluation of phase I metabolism showed that enhanced Cytochrome P4501A1 activity was dose-dependent ($p < 0.001$). Test toxins decreased reactive oxygen species levels, and significantly hyperpolarised the mitochondrial membrane potential ($p < 0.001$). Assessment of the mode of cell death revealed a significant elevation of caspase-3 levels, with DDD proving to be most potent ($p < 0.001$). Interestingly, only DDT induced dose-dependent loss of membrane integrity ($p < 0.001$).

Conclusion:

These results indicate that the tested compounds produce apoptotic death probably due to hyperpolarisation of the mitochondria, resulting in Cytochrome c release with subsequent caspase-3 activation. From seeding of cells to obtaining the final raw data took 4 days showing that the developed *in vitro* method reduces the time it would take to assess the tested parameters separately and lays a good foundation for further development of this type of *in vitro* toxicity testing.

11.1.11.4 Van Zyl, J

A Synthetic Peptide-Containing Surfactant: Secondary Structure and Efficacy as Therapeutic Agent for Respiratory Distress Syndrome

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Purpose:

Pulmonary surfactant which is essential for breathing is a thin lipid-protein coating of the alveolar epithelium at the air interface of the mammalian lung. It consists of a complex mixture of phospholipids and apoproteins that reduces surface tension at the alveolar surface. It is well established that surfactant proteins B (SP-B) and C (SP-C) are of significant importance for respiratory function. Although commercially available pulmonary surfactants have been available for many years, they are very expensive. This led to the development of newer synthetic surfactants such as Surfaxin[®], containing a synthetic peptide which mimics the action of SP-B. For the purpose of our study we developed a polymer containing surfactant (Synsurf[®]) consisting of phospholipids combined with poly-L-lysine electrostatically bonded to poly-L-glutamic acid. We analysed aspects of its secondary structure and tested its efficacy on the pulmonary function in adult NZW rabbit and in preterm lamb models.

Methods:

Preparation of Surfactant: Synsurf[®] was prepared by mixing DPPC, cetyl alcohol and PG in chloroform and combining the dried phospholipid with a poly-L-lysine poly-L-glutamate complex in a NaCl solution. After ultrasonication tyloxapol was added and preparations were vialled under nitrogen.

Circular dichroism infrared spectroscopy and surface tension measurements: Analysis of the secondary structure of the poly-L-lysine poly-L-glutamic acid complex was done with a Jasco-810 spectropolarimeter. FT-IR spectroscopy of aqueous solutions of poly-L-lysine and poly-L-glutamic acid were carried out in D₂O in a Thermo-Nicolet Nexus 670 spectrometer. Surface tension analyses were done using a Digidrop operating system.

In vivo surfactant activity: Animal care and experimental procedures were performed under approval from the Faculty of Health Sciences Research Committee of Stellenbosch University. Adult NZW rabbits were used as a model of surfactant depletion after repeated lung lavage. We studied rescue surfactant treatment for respiratory distress syndrome in preterm lambs. Antero-posterior chest radiographs were taken prior and at the end of the study.

Results:

Assessment of systemic oxygenation and lung mechanics in animal models after instillation of Synsurf[®] showed a sustained improvement in oxygenation over the study period. CD spectra of the poly-L-lysine, poly-L-glutamic acid complex was indicative of a random coil conformation in addition to a conformational transition into a stacked antiparallel β -sheet network.

Conclusion:

The data demonstrate that the synthetic surfactant containing poly-L-lysine/poly-L-glutamic acid mimics the activity of SP-B when formulated in a phospholipid mixture. In both animal models of surfactant depletion Synsurf[®] improves oxygenation.

11.1.11.5 Samant, BS

Synthesis and Structural Activity Relationship Study of Halogenated Aromatic Compounds against Human African Trypanosomiasis

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Purpose:

The threat of protozoan diseases such as malaria and human African trypanosomiasis (HAT, sleeping sickness) affects the life style of millions of people in 36 countries of sub-Saharan Africa. Synthesis of active pharmaceutical ingredients (API) and their Structural Activity Relationship Study (SARS) are important steps in the drug discovery projects. With an intention to enhance the activity of synthesized compounds against these diseases, SARS was undertaken. Most of the organic compounds have a very low solubility in water and hence, organic solvents with costly catalyst systems are often used for various chemical reactions. These organic solvents and catalysts were replaced by a cheap and eco-friendly alternative of water by the use of micellar aggregates as microreactors. Halogenated aromatic compounds have various applications in the field of medicine; especially fluoroaromatics are representative of 20% of API. These are the potential API against HAT.

Method:

Halogenation reactions (chlorination, bromination, iodination and fluorination) of various substituted aromatics were done in the micellar medium in order to enhance reaction rate and selectivity. Synthesis of various derivatives of chloro, bromo, iodo and fluoro aromatics, which are potential API against malaria, were prepared using simple reaction conditions in the micellar media. For chlorination, bromination and iodination eco-friendly oxyhalogenation method was used in which in situ halogen species was generated in water by the action of hydrogen peroxide on the corresponding halogen salt. These three halogenation reactions followed electrophilic substitution mechanism. On the other hand fluorination followed nucleophilic substitution reaction mechanism in which bromoaromatic compounds were fluorinated using the biarylphosphine ligand *i.e.* cyclohexyl BrettPhos ligand, along with [cinnamylPdCl]₂ and CsF as the fluoride source in reverse micellar media.

The series of compounds were tested for its biological activity against HAT. These compounds were evaluated on the basis of their ability to inhibit cell proliferation of *T. brucei rhodesiense* in culture. The growth inhibitory activity against L-6 rat skeletal muscle myoblast cells was determined to establish a cellular therapeutic index.

Results:

This approach not only increased the conversion rate but also widened the scope of the particular reaction for various aromatics with an electron withdrawing as well as donating functionalities. The data also proved to be the cost effective and eco-friendly option of micellar aggregates as microreactors.

Some halogenated aromatics showed effective *T. brucei rhodesiense* inhibitory activity and low cytotoxicity. The exact mechanism of action for this series of API is underway. However, the above results indicate that the compounds with halogen (with electronegative nature) have an effective *T. brucei* inhibitory activity.

11.1.12 Session I

11.1.12.1 Mansoor, LE

Safety and Effectiveness of 1 % Tenofovir Gel in Preventing HIV Infection: Results of the CAPRISA 004 Trial and Next Steps

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The results of the CAPRISA 004 trial, released in July 2010, showed that 1% tenofovir gel reduced the risk of HIV infection in women by 39% compared with placebo, and by 54% in the women who reported more consistent gel use. This landmark study has been heralded as one of the most significant scientific breakthroughs in the fight against AIDS by the World Health Organisation, UNAIDS and several leading international research organizations. After nearly two decades of research, this was the first clinical trial to show that a vaginal microbicide could provide a safe and effective way to prevent sexual transmission of HIV. The gel also provided a 51% protective effect against herpes simplex virus type 2 infection (HSV-2).

The announcement raised questions about the most appropriate next steps, leading to WHO and UNAIDS convening a meeting in August 2010 where high priority was accorded to studies which support licensure and generate the urgently needed evidence for implementation.

A major gap exists between the prevention effectiveness achieved in clinical trials and subsequent performance of the health system in real-life clinical settings. The implementation of tenofovir gel through the health services is the next biggest challenge once tenofovir gel is registered as a medicine and can be legally dispensed in each country. It is anticipated that regulatory approval and licensure of tenofovir gel will take place in late 2013. Hence, the next 2-3 years are a critical window of opportunity to prepare.

Two of the studies that were deemed high priority at the WHO/UNAIDS consultation were 1) a post-trial access protocol which set out to devise and assess an implementation strategy of tenofovir gel provision through Family Planning Clinic (CAPRISA 008) and 2) a study to assess long-term treatment outcome and resistance to tenofovir gel (CAPRISA 009).

11.1.13 Session J

11.1.13.1 Du Toit, L

Conceptualisation and Experimental Optimisation of an Intelligent Intraocular Implant for Exemplification of the Bioresponsive Potential

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Purpose:

The concept of an autofeedback polymeric platform was intrinsically implemented in the design of an intelligent intraocular implant – the I³ – employing stimuli-responsive polymers, having application as a smart release system capable of delivering controlled therapeutic levels of an anti-inflammatory agent and antibiotic for posterior segment disorders within the vitreous cavity in response to inflammation. Bioresponsive polymeric matrices (BPM) were designed that released the incorporated drugs in a fashion responsive to a stimulus, namely the highly reactive hydroxyl radicals (OH \cdot) that are released from activated leukocytes both *in vitro* and during acute and chronic intraocular inflammatory reactions *in vivo*.

Methods:

The I³ was formulated as a 3 component system incorporating two differential release BPMs and a nanosystem. The outer BPM was designed for fast to intermediate release of antibiotic (ciprofloxacin) for the therapeutic management of the initial infection. The inner BPM was chemically modified to release an indomethacin-loaded nanosystem at a slower rate than the outer BPM. The differential release BPMs were simultaneously originated from polymers susceptible to free radical degradation, thus inflammation-responsive (e.g. hyaluronic acid, HA, alginate and chitosan). Carbodiimide coupling chemistry was exploited to increase the interconnectivity of the matrix, employing *N,N'*-dicyclohexylcarbodiimide (DCC) as an activator/ coupling agent, *N*-hydroxysuccinimide (NHS) as a reagent, and AlCl₃ as an additional catalyst, to facilitate coupling between the HA, alginate, and polyacrylic acid. For the inner BPM, a chitosan solution incorporating the indomethacin-loaded nanosystem was prepared. Optimisation of the I³ was conducted by constructing and analysing a four-factor, three-level (3⁴) Box-Behnken statistical design on MINITAB[®], (V15, Minitab, USA). The *in vitro* bioresponsive drug release behaviour from the experimentally-derived BPMs in the presence and absence of inflammatory mediators (hydroxyl radicals) was evaluated and the mean dissolution time (MDT) at 28 days calculated for each formulation, as well as the change in MDT from normal to pathological conditions (Δ MDT). Investigation of the transitional textural attributes (resilience, hardness and deformation energy) and fluid uptake (expressed as the water absorption capacity, WAC) of the experimentally-derived intelligent intraocular implants was also undertaken.

Results:

Various degrees of bioresponsiveness were attained for the experimentally-derived I³ devices, with Δ MDT ranging from 0-32.606 for indomethacin, and 5.109-25.956 for ciprofloxacin. The interaction between [HA] and [DCC] emanated in a significant effect on the Δ MDT of indomethacin ($p=0.050$). [AlCl₃] also had a significant impact on the WAC of the I³ under normal conditions ($p=0.023$), whereas the effect of [NHS] was significant when considering the resilience of the I³ under pathological conditions ($p=0.047$).

Conclusion:

Optimisation of the formulation process was achieved to obtain the levels of independent variables that would exemplify the bioresponsive capabilities of the I³ i.e. minimisation of individual MDTs, but maximisation of the Δ MDT and minimisation of the WAC.

11.1.13.2 Khamanga, SMM

A Novel Approach based on *Eigenvalues* of *Hessian* Matrix to Solve Optimization Problems in Analytical Method Development

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Purpose:

One of the most commonly asked questions about *Eigenvalues* is, “What is its relevance in Pharmaceutical analysis and why is it not getting prominence like other statistical/mathematical tools that are used in method development?” The purpose of this work is to describe how *Eigenvalues* of a matrix obtained using design of experiments (DOE) can be used to solve 2nd order differential optimization problems.

Method:

A modular HPLC system consisting of a Waters Model M 6000A pump, an automated Waters Intelligent Sample Processor Model 710B, a Coulochem dual electrode electrochemical detector with an analytical cell operated in the “oxidative-screen” mode were used in the development of an analytical method. Central composite design (CCD) of experiments was used to construct 2nd order response surfaces with three design factors, buffer pH (X_1), buffer molarity (X_2) and organic solvent composition (X_3). Twenty experiments were conducted in this work. All experiments were performed in a randomized fashion in order to minimize the effects of uncontrolled factors that may introduce bias to the response. The selected optimization parameters were retention time (R_t), (Y_1), peak symmetry (Y_2) and peak resolution (Y_3). A *Hessian* matrix, $H(f)_{ij}(x) = D_i D_j(x)$ was generated from the 2nd order partial derivatives of the final CCD functions. This was used to describe the local curvature of X_1 , X_2 and X_3 . The *Eigenvalues* (λ_1 , λ_2 and λ_3) of H were computed using an online matrix calculator (Blue Bit) powered by the Net matrix Library (500).

Results:

A classical 2nd degree model with a cubic experimental domain was postulated. The coefficients for the 2nd order polynomial model were estimated by least squares regression. The equation for Y_1 was; $Y_1 = 3.54 - 0.077X_1 + 0.011X_2 - 0.26X_3 - 0.061X_1X_2 - 0.061X_1X_3 + 0.054X_2X_3 - 0.019X_1^2 + 0.00704X_2^2 + 0.076X_3^2$ and this was further differentiated to obtain H and its associated λ_s ($\lambda_1 = -0.00056$, $\lambda_2 = 0.92$ and $\lambda_3 = 6.06$). Upon manipulation of independent variables the optimized conditions obtained were buffer pH=2.4, molarity =56 mM and organic solvent content of 33%. These conditions were then applied to the analysis of commercial dosage forms. This study proves that mathematical and statistical designs offer an efficient and feasible approach for analytical method development and optimization.

Acknowledgements:

The authors would like to thank the Andrew Mellon Foundation (SMK), National Research Foundation (RBW), Joint Research Committee (SMMK, RBW) for financial support.

11.1.13.3 Lombard, MC

Artemisinin-quinoline Hybrids and Hybrid-dimers: Synthesis, *in vitro* and *in vivo* Antiplasmodial Activity

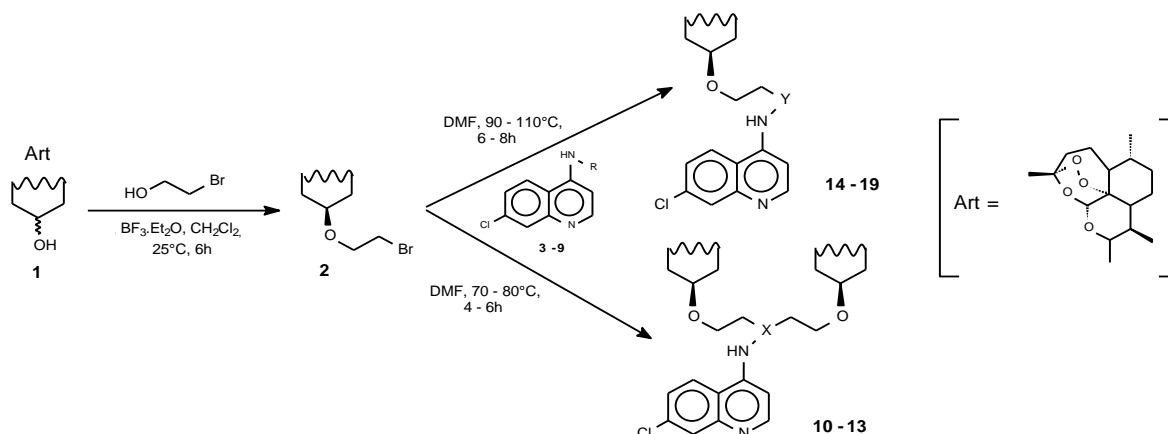
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Introduction:

The synthesis of hybrid molecules developed into an emerging strategy within medicinal chemistry and drug discovery. Hybrids offer a simpler and more effective way to deliver two agents, especially when differences like elimination times occur, as in the case of artemisinin and quinolone. Dimers containing a 1,2,4-trioxane unit exhibit potent *in vitro* antimalarial, antiproliferative, antitumor and anticancer activities. The aim of this study was therefore to chemically link one or two artemisinin moieties with quinoline, the pharmacophore of classic antimalarial drugs, forming hybrids and hybrid-dimers and to determine their antimalarial activity.

Methods:

In vitro antiplasmodial activity of free bases and their oxalate salts were determined against the chloroquine sensitive D10 strain (CQS) and resistant Dd2 strain (CQR) of *P. falciparum* using DHA and chloroquine (CQ) as reference drugs. *In vitro* cytotoxicity was evaluated using a mammalian cell-line, Chinese Hamster Ovarian (CHO). Female Swiss mice were treated intraperitoneally for *in vivo* antimalarial activity and toxicity with doses varying from 50 – 15 mg/kg.

Results and Conclusion

All compounds were obtained as 10β -isomers and showed good selectivity towards *P. falciparum* ($\text{SI} \geq 20$). Hybrids as well as hybrid dimers with linkers of 2-3 carbon atoms between the artemisinin and quinoline moieties displayed the best antiplasmodial and cytotoxic profile. Cyclic groups do not appear to be good linkers. The oxalate salt of hybrid 17 containing an isopropyl linker exerted a very potent antimalarial effect against *P. vinckei* *in vivo* in mice. A dose of 15 mg/kg was sufficient to induce a rapid and total clearance of the parasites, without recrudescence and no visible toxicity was observed up to 30 mg/kg. These artemisinin-quinoline hybrids and hybrid-dimers displayed promising antimalarial activity and merit further investigation.

11.1.13.4 Mufamadi, MS

An Implantable Nano-enabled Biorobotic Intracranial Device for Neurotherapeutic Applications

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Purpose:

The purpose of this study was to design a nano-enabled Bio-Robotic intracranial Device (BICD) that is capable of delivering neuroactive drugs to a specific site in the brain in response to condition present in Alzheimer's disease (AD).

Methods:

Nanoliposomes (NLPs) formulated with distearoyl-sn-glycero-phosphatidylcholine (DSPC), cholesterol, 1,2-distearoyl-sn-glycero-3-phosphatidyl-ethanolamine-methoxypolyethylene-glycol 2000 conjugate (DSPE-mPEG2000) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[maleimide(polyethylene glycol 2000)] (DSPE-PEG-Mal) using an adapted reverse phase evaporation technique. Galantamine or Fluorescein isothiocyanate (FITC)-loaded NLPs were produced by hydration of NLP film with 48mM of FITC or 5mg of galantamine in 130mM ammonium sulphate and 4mL PBS buffer (pH4.0 or pH7.4), and thereafter functionalized with synthetic peptide ligands. Both NLPs and functionalized NLPs (FNLPs) were prepared and subjected to in-depth physicochemical characterisation. Cytotoxicity and PC12 cell uptake of FNLPs were performed using confocal laser scanning microscopy (CLSM) and VictorTMX3 Perkin Elmer 2030. BICDs produced by embedding drug-loaded FNLPs within depot "intelligent" scaffolds were followed by lyophilisation technique. Controlled release of drug-loaded FNLPs release from BICD was investigated using orbital shaker bath (25rpm, 37°C) and at predetermined time intervals samples were withdrawn and subjected to UV analysis.

Results:

Formation of FNLPs were validated by Fourier Transmission Infrared (FTIR) spectra which showed new absorption bands at in 1590cm⁻¹ and 1155cm⁻¹ associated with amine bond formation during NLP and ligand interactions. The particles size and zeta potential of FNLPs produced were in the range of 110-180nm and -27 to -35mV, respectively demonstrating superior stability. The FNLPs produced showed no notable impact on PC12 cell viability (80% cell viability in 48hours). FNLPs demonstrated improved cell uptake (70-85% FITC uptake within 48hrs) and drug release capacities (60-85% drug uptake within 48hrs) by targeting PC12 cells of Alzheimer's disease when compared to non-functionalized NLPs (20% drug within 48hrs). Microscopic profiles confirmed that labelled FNLPs (spherical in shape) were entrapped within the BICD. These results demonstrate that controlled release of FNLPs from the BICD was influenced by the BICD porosity, diffusion pathway and size of the FNLPs.

Conclusion:

Novel BICD may provide an improvement to existing drug delivery systems and may confer satisfactory management of ND particularly in AD in terms of therapeutic efficacy, long-term pharmaceutical stability, targeted drug delivery and less frequent drug dosing intervals.

11.1.14 Session K

11.1.14.1 Richmond, BJ

The Organization of Brain Circuits Underlying Motivated Behaviour

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Motivation, that is, the incentive to act, underlies all goal-seeking behaviour. Disturbances of motivation are seen in many mental illnesses. For example, in depression no goals seem worth pursuing, in drug abuse the drugs seem so compelling that the subject will pursue them even if the pursuit leads to great personal or societal damage, or in obsessive-compulsive disorder where completion of compelled tasks leads to no sense of reward. We seek to identify the circuits and mechanisms underlying how values of rewards and outcomes are assessed.

Dopamine and noradrenaline, two chemically related neuromodulators, play a large role in arousal and reward evaluation. We have therefore paid special attention to dopamine and dopamine rich brain regions, both cortical and subcortical, and more recently to noradrenaline. We study motivation in monkeys by manipulating the outcome values of simple behaviour during neuropsychological and neurophysiological experiments. In normal monkeys and in monkeys given selective ablations we seek to identify which brain regions participate in assessing reward values, and how they interact to assess the value of a future action. In particular, we present visual stimuli that can be converted into subjective value, i.e., the likelihood, associated with a possible impending action. In recent work we compare the activity of dopamine and noradrenergic neurons during reward seeking. We find evidence that dopamine neuronal responses reflect the reward value and that noradrenergic responses reflect the cost needed to reach the desired outcome. We also examine the interaction between prefrontal and medial temporal lobe in setting subjective values.

A continuing difficulty in systems neuroscience is methodological. We want to learn more about brain circuits giving rise to behaviour. However, until recently, targeting specific components of circuits such as particular cell types or particular receptors for manipulation has not been as controllable as one would desire. In a peek at the future, one area that holds great promise for controlled selective targeting is molecular biology, that is, molecular pharmacology. We have been working to apply these tools in nonhuman primates.

Finally we can speculate together about how disorders in the brain circuits that we have identified might play a role in disorders with abnormal motivation as a symptom.

11.1.15 Session L

11.1.15.1 Ndlovu, BC (SANS Young Scientist)

The Effects of Oleanolic Acid on Dopamine Cell Lines following a Toxic Insult: Implications for Parkinson's Disease

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Purpose:

Parkinson's disease is a neurodegenerative disease of unknown cause. The leading hypothesis in the aetiology of Parkinson's disease is the increase in oxidative stress which is believed to lead to cell death by apoptosis. Compounds having the ability to scavenge the reactive oxygen species hold great promise as therapeutic interventions in Parkinson's disease. The compound of interest in this study is oleanolic (OA), a triterpenoid extracted from more than 120 medicinal plants e.g. *Syzygium aromaticum*. In our study we looked at the effects of OA in oxidatively stressed PC-12 cells.

Methods:

The cells were exposed to a neurotoxin 6-OHDA at different doses; 150 μ M, 300 μ M and 600 μ M for 1hour. The cells were also pre-conditioned with 6-OHDA (50 μ M) for 30minutes and then later on with a higher dose (600 μ M) to determine if pre-conditioning primes the cell to combat high levels of reactive oxygen species. Where indicated, cells received OA (5 μ M) before, during and after 6-OHDA treatment. The metal zinc (26 μ M) was also used in combination with 6-OHDA to see what effect does zinc have on PC-12 cells. Cells treated with zinc also received OA. This was done to test the antioxidative ability of OA in the presence of a metal. The cell viability was tested by MTT assay 24hours after cell treatment. Annexin V (PI) and mitoscreen tests were used to detect apoptosis.

Results:

The results showed that OA increases the cell viability of oxidatively stressed cells, pre-conditioning increases cell viability whether the cells are treated with OA or not. The results also showed that when zinc is combined with 6-OHDA it increases cell viability and if these cells are treated with OA, the cell viability is increased even more. OA alone has no effect on cell viability. These results show that OA is a good antioxidative compound and therefore a good neuroprotector that can be used to prevent, manage and treat the oxidative stress seen in Parkinson's disease.

11.1.15.2 Mohamed Moosa, Z (SANS Young Scientist)

The Effects of Methylmercury Exposure in a Parkinsonian Model

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Purpose:

Methylmercury (MeHg) is a metal toxin commonly found in the environment due to industrial contamination. MeHg poisoning has been shown to cause neurodevelopmental disorders such as mental retardation as well as motor and cognitive dysfunction. MeHg poisoning is especially hazardous during pregnancy as it affects the developing foetal brain, resulting in severe neurobehavioural deficits in offspring. In our study we investigated the effects of prenatal MeHg exposure (2.5mg/kg s.c.) on the locomotor behaviour of a 6-hydroxydopamine (6-OHDA)-lesioned (5 µg/4µl) rat (Ethical clearance reference: 046/09/Animal). We also studied the effect of the neurohormone melatonin (10mg/kg i.p.) in alleviating neurobehavioural deficits in this model.

Methods:

Two tests were used to assess the behaviour of the rat pre- and post-lesion. These included the step test and the cylinder test.

Step test: The step test measures movement initiation. The animal was held by its torso such that the hindquarters and forelimb not being tested were elevated by the experimenter and the weight of the animal is supported on the forelimb being tested. The animal was then propelled forward on a surface covered with sandpaper and the adjusting steps made by the forelimb were measured using a ruler attached adjacently.

Cylinder test: The cylinder test examines forelimb use during explorative behaviour. The animal was placed in a Plexiglas cylinder (20 cm diameter and 30 cm height) for 5 min and its behaviour was videotaped and assessed. The animal was tested with each forelimb for wall exploration, contact with the wall as well as landing after wall contact. This is assessed as a score and the scores are calculated as a percentage.

Glutathione assay: Plasma glutathione levels were measured using a Glutathione Assay kit.

Results:

In the step test, exposure to MeHg did not seem to affect the size of the lesion as there was no significant difference between the lengths of the step taken by the metal-exposed group compared to the control group post-lesion. Melatonin had a positive effect by decreasing neurobehavioural deficits caused by the toxin. Results of the cylinder test showed no significant difference in the percentage limb use of the impaired limb in control and metal-treated groups. Results also showed that groups treated with melatonin had the greatest percentage limb use of the impaired limb. Plasma glutathione levels were measured as an indicator of oxidative stress. Results showed no significant differences in glutathione levels between all groups.

11.1.15.3 Strydom, B

8-Aryl- and Alkyloxycaffeine Analogues as Inhibitors of Monoamine Oxidase

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Purpose:

Monoamine oxidase (MAO) is an important drug target for the treatment of Parkinson's Disease (PD) and consists of two isoforms, MAO-A and MAO-B. MAO is responsible for dopamine metabolism in the brain. During this metabolic process, H₂O₂ is released which can contribute to neuronal cell damage. Inhibitors of MAO may thus conserve dopamine in the Parkinsonian brain as well as provide neuroprotection by preventing the formation of H₂O₂. Currently the irreversible MAO-B inhibitor, selegiline, is being used for the treatment of PD. The use of selegiline has been associated with cardiovascular and psychotoxic side effects.

Although there is a higher concentration of MAO-B in the aged Parkinsonian brain, selective inhibition of MAO-B may not effectively reduce dopamine metabolism. This can be attributed to the fact that MAO-A, although lower in concentration, can still metabolize dopamine sufficiently in the brain. Therefore, the development of non-selective, reversible inhibitors of MAO might be of value.

Rationale:

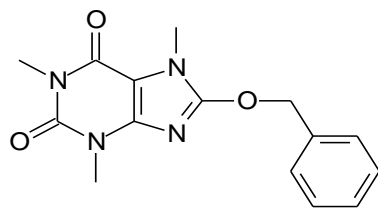
A previously synthesized series of 8-benzyloxycaffeine analogues proved to be potent, reversible inhibitors of both MAO-A and MAO-B with a slight preference for MAO-B. In the current study we propose to synthesize a series of related 8-aryl- and alkyloxycaffeine analogues in an attempt to discover additional, potent MAO inhibitors. These inhibitors will be compared to the series of 8-benzyloxycaffeines.

Methods:

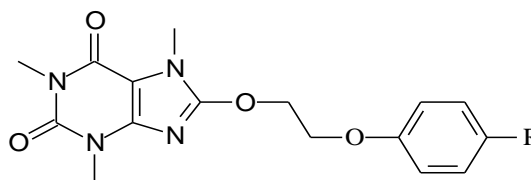
The 8-aryl- and alkyloxycaffeine analogues were synthesized by condensing 8-chlorocaffeine with the appropriate alcohol at high temperatures in the presence of metallic sodium. For the biological assays recombinant human MAO-A and MAO-B were used as enzyme sources while kynuramine was utilized as substrate. The inhibitor potencies were expressed as IC₅₀ values.

Results and Conclusion:

The 8-aryl- and alkyloxycaffeine analogues proved to be potent, non-selective inhibitors of MAO, but displayed greater selectivity towards MAO-B. One of the most potent inhibitors toward MAO-B was 8-(2-phenoxyethoxy)caffeine with an IC₅₀ value of 0.38 μM. This is approximately 4.6 times more potent than 8-benzyloxycaffeine (1.77 μM). It was also found that halogen substitution on the phenyl ring enhanced the inhibitory activity of 8-(2-phenoxyethoxy)caffeine. For example, 8-[2-(4-bromophenoxy)ethoxy]caffeine was the most potent inhibitor for this series with an IC₅₀ value of 0.166 μM towards MAO-B



8-Benzyloxycaffeine



8-(2-Phenoxyethoxy)caffeine

R = H, Br

11.1.15.4 Poka, MS

Investigation of Natural Polymer Systems to Control Nicotinic Acid Release

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Purpose:

An extended release dosage form of nicotinic acid is currently marketed as Niaspan® in the United States. The development of a generic product would provide a cost effective and an equivalent treatment option for the South African population suffering from hyperlipidaemia. Therefore, development of a natural polymer based matrix tablet was undertaken to produce an extended release dosage form of nicotinic acid. Natural polymers are widely available and economical to produce on commercial scale.

Methods:

Preformulation studies were conducted to identify suitable polymers from the available options and evaluated for physico-chemical compatibilities with nicotinic acid using differential scanning calorimetry and short term accelerated stability studies. Using the selected polymers and excipients, a generic version of Niaspan® tablets were formulated by matching the *in-vitro* dissolution profiles. A trial and error approach was used to match the dissolution profiles to the innovator product. Formulations were developed by changing the different tablet variables, such as polymer ratios, binder concentration and type of granulating agent. The drug release profiles were evaluated statistically by means of the similarity (f1) and difference (f2) factors. The developed formula was subjected to scale-up, film coating and placed on real time and accelerated stability.

Results:

From the preformulation studies sodium alginate and rosin were identified as suitable polymers as they offer flexibility in drug release over a wide pH range and were found to be compatible with nicotinic acid. From the preliminary development studies a prototype formulation with sodium alginate to rosin ratio of 10: 90 (w/w) was found to have satisfactory similarity (f2) and difference (f1) factor values, suggesting pharmaceutical equivalence to the marketed product. The prototype formulation was successfully scaled-up and subjected to stability studies. The results of preliminary stability testing indicated potential stability for 24 months when stored under long term conditions, however, under accelerated conditions it failed due to physical instability.

Conclusions:

This study proved that it is possible to develop a stable once daily dosage form of highly water soluble drugs using natural polymers. Stability studies need to be repeated with a different packing material in order to improve the stability of final formulation when stored under accelerated conditions.

11.1.15.5 Hayeshi, R

Nanomedicine for Improved Efficacy of TB Drugs

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Introduction:

South Africa currently has the highest incidence of Tuberculosis (TB) per 100 000 people in the world. In addition, current TB treatment experiences low success rates due to poor pharmacokinetics and thus reduced efficacy. The TB drugs are administered daily in high doses due to poor bioavailability, induced by low solubility and premature degradation of the drugs before reaching the target sites. This leads to poor patient compliance and the emergence of drug resistance. Nanomedicine offers a possible solution by presenting the ability to alter the pharmacokinetics of the conventional drugs to enhance bioavailability, increase the half-life of the drugs and reduce the toxicity. The aim of this work is to reduce the dose of TB drugs, as well as the dose frequency using nanomedicine.

Methods:

Isoniazid and Rifampicin were nano encapsulated in Poly (lactide-co-glycolide), (PLGA) using a novel multiple emulsion spray-drying technique. The nanoencapsulated drugs were administered to TB infected mice once a week for 4 weeks compared to the conventional drugs which were administered every day for 4 weeks#. The bacterial burden in the lungs and spleen was measured as colony forming units (cfu). Pulmonary pathology was also observed. Ethical clearance number: REC REF: 008/036, University of Cape Town.

Results:

Nanoparticles of 250 nm to 400 nm were obtained with an encapsulation efficiency varying from 50 % to 65 %. The once weekly treatment with nanoparticulate drug resulted in a similar reduction in cfu as the daily treatment with conventional drug in both lungs and spleen. This was supported by the lung pathology.

Discussion and conclusion:

The results suggest that there is a slow release of drug from the nanoparticles and hence the comparative efficacy with the low frequency dose. This slow release from the nanoparticles results in an increase in the half-life and hence the lower dose frequency has a similar effect as the higher dose frequency. There is therefore the potential to develop a low dose, low frequency TB nanomedicine with the aim to improve efficacy and patient compliance. Once the system has been optimised for TB drugs, it is envisaged that the technology will be applied for Malaria, HIV/AIDS as well as other diseases, which suffer from patient non-compliance and are major burdens in Africa.

11.1.16 Session M

11.1.16.1 Jobson, R

Through the Gaps: Paradigm Hopping

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Introduction:

Complementary and Alternative Medicines (CAMs) manufacturers and sellers often suggest that CAMs cannot be assessed or evaluated using the same methodologies as conventional medicines. It is claimed that paradigm shifts are needed to assess or evaluate these products.

Methods:

Definitions and understandings of “paradigms” are explored. An example of a website advertising a health product is deconstructed, wherein it is stated that the product functions “in terms of the Chinese Herbal System and does not imply that [it] is recommended for any western disease diagnosis.” The original version of the advertising was aimed at HIV/AIDS sufferers, but this was changed after a complaint to the Advertising Standards Authority (ASA) was upheld. The manufacturer and seller of the product conducted a survey of users and the results of this survey as published on-line on the website are analysed.

Results:

The advertising incorporates both Chinese Medicine concepts and biomedical pathophysiological concepts. Innuendo that the product will assist HIV/AIDS remains in the several references to the product’s effects on the immune system.

The report on the survey of users’ responses only address “western diseases” and no diagnoses or descriptions from a Chinese medicine discipline or paradigm are included.

Discussion:

The term “paradigm shift” when used to promote CAMs may be extremely misleading and could possibly more accurately be referred to as “paradigm hopping” or “paradigm confusion”.

The regulatory consequences of this are also discussed.

11.1.16.2 Kapp, E

Interventions to Maintain Quality Pharmaceutical Care within the Public Health Care Sector: The Changing Role of the Pharmacist

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Purpose:

Dramatic increases in the prevalence of HIV, TB and related diseases has resulted in drastic changes in the number of patients as well as the profile of patients requiring pharmaceutical care within the public health care sector. The critical shortage of pharmacists combined with the need for additional training and experience in the field of HIV and TB is making it increasingly difficult to provide adequate pharmaceutical care to an ever increasing pool of patients. Kheth'Impilo has over the last 4 years developed methods, tools and models of care in an effort to maintain good quality pharmaceutical care as required by such a complex program.

Methods:

Additional Training: The Health Care Services Cluster Training department within Kheth'Impilo has developed comprehensive HIV/AIDS didactic training programs presented to the full health care team including pharmacists, pharmacist assistants, nurses and doctors. Didactic training is followed up by on-site mentoring to facilitate translation of knowledge into practice.

Roving Care Teams: Kheth'Impilo has developed a Roving Team model of care where a number of smaller Anti-Retroviral Therapy (ART) initiation facilities are visited by a team of experienced health care workers who will take responsibility of the initial ART initiation of patients at these facilities. Roving team members will mentor facility based health care workers to take responsibility for the comprehensive management of HIV-positive patients to allow roving teams to take on a supportive role while facility based health care workers manage patients.

ISPA Model of Care: The Pharmacy Act (53 of 1974) allows Post Basic Qualified Pharmacist Assistants (PBPA) to work under the Indirect Supervision of a pharmacist at Primary Health Care Clinics (PHC) under conditions as stipulated by the act. Kheth'Impilo (KI) has developed a model of care where PBPAs undergo additional training and mentoring to better equip them to work under indirect supervision. The Indirect Supervision of PBPA model or ISPA model goes beyond what is required by good pharmacy practice (GPP) and prepares the assistant to take responsibility for the full functioning of the dispensary as well as provide good pharmaceutical care to patients served by the facility. Pharmacists do monthly audits and continuously mentor, support and supervise pharmacist assistants during regular support visits.

Results:

Through implementation of these models KI supported facilities currently care for more than 90 000 patients. 79.5% of patients in a 24 month cohort are maintaining Viral suppression showing good quality of care despite increasing patient numbers. A comparison of patient outcomes between pharmacist-supported facilities and facilities supporting the ISPA model of care showed equivalent patient outcomes.

Conclusion:

Pharmacists will remain in critical shortage within the public health care sector for the foreseeable future. The scarce skill set of pharmacists should be used more effectively in supporting, mentoring and training lower level pharmacy workers and allied health care professionals. Pharmacists have the ability to introduce innovative viable cost effective solutions to provide quality pharmaceutical care to patients in order to reach the targets being set in the new HSP. Pharmacist should be a key part of the solution to the re-engineering of Primary Health care services.

11.1.16.3 Meyer, R

Restructuring of Practical Training at the NWU School of Pharmacy

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Introduction:

The NWU School of Pharmacy have traditionally relied on provincial hospitals, pharmacies and PHC clinics locally and in the surrounding districts for training and exposure of students to pharmacy practice and primary healthcare. Clinic and hospital visits traditionally had to be scheduled during practice sessions of Pharmacy Practice theory modules. In 2010, however, a pilot process was introduced, in which most of the clinical and workplace-related, off-campus activities were moved to a consolidated block of 2 weeks before the official start of the normal academic year. This was intended to relieve time pressure on the stressed academic roster. The pilot programme initiated in 2010 proved to be an improvement on previous methods and was further refined in 2011, supported by a grant from the Department of Health.

Program content and logistics:

The logistics were complex, considering that 196 students had to be rotated and transported in small groups of 4-8 among 55 destinations (some as far as 50 km from Potchefstroom) within the course of 8 days. The clinical training programme involved only 3rd and 4th year pharmacy students, but if possible, depending on cost and logistics, we believe that 2nd year students should be included in future. The first two program days were dedicated to training and preparation of students on campus for the activities that would follow. Three days were also dedicated to emergency care training for 3rd years. Thereafter 3rd and 4th year students in groups of 2-8 were rotated according to a roster which allowed each student 1-2 days practical exposure to different types of medical facilities depending on the needs and logistics at a particular clinic or hospital. For certain activities 3rd years accompanied 4th years who acted as mentors. In a local private hospital, student groups participated in ward rounds with a medical practitioner, and group feedback on each case had to be presented afterwards. The program also included at least one exposure per student to one each of the 10 participating PHC clinics and 10 private and/or provincial hospital pharmacies in the region, where quality assurance of medication and prescriptions were performed in wards and outpatient departments, and practical assignments completed to demonstrate integration of their theoretical knowledge and practice skills.

Community outreach programmes were planned and executed independently by individual groups of 8 students. In total approximately 5 000 screenings for cholesterol, glucose, blood pressure, BMI and peak flow were performed at the abovementioned PHC clinics, 7 schools and 28 community pharmacies in Potchefstroom, Parys, Carletonville, Klerksdorp, Orkney and Ventersdorp. Health education was provided to the public and to learners at all the sites.

Conclusion:

In conclusion we can state that, although logistically challenging, especially in view of increasing student numbers at the NWU School of Pharmacy, we believe that a program like this can be executed successfully notwithstanding the limits of facilities available to us, to the benefit of both the training institution, the medical facilities and the local community, creating a win-win situation for all.

11.1.16.4 Moch, S

Does Exit-Level Assessment of Wits Medical Students Test Rational Prescribing Skills?

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Introduction:

Prescribing medicines is the primary intervention that doctors offer to influence their patients' health; however concerns have been expressed about the extent to which graduates are prepared by medical schools to assume prescribing responsibility.

Aim:

To analyse the exit-level written assessment component of final-year students in the Graduate Entry Medical Programme (GEMP) at the University of the Witwatersrand, Johannesburg with respect to fitness-for-purpose (validity) and constructive alignment.

Methods:

Permission to conduct the study was secured from the Human Ethics Research Committee (Medical) of the University of the Witwatersrand, Clearance Number M080949. Examination questions were selected via an adjudicative process to determine a prescribing mark. Question items were then analysed according to Bloom's Revised and the SOLO Taxonomies. The theoretical framework of constructive alignment was used to interrogate fitness-for-purpose and the knowledge structures of the skills were explored using a Bernsteinian lens.

Results:

A comparison of "A-Type" (single best answer) multiple choice questions (MCQs) with "R-Type" (extended matching) MCQs paradoxically highlighted students' greater proficiency in the R-Type questions ($p < 0.0001$). Both Bloom's and SOLO taxonomies indicated that students scored well on questions which tested recall and application of knowledge, but struggled with questions involving evaluation. Questions were not well distributed according to Harries' delineation of prescribing skills to be tested. Examination marks showed that 83.6% of students were competent to prescribe according to the graduating standards of the University.

Conclusions:

Despite high examination scores, this study illustrates a lack of constructive alignment between assessment requirements, curriculum delivery and objectives of the course. Curricular components including problem-based learning and horizontal integration constrained epistemic access to the structure of rational prescribing knowledge and the exit-level written assessment does not sufficiently test rational prescribing skills.

11.1.16.5 Suleman, F

AIDS Online International (AOI): An internationally Synchronized Online University course on HIV/AIDS Education, Prevention, and Behavioural research - A Pilot Study for International Collaboration

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Purpose:

Because the rate of new HIV infections among youth is a global crisis and youth ages 13-24 are at high risk of HIV infection, more international HIV/AIDS intervention initiatives targeting young adults are needed to help decrease the rate of HIV infections among young adults. In this study, we describe the impact of an online college-credit HIV/AIDS course on the HIV-related risk behaviours, attitudes, beliefs and knowledge of student course participants at UKZN and Purdue University.

Methods:

Online anonymous surveys, the Pre-CAS and Post-CAS, were administered through an online survey tool, SurveyMonkey (SurveyMonkey.com, Portland, OR). The Pre-CAS was given online during first week of class and the Post-CAS was given online during the last week of class.

Results:

70 percent of the students were female, and 80 percent were in the 15-19 years age group. 60 percent of the students were Indian, and 35 percent were African. Generally, student knowledge about the correct methods of transmission of HIV improved during the course. Overall, students indicated more confidence in their knowledge of HIV prevention. Students also indicated that as a direct result of taking AOI they were more reluctant to have unsafe sex (83%), more likely to ask their partner to use a condom (31%) or ask his/her HIV status (27%), and more confident in how to protect themselves (93%). Most students enrolled in the course had little to no knowledge about the science of HIV disease; thus the course had a significant impact on knowledge acquired in this area. After taking the AOI, students at UKZN reported having more discussions about HIV with their peers/partners.

Conclusion:

The implementation of an online college-credit HIV/AIDS course has proved to be an effective method of HIV/AIDS education, prevention, and behavioural research and may also be a viable HIV intervention initiative.

11.1.17 Session N

11.1.17.1 Osman, L

Putting the Puzzle Pieces Together

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The publication of new policies and new legislation can be confusing. It is sometimes unclear as to if or how they interrelate. New national health policies and legislation, as well as national health discussion documents, all affect pharmacy. In addition, new pharmacy legislation is about to change the pharmacy environment.

This presentation shows the relationship between recent developments in national health policies and new developments in scopes of practice of pharmacists and pharmacy support personnel. The implications for pharmacy education and training are discussed.

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11.2.1 Session One

11.2.1.1 Ahmad, T

PK/PD and Safety Evaluation of 20kDa Peginterferon Alpha-2a from “Unipeg®” in Healthy Human Subjects

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Purpose:

Peginterferon alfa 2a (20 kDa) derived from E. coli is a distinct variety of peginterferons. A pilot study of this drug (UNIPEG®) was conducted on healthy human subjects to evaluate its safety and pharmacokinetic (PK) and Pharmacodynamic (PD) behaviour in local population.

Methods:

With due approval granted by IEC of ICCBS, University of Karachi via letter “ICCBS/AEC/Lett-17/10 dated July 9, 2010” ten healthy male subjects (Age: Years 25.2±5.33 (20-32); Weight: Kg 59.60±7.71 (60-74) were selected randomly from the Pakistani population after thorough screening and signing of the Informed Consent Document for an Open label, Single Dose study. Each subject received a subcutaneous injection of the drug (180 µg) in abdominal skin and blood samples were collected at 0 (pre-dose) and 1, 2, 3, 6, 12, 24, 36, 60, 84, 108, 132 and 156 hours, and analysed by a validated ELISA for Unipeg®. The assay with a dynamic range of 900 to 7pg/mL gave an inter assay mean accuracy of 98.31% and precision of 8.59% (n=18). The samples are stored at -80°C for the PD evaluation through analysis of biomarkers neopterin and β2-microglobulin. The PD work is in progress and has not concluded yet. The safety and tolerance of the drug was evaluated by observation of Adverse Events and evaluating the change in general health parameters, haematological and biochemical test results during and after the study.

Results:

Pharmacokinetics presented as Mean±SEM (range). C_{max}: 18.67±2.92 ng/ml (7.05 -34.51); AUC_{0-∞}: 1440±113 h.µg/l] (969 - 2101); Absorption Half-Life: 17.02±2.06 h (10.37 -29.26); Volume of Distribution: 8.933±1.72 L (4.81 - 18.34); Clearance: 0.112±8.21 ml/h (71.96 - 155.96).

Safety: No severe adverse effect was observed however the most common Adverse Event (AE) was the fever; observed in all volunteers (n=10), headache (6), Fatigue (5), Vomiting (4) and diarrhoea, loss of appetite, body ache was observed in 3 volunteers. Three out of ten volunteers demonstrated decrease in WBC and platelets count. Insignificant changes in haematology returned to normal values within 16 days.

Conclusions:

The pharmacokinetic results of UNIPEG® correlate very well with those found for other pegylated interferons generally used in therapy. The safety profile of UNIPEG® was found very similar to those of reported in literature for unmodified IFNs and other pegylated interferons generally used in therapy. Future clinical trials are recommended to further establish the safety profile and pharmacokinetics.

11.2.1.2 Bhika, F

Monitoring of the Stability of Promethazine Hydrochloride in a Fixed-Dose Combination Syrup

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Purpose:

Stability studies are required to determine the shelf-life of pharmaceutical products. The active pharmaceutical ingredients (APIs) are assayed and the degradation products of the APIs in the products are detected and quantitated over the proposed shelf-life of the product and stability trends are determined therefrom. Promethazine hydrochloride is an anti-histamine used for the treatment of allergy. Thermo- and photo-degradants of promethazine hydrochloride include promethazine sulphoxide and isopromethazine hydrochloride. The purpose of this study was to ascertain whether promethazine hydrochloride was present within acceptable limits and whether the degradants were either absent or present below acceptable limits, in syrup containing 6.5 mg/5 ml promethazine hydrochloride, when stored under standard conditions over the proposed shelf-life of the product.

Methods:

The product which is packaged in 100 ml amber bottles with screw-on caps, each in a cardboard secondary packaging was stored at 25°C and 60% RH over a 36 month period each at 0, 3, 6, 9, 12, 18, 24 and 36 months. A validated liquid chromatography method with ultraviolet detection was used to assay for the API, promethazine hydrochloride as well as the degradants, promethazine sulphoxide and isopromethazine hydrochloride. The assay for promethazine hydrochloride was accepted if the concentration fell within 5.85 – 7.15 mg/ml (90 – 110 % of the label claim) whilst the assays for the degradants, promethazine sulphoxide and isopromethazine hydrochloride were accepted if these were present at no more than 2.5 and 1.0 % of the promethazine hydrochloride content, respectively.

Results:

The results showed that at each interval, promethazine hydrochloride concentrations ranged from 6.34 mg/ml to 6.82 mg/ml (95.2 – 102.4 % of the label claim). The degradants were present between 12 and 36 months, but at concentrations which were below the limits set.

Conclusion:

A shelf-life of 24 months is appropriate for this promethazine hydrochloride-containing syrup packaged in 100 ml amber bottles with screw-on caps, each in cardboard secondary packaging.

11.2.1.3 Boschmans, S-A

The Emergence of “the ESKAPE bugs” and the Management of Patients with Positive Bacterial Cultures in the General Medical Wards of a Tertiary Level Hospital

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Purpose:

The Infectious Diseases Society of America (IDSA) has expressed their awareness regarding the problem of resistance and is concerned because the development of new antibiotics has been abandoned by pharmaceutical companies and the decline in development has carried on for greater than a decade. In 2006 the cost to treat resistant nosocomial infections in the USA was estimated to be more than \$1.87bn (ZAR 13.1bn), illustrating just how serious the problem of resistance has become. Given the problem of limited antimicrobial drugs, health care professionals should appreciate the available drugs and use them correctly so that their efficacy can be preserved for generations to come. Resistant bacteria have been narrowed down to a group of bacteria known to “escape” the action of antibiotics and have been named “the ESKAPE bugs” (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species). In addition, the UK has also labelled methicillin-resistant *Staphylococcus aureus* (MRSA) and extended spectrum β -lactamases as a problem. Antibiotic resistance can also be expected to be as much of a problem in developing countries as it is in the developed world.

Methods:

The microbiological data of all patients in the general medical wards who were older than 18 years and for whom IV antibiotics had been prescribed was obtained from the National Health Laboratory Service (NHLS) database at a local tertiary level government hospital over a period of 18 weeks in 2009/10. Three separate audits consisting of 150 patients each were conducted using a total population sample. Ethical approval was obtained from the Nelson Mandela Metropolitan University Ethics Committee (Human) (Reference number: H09HeaPHA001). Access to medical records was granted by the Medical Superintendent and thus informed consent was not obtained from individual patients.

Results:

Twenty one different organisms were identified during the study including *Klebsiella pneumoniae* (22%; 13; n=59), *Enterobacter cloacae* (10.2%; 6; n=59), *Staphylococcus aureus* (10.2%; 6; n=59) and *Pseudomonas aeruginosa* (6.8%; 4; n=59). During each audit there were patients who were not appropriately managed according to susceptibility results (44.4%; 4; n=9, 56.3%; 9; n=16, 52.6%; 10; n=19). Bacteria were highly resistant to penicillins (ampicillin, amoxicillin & penicillin G) (with resistance ranging from 76.2%; 16; n=21 to 100%; 10; n=10) and the only organism that showed acceptable susceptibility to penicillin was *Streptococcus pneumoniae*.

Conclusion:

The results confirm that resistance from organisms already known in other countries to “escape” the action of antibiotics is also arising in South Africa. Antibiotic resistance to commonly prescribed antibiotics and inappropriate prescribing should draw attention to the need for antibiotic guidelines and educational measures, which in turn, would lead to a reduction in morbidity and mortality and health care costs.

11.2.1.4 Boschmans, S-A

Implementation of Intravenous to Oral Antibiotic Switch Therapy Guidelines in the General Medical Wards of a Tertiary Level Hospital

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Purpose:

Studies have shown that cost and pharmacoeconomic aspects are becoming increasingly important in antibiotic therapy. Switching from IV to oral antibiotics as soon as patients are judged clinically stable, according to specified criteria, has pharmacoeconomic benefits without compromising antimicrobial efficacy. In many countries pharmacists are responsible for the implementation of antibiotic IVOST (IV to oral switch therapy) guidelines. The benefits of IV to oral switch are: reduced workload and hospital staff time required; decreased length of hospital stay; reduced waste disposal; a reduced number of IV administration equipment, cannulae and infusion bottles required; oral antibiotics are cheaper than parenteral therapy; reduced storage costs for IV drugs; increased patient comfort and mobility; and the elimination of intravenous line complications.

Methods:

The study employed a before-and-after intervention design and consisted of a pre-implementation phase (three weeks), before the introduction of any guidelines, an implementation phase (a ward pharmacist visited four general medical wards two to three hours per day, three to five days per week, Monday to Friday over a period of seven weeks) and two post-implementation audits (conducted immediately after implementation over three weeks (in 2009), and three months after implementation over five weeks (in 2010)). Using a total population sample, data was collected from the medical records of all patients in the general medical wards during each study period who were older than 18 years and for whom IV antibiotics had been prescribed. The three audits consisted of 150 patients each. Ethical approval was obtained from the Nelson Mandela Metropolitan University Ethics Committee (Human) (Reference number: H09HeaPHA001). Access to medical records was granted by the Medical Superintendent and thus informed consent was not obtained from individual patients.

Results:

The incidences of switch for the three audits were: pre-implementation, 16% (19; n=119); immediate post-implementation, 43.9% (47; n=107); and three-month post-implementation, 20.8% (25; n=120). There was a statistically significant increase in the number of patients switched which then significantly decreased again ($p < 0.0005$). There was also a significant difference between the average length of IV therapy for the three audits (7.3 ± 3.5 days pre-implementation; 5.2 ± 3.0 days immediate post-implementation; 6.5 ± 3.5 days three-month post-implementation) ($p < 0.0005$). The estimated financial saving for the switched groups during each audit were: R9004.07 (pre-implementation; n=19), R15715.68 (immediate post-implementation; n=47) and R5787.25 (three month post-implementation; n=25).

Conclusion:

The design, presentation and distribution of an institution specific IVOST guideline followed by the placement of 'iv to oral' stickers on patients' drug prescription charts and physicians' daily assessment sheets (once patients were deemed eligible for switch according to IVOST criteria) by a ward pharmacist can lead to positive IVOST outcomes. In this study the intervention increased the number of switched patients which also contributed towards saving medication costs by preventing unnecessary IV treatment days.

11.2.1.5 Boschmans, S-A

Response to Treatment in HIV Positive Women versus HIV Negative Women Diagnosed with Cervical Cancer

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Purpose:

Cervical cancer is the second leading cancer among South African women. Up to 30% of these patients are HIV positive. There is limited literature available regarding the response of HIV positive women to cervical cancer treatment. Therefore, the aim of the study was to assess the extent to which HIV positive women with cervical cancer respond to cervical cancer treatment.

Methods:

A historical cohort design was used to conduct the study at an Eastern Cape tertiary level hospital (ethics clearance number: H10-HEA-PHA-001). The sample consisted of 196 medical records of women diagnosed with cervical cancer between 2005 and 2008. One hundred women were HIV negative, 83 were HIV positive and the HIV status of 13 women could not be determined. The records were audited over a period of two years from the date of diagnosis.

Results:

The term 'complete response' referred to patients who had no recurrence of cervical cancer and no evidence of metastases after undergoing treatment. At one month following treatment there was a significant difference in the incidence of complete response between the HIV positive patients and the HIV negative patients ($\text{Chi}^2 = 16.4$, d.f. = 1, $p = 0.00005$, $V = 0.31$). The significant difference in response to treatment between the HIV positive patients and the HIV negative patients was maintained at six months after treatment ($\text{Chi}^2 = 15$, d.f. = 1, $p = 0.00011$, $V = 0.34$), 12 months after treatment ($\text{Chi}^2 = 20.5$, d.f. = 1, $p = 0.00001$, $V = 0.37$), 18 months after treatment ($\text{Chi}^2 = 9.8$, d.f. = 1, $p = 0.00173$, $V = 0.28$) and 24 months after treatment ($\text{Chi}^2 = 5.0$, d.f. = 1, $p = 0.02571$, $V = 0.26$). At each of these intervals, the response to cervical cancer treatment was poorer among HIV positive women.

Conclusion:

Cases of treatment failure and metastases were significantly higher in HIV positive women than in HIV negative women.

11.2.1.6 Breytenbach, JC

New Antimalarial Compounds

Jaco C. Breytenbach^{1*}, **David D. N'Da**¹, **Marli Lombard**¹ and **Wilma J. Breytenbach**²

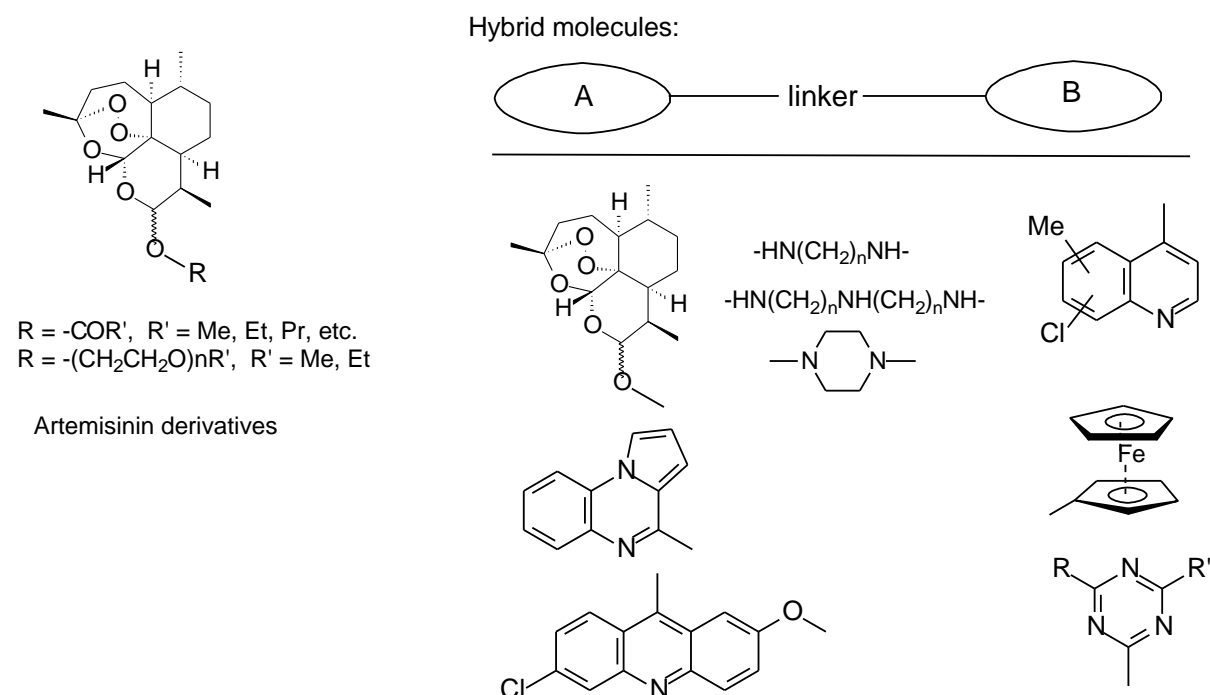
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Malaria is with HIV and TB one of the three diseases of priority declared by the SA Department of Health. World-wide there are each year more than 225 million cases and 781,000 deaths because of malaria. Ninety percent of malaria-related deaths occur in sub-Saharan Africa of which the majority are young children. Despite international efforts to 'roll back malaria' the disease still affects approximately 3 billion people in 109 countries; 45 within the WHO African region. The disease also has a devastating economic effect on regions where it is widespread and it has been estimated that malaria costs Africa US\$12 billion every year and in some countries with a heavy malaria burden, the disease may account for as much as 40% of public health expenditure.

The problem is currently handled by prevention of infection, vector control, prophylaxis and chemotherapy. All the drugs currently in use suffer from shortcomings and all attempts to produce a vaccine have to date not delivered any solution, which stresses the need for new and effective medicinals.

By preparing derivatives of existing antimalarial agents or hybrid molecules consisting of two antimalarial moieties we investigate the effects of molecular modification on the activity of these compounds against *Plasmodium falciparum* in an attempt to address the current shortcomings.



Activity against both sensitive and resistant strains of *P. falciparum* better than that of chloroquine and in the range of that of artemether and dihydroartemisinin was obtained.

11.2.1.7 Breytenbach, WJ

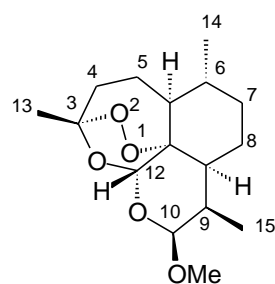
Statistical Analysis of Antimalarial IC₅₀ Values of Ethylene Glycol Ethers of Artemisinin

Wilma Breytenbach^{1*}, **Minette Steyn**², **David N'Da**², **Peter Smith**³, **Jaco Breytenbach**² and **Sandra Meredith**³

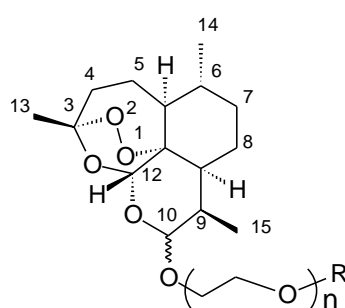
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The antimalarial activity of ethylene glycol ethers (MS01 – MS06) against the chloroquine sensitive D10 strain and chloroquine resistant Dd2 strain of *P. falciparum* was statistically analysed using one-way analyses of variances (ANOVA) to determine whether statistically significant differences existed between the means of the IC₅₀ values of the new compounds and that of Artem and CQ, respectively.



Artemether (Artem)



Ethylene glycol ethers

Ether	Isomer	R	n
MS01	10β	Me	1
MS02	10β	Me	2
MS03	10β	Me	3
MS032	10α	Me	3
MS04	10β	Et	1
MS042	10α	Et	1
MS05	10β	Et	2
MS06	10β	Et	3

These statistical methods, contrary to inspection, allow reliable conclusions on the differences in the antimalarial activity of all tested compounds limiting the risk of error to 5%.

Table: Descriptive statistics of IC₅₀ values, results of ANOVA and Dunnett's tests, and antimalarial activity of ethylene glycol oligomeric ethers of artemisinin, artemether and chloroquine

Ether	<i>P. falciparum</i> CQ sensitive D10 strain						<i>P. falciparum</i> CQ resistant Dd2 strain					
	n ^a	Mean IC ₅₀ (nM)	Std. dev.	p value: ANOVA	p-value: Dunnett		n ^a	Mean IC ₅₀ (nM)	Std. dev.	p-value: ANOVA	p-value: Dunnett	
					Artem	CQ					ARM	CQ
MS01	^b						3	0.510	0.007		0.000*	0.000*
MS02	3	0.045	0.002		0.000*	0.020*	3	0.039	0.001		0.000*	0.000*
MS03	3	0.094	0.005		0.000*	0.000*	3	0.061	0.002		0.000*	0.000*
MS032	3	0.050	0.006		0.000*	0.203	3	0.030	0.002		0.000*	0.000*
MS04	3	0.090	0.003		0.000*	0.000*	^b					
MS042	3	0.051	0.007		0.000*	0.230	3	0.025	0.002		0.000*	0.000*
MS05	3	0.051	0.005		0.000*	0.230	3	0.032	0.004		0.000*	0.000*
MS06	3	0.030	0.003		0.162	0.000*		0.023	0.001		0.000*	0.000*
Artem	3	0.021	0.008	0.00*			3	0.004	0.000	0.00*		
CQ	2	0.060	0.004	0.00*			2	0.466	0.005	0.00*		

* Statistically significant at 0.05 level, ^a number of replicates, ^b omitted from statistical analysis

Statistical inference confirms (error ≤ 5%) that all the ethers, except MS01, had statistically significantly better activity than CQ on the Dd2 strain, while MS02 and MS06 were also better than CQ on the D10 strain. Except for MS06, whose activity did not differ statistically significantly from that of Artem on the D10 strain, all compounds performed worse than Artem on both strains of *P. falciparum*.

11.2.1.8 Burton, S

Numerical Skills of Pharmacy Students: Requirements, Abilities and Attitudes

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Purpose:

For pharmacists, the ability to perform routine calculations accurately is essential to delivering pharmaceutical care and ensuring the well-being and safety of patients. However worldwide, there is mounting concern regarding both the calculation capabilities of pharmacy graduates and the poor numeracy skills of first year pharmacy undergraduate students. The aim of this study was to assess the numerical capabilities, with and without the use of calculators, of entry level pharmacy students at the Nelson Mandela Metropolitan University (NMMU).

Methods:

Pre-testing of numeracy skills of all entry-level BPharm students, using purpose-designed test instruments, was completed at the start of the 2010 academic year. An attitudinal survey-based evaluation of the students' perceived competence and self-confidence in their numerical capabilities was also conducted. With the permission of the South African Pharmacy Council an analysis of five years of pre-registration exam papers, and examiners' reports (2004-2008), was carried out, in order to identify both the nature of numeracy skills involved in calculations required of pharmacy graduates, and the examiners' perceived ability of interns to perform these calculations. Ethical approval (reference H10HEAPHA002) was granted by NMMU's Research Ethics Committee (Human) prior to commencement of the research.

Results:

The mean score for the non-calculator test was 59,63% (n=98, sd=18,43) and for the calculator test, 82,39% (n=100, sd=9,29). In the non-calculator test 34 students had a score of less than 50% and 69 less than 70%. In an equivalent test where calculators were used, all students achieved scores of greater than 50% and only 8 had a score less than 70%. Of the students who completed the attitudinal survey (n=84), 79,8% rated their ability to do basic calculations as good or excellent, however 94,0% admitted to routinely using a calculator to perform calculations. Although 71,4% of respondents agreed that pharmacists should be able to perform calculations without a calculator, only 19,0% agreed that calculators should not be permitted in pharmacy examinations. On analysis of the preregistration examination papers, it was found that on average only 12% of the marks in the papers required calculations, and that interns may easily pass the examination without demonstrating mastery of calculations. The examiners' reports consistently express concern about poor competence in performing calculations and especially the inability of many interns to recognise, using common sense, illogical numerical answers.

Conclusion:

Pharmacy students are reaching university with low-levels of non-calculator based numerical capabilities, and with an almost total reliance on calculators. If not addressed during their training this could have serious consequences on their future numerical reasoning abilities and computational skills and therefore impact on their competency as pharmacists.

Acknowledgements:

The South African Pharmacy Council for permission and access to the examination papers and examiners' reports.

11.2.1.9 Butler, N

An Investigation into Preferences for Marketing Messages for the Pharmaceutical Society of South Africa (PSSA)

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Background and purpose:

According to the South African Pharmacy Council (SAPC), there are currently more than 11 000 pharmacists registered in South Africa. In contrast, the Pharmaceutical Society of South Africa (PSSA), a voluntary professional organisation has around 5 000 pharmacist members. This study was motivated by this significant difference. The main purpose of this study was to develop marketing messages for PSSA and to assess the relationship between preferences for the various messages and demographic variables of the study respondents. Since the PSSA is engaged in the implementation of a strategic plan aimed at increasing membership, recommendations from this study would feed into that process.

Methods:

A series of 14 marketing messages were developed, encompassing both ideas congruent and incongruent with the mission statement of the PSSA; these were disseminated to participants in questionnaire format. Respondents provided demographic information and rated preferences for the messages on a 5-point Likert scale. Preferred media channels for dissemination of marketing messages were also determined. This study was conducted in three phases: viz. two quantitative studies targeting potential members (current fourth year pharmacy students, n=258) and pharmacists (both members and non-members of the PSSA, n=338) and a qualitative study focus group (n=6) in which pharmacists from different practice sectors participated. The pharmacist questionnaire was administered via the PSSA e-newsletter.

Results:

The most preferred marketing message amongst potential members and members was “PSSA keeps you updated with the latest pharmaceutical developments”, with high positive response rates of 70% and 80% respectively. Not only plausible messages were rated positively; incongruent messages, for example, “PSSA maximises your profits” which received favourable responses of 50% and 40% respectively, were also preferred. The future utilisation of newer social networking media was recommended by both potential and actual members. There was a general consensus that the PSSA’s marketing amongst students and some hospital pharmacists is non-existent.

Conclusion and Discussion:

The PSSA lacks a viable marketing strategy which possibly explains the disparity in PSSA membership and registered pharmacists. This study recommends that PSSA embarks on focussed marketing campaign and uses appropriate channels that comprise clear and understandable messages that relate to what the PSSA actually has to offer, because it is clear that there is much confusion about what PSSA is and what the benefits of membership would be.

11.2.1.10 Charles, B

Interim Report from the Medunsa National Pharmacovigilance Centre: A Phase IV Study Monitoring Anti-Retroviral Therapy in the South African Public Sector

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Elzbieta Osuch¹

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Introduction:

The Medunsa National Pharmacovigilance Centre (MNPC) operates through a prospective, observational, longitudinal cohort event monitoring system for Anti-Retroviral Therapy (ART) in the South African public health sector. Situated on the Medunsa Campus of the University of Limpopo, the Centre works through sentinel sites at five major ART health centres, in three provinces. Active since October 2006, its database covers over 2500 HIV positive patients on ART.

Objectives:

To report selected statistics generated by the MNPC. Demographics, ART initiation regimens, age and gender at ART initiation and the MNPC's enrolment strategy were reviewed.

Method:

The cohort includes HIV positive subjects on ART, 15years and older. Random sampling was undertaken at the sites. Informed consent was obtained. Relevant data from subjects' files were captured onto Case Report Forms (CRF) by on-site coordinators at every visit and subsequently entered into the database at the MNPC. A data dump of the current database was done into Microsoft Excel. Four spread sheets were generated: Patient (2590 records with 22 variables); pharmacy (17461 records with 48 variables); laboratory (30021 records with 5 variables) and clinic (7147 records with 19 variables). Validation checks were done and inconsistencies corrected. Epi Info 3.5.1 and STATA 11 (StataCorp ®) were used for data analysis. Ethical clearance was obtained from University of Limpopo, Medunsa campus: MREC: MP119/2006.

Results:

From October 2006 to March 2011, 2590 subjects were enrolled. There is a higher proportion of females (70%) than of males. Almost all subjects are of black ethnicity. The peak age group of enrolled subjects is 35 to 39. Gauteng Province has the largest representation of subjects (41.3%), followed by Limpopo Province (39.9%) and Mpumalanga Province (18.8%). A large majority of subjects were initiated on stavudine, lamivudine and efavirenz (79.3%). The peak age group at initiation of ART is 30 to 34 years with males initiating ART later than females. The Centre achieved its highest enrolment figures in 2009. Most subjects were enrolled into the study five to nine months after initiating ART.

11.2.1.11 Chanakira, TJ

Development and Validation of a RP-HPLC Method for the Determination and Stability Testing of Mometasone Furoate in Pharmaceutical Dosage Forms

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Purpose:

To develop a simple, sensitive, selective, accurate, linear and precise RP-HPLC method for the quantitative analysis of mometasone furoate in pharmaceutical dosage forms and to monitor the release of mometasone furoate from topical formulations during *in vitro* testing.

Method:

An isocratic RP-HPLC system consisting of a Spectra Physics[®] SP8810 solvent delivery system (San Jose, USA), Waters[®] Associates WISP 710B auto-sampler (Milford, MA, USA), a Linear[®] UVIS 200 detector (Reno, Nevada, USA) and a Perkin-Elmer[®] 561 Chart recorder (Tokyo, Japan). A Nova-Pak[®] C₁₈ (150mm x 3.9mm x 4µm) column was used as the stationary phase and the mobile phase consisted of acetonitrile and water in a ratio of 55:45 v/v. The eluant was monitored at a wavelength of 240nm. The injection volume was 15µl at a flow rate of 1ml/min. Fresh stock solutions of mometasone furoate and betamethasone 17-valerate (internal standard) were prepared daily by weighing 10mg of each compound into different A-grade volumetric flasks (100ml) and making up to volume with mobile phase to produce solutions of a final concentrations of 100µg/ml. Calibration standards were prepared by serial dilution of the stock solution and the calibration curve was constructed over a range of 0.1-20µg/ml by plotting the peak height ratio of mometasone furoate and betamethasone 17-valerate versus concentration. The concentration of betamethasone 17-valerate was constant for all samples (6.25µg/ml). The effect of flow rate, injection volume and mobile phase composition on the retention time of these compounds was also monitored. The peak tailing, asymmetry and resolution factors were also established. The method was then validated using ICH guidelines for linearity, precision, accuracy and the limits of quantitation and detection.

Results:

An increase in the percentage of acetonitrile in the mobile phase decreased the retention time of mometasone furoate and betamethasone 17-valerate, whereas a decrease in the flow rate increased the retention times of these compounds. The method was linear over the range 0.1µg/ml-20µg/ml with an R² value of 0.9995. The equation of the line was $y = 0.1161x + 0.0041$. The peaks were sharp, symmetrical and narrow without evidence of tailing. Mometasone furoate is unstable in strong oxidising, acidic and alkali conditions. It is unstable at high temperatures.

Conclusion:

A simple, sensitive and precise RP-HPLC method has been successfully developed and validated and applied to the analysis of commercially available mometasone containing creams.

Acknowledgements:

The authors acknowledge funding from Rhodes University Joint Research Committee (KWK and RBW) and National Research Foundation (RBW).

11.2.1.12 Dagnolo, B

Development and Assessment of Fast Dissolving Sildenafil Citrate Tablets for Paediatric Use

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Purpose:

Sildenafil citrate is a phosphodiesterase-5 inhibitor that is used to treat pulmonary hypertension (PH) in paediatric patients. The purpose of this study was to formulate a fast dissolving tablet (FDT) that can be easily administered to neonates and children with PH. The advantages of FDT's include ease of administration, rapid dissolution of sildenafil and the FDT can be taken without water which is beneficial to patients without immediate access to potable water.

Method:

Several batches of tablets were manufactured to produce dosage forms that contained 3mg of sildenafil citrate. Mannitol or fructose, were used as the primary diluents with combinations of starch and/or microcrystalline cellulose (MCC) as additional diluents. Crospovidone, croscarmellose sodium and sodium starch glycolate were included in the formulation as disintegrants and were used in the same proportions for each batch manufactured, viz., 3.5%, 8.5%, and 8% m/m, respectively. Magnesium stearate, talc and colloidal silicon dioxide were used as anti-frictional agents. Sildenafil citrate, the diluents and disintegrants were screened through a #20 sieve separately from the magnesium stearate, talc and colloidal silicon dioxide which were screened through a #44 mesh. The mixture, without the magnesium stearate, was blended for 10 minutes, and then blended for a further 3 minutes with the magnesium stearate. The mixture was compressed to form 7mm diameter flat-faced tablets using a Manesty® F3 single punch press.

Results:

The optimum formulation was found where 15% m/m and 61.4% m/m of MCC and mannitol respectively were included in the formulation. This batch showed a wetting time of 8.2s and a disintegration time of 20±2s. When starch was substituted for MCC the wetting time increased to 113.6s and the disintegration time increased to 80±3s. The tablets manufactured with fructose were generally more variable than those manufactured with mannitol with respect to their hardness, disintegration and sildenafil content. This is most likely due, in part to the larger crystal size of the fructose used compared to that of mannitol which was confirmed with Scanning Electron Microscopy. When mannitol and fructose were used in large amounts in the formulation, the disintegration times were shorter which was attributed to the good aqueous solubility of both excipients.

Conclusion:

The addition of three super-disintegrants in addition to the use of high concentrations of mannitol produced tablets that were acceptable in terms of their hardness, friability and disintegration times. Therefore a suitable optimized formulation for an FDT of sildenafil citrate should include appropriate proportions of disintegrants and diluents to ensure rapid delivery of the API to patients with PH.

Acknowledgements:

The authors acknowledge financial support from the Joint Research Committee and NRF (RBW).

11.2.1.13 Danckwerts, MP

The Prevalence of Hyperlactatemia in Adult Patients on an Anti-retroviral Therapy Programme in a Public Sector Clinic in the Free State Province

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Purpose:

The national programme of expanded access to antiretroviral therapy in the South African public health sector has resulted in thousands of South Africans being subjected to prolonged antiretroviral therapy (ART) with the associated risks of adverse drug effects. Among the most common adverse effects are metabolic disorders, one of which is mitochondrial toxicity. Mitochondrial toxicity may manifest as hyperlactatemia. The study was designed to determine the frequency with which hyperlactatemia occurs in HIV – infected adults on long-term ART. The objective was to determine the proportion of patients with blood lactate levels that exceed a predetermined cut-off level and to attempt to relate hyperlactatemia to a set of factors namely, gender, age, obesity, symptoms, type of ART regime and duration of ART use.

Methods:

The study was conducted at an ART clinic in the provincial state hospital of Bongani in the town of Welkom in Free State (Ethics approved by the University of the Witwatersrand on the 16th July 2008 Number M080622). The target population was male and female adult patients (18 years and above) on ART for a duration of 1 year or longer. Participants were selected by a random sampling of hospital case file numbers using random table numbers. The patients answered a set of 7 questions on symptoms, underwent weight and height measurements, before having blood drawn for lactate assay. Blood specimens for lactate assays were processed at the local National Health laboratory.

Results:

Hyperlactatemia was picked up in 33.4% of the patients. Most cases were mild with few or no symptoms. The combination of efavirenz, stavudine and lamivudine (regime 1A) emerged as the strongest risk factor and predictor for hyperlactatemia. The most significant finding was a higher than usual frequency of hyperlactatemia and the strong influence of the specific combination of efavirenz, stavudine and lamivudine. There was no significant correlation between lactate levels and level of symptoms reported, age, BMI, and gender.

11.2.1.14 Dimatelis, JJ

Protein Role Players in “Despair” – Rat Models of Depression / Developmental Stress

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Purpose:

Early life adversity has been continually associated with adult psychopathologies which include mood- and anxiety-related behavioural disorders. The maternal separation (MS) paradigm is an animal model that has been successfully used to study the long term effects of early adversity. Experiments showed that animals subjected to trauma and stress early in life display behavioural, endocrinological and growth factor abnormalities that mirror clinical findings.

Methods:

The present study also included an additional group of MS animals that were also subjected to a bout of stress which consisted of constant light exposure over a period of 3 weeks (postnatal day 42-63) in adolescence, to enhance the depressive effects of MS and provide a more robust model of depression/anxiety (ethical clearance no: 010/007). What is not known is the underlying neural change or differential protein expression that gives rise to depression or the lifting of despair.

Results:

Behaviourally, we found MS rats have increased immobility time in the forced swimming test, indicative of depressive-like behaviour. Interestingly, MS rats exposed to constant chronic light show similar immobility time compared to control animals. Chronic constant light stress thus having beneficial effects on depressive-like behaviour in the forced swimming test. As the opioid system is affected by early life stress, we measured mu- and kappa-opioid receptor levels in the nucleus accumbens and found decreased mu-opioid receptor levels in animals subjected to MS. We also measured mitogen-activated protein kinase (MAPK) phosphatase-1 (MKP-1) and brain-derived neurotrophic factor (BDNF) levels in the ventral hippocampus. MKP-1 levels in the MS groups tended to be higher. BDNF being one of the downstream proteins affected by MKP-1, MS rats had increased BDNF levels.

Conclusion:

We wish to expand on these findings in order to have a clearer understanding of the molecular protein machinery involved in depression and the anti-depressive effect of light exposure which would ultimately lead to the development of more effective treatment of depression as the conventional treatment of depression relies on altering the monoamine systems which have been unsuccessful in relieving depressive symptoms in all affected.

11.2.1.15 Dube, T

Development of a RP-HPLC Method for the Analysis of Tenofovir Disoproxil Fumarate in Pharmaceutical Dosage Forms

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Purpose:

The purpose of this research was to develop a simple, selective, accurate, linear and precise RP-HPLC method for the quantitative analysis of tenofovir disoproxil fumarate in pharmaceutical formulations using nevirapine as an internal standard.

Method:

An isocratic RP-HPLC system consisting of a SpectraPhysics[®] Iso-chrom solvent delivery module (San Jose, USA), Waters[®] Associates WISP 710B auto-sampler (Milford, MA, USA), Linear[®] UVIS 200 detector (Reno, Nevada, USA) and a Perkin-Elmer[®] Model 561 strip chart recorder (Tokyo, Japan). A 5 μ M Phenomenex[®] Luna[®], C₁₈ (2) 150 x 4.60mm column was used as the stationary phase and the mobile phase was comprised of acetonitrile:water in a ratio of 40:60 v/v with UV detection at 259nm. The injection volume was 10 μ l and the flow rate was 0.9 ml/min. Fresh stock solutions of tenofovir disoproxil fumarate and nevirapine were prepared daily by dissolving approximately 30 mg of tenofovir and the nevirapine in 100ml mobile phase in A-grade volumetric flasks to produce solutions of a final concentration of 300 μ g/ml. A calibration curve was constructed over a range of 1.0-180 μ g/ml following serial dilution of the stock solution and plotting the peak height ratio of tenofovir disoproxil fumarate and nevirapine versus concentration. The concentration of nevirapine was kept constant (44 μ g/ml) for all samples. The effects of flow rate, injection volume and mobile phase composition on the retention time of the compounds were assessed and the peak tailing, asymmetry and resolution factors were calculated.

Results:

An increase in the percentage of acetonitrile in the mobile phase decreased the retention time of tenofovir disoproxil fumarate and nevirapine whereas a decrease in the flow rate increased the retention times of tenofovir disoproxil fumarate and nevirapine. The method was linear over the range 1.0 μ g/ml - 180 μ g/ml with a resultant R² value of 1. The peaks were sharp, symmetrical and narrow without evidence of tailing.

Conclusion:

A simple and precise RP-HPLC method has been successfully developed and will be validated using ICH guidelines and applied to the analysis of pharmaceutical dosage forms.

Acknowledgements:

The authors acknowledge funding from Rhodes University Joint Research Committee (SMMK and RBW) and National Research Foundation (RBW).

11.2.1.16 Ebrahim, N

Direct Inhibition of Cyclooxygenase-2 Enzyme by an Extract of *Harpagophytum procumbens*, Harpagoside and Harpagide

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Purpose:

Devil's Claw (*Harpagophytum procumbens*) is a plant geographically located in many regions throughout Southern Africa. The plant root has been used for the treatment of osteoarthritic conditions and has analgesic properties. The present study demonstrates direct inhibition of COX-2 enzyme by an extract of *Harpagophytum procumbens* as well as harpagoside and harpagide.

Methods:

A methanolic extract of *Harpagophytum procumbens* as well as harpagoside and harpagide were tested as direct inhibitors of Cyclooxygenase-2 enzyme (COX-2). DuP-697 [5-bromo-2-(4-fluorophenyl)-3-(4-(methylsulfonyl)-thiophene)] a member of the diaryl heterocyclic group of selective COX-2 inhibitors, which includes MK-966 rofecoxib and celecoxib, was used as the reference inhibitor.

Preparation of *Harpagophytum procumbens* extract. Dried *Harpagophytum procumbens* was powdered using a hammer and methanol was added to a final powder concentration of 100 mg/ml. The samples were vortexed followed by shaking at 400 rpm at room temperature. Thereafter, it was centrifuged at 800 rpm. The supernatant was concentrated by the removal of the methanol at 35° C under a gentle stream of nitrogen and a digital dry bath.

Determination of Cyclooxygenase-2 inhibition. Twenty-five units of purified COX-2 (ovine) enzyme was added to 700 µl of enzyme reaction buffer. The buffer consisted of 100 mM sodium phosphate pH 6.5, 0.5 µM hematin, and 1mg/ml gelatin. The enzymatic reaction was initiated by adding 100 µM of arachidonic acid and 50 µM N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD) to a final reaction mixture of 1ml. COX-2 activity was determined by measuring UV absorption change of the reaction mixture at wavelength 610 nm exactly eight minutes after initiating the reaction, against the appropriate blank. No change of initial absorption values indicated 100% inhibition and maximum absorption change in the reaction without added inhibitors indicated zero inhibition. Data was fitted into GraphPad Prism version 5 software for the calculation of IC₅₀ values.

Results:

The *Harpagophytum procumbens* extract demonstrated direct inhibition (68%) of COX-2 enzyme. The concentration of harpagoside and harpagide equivalent to that found in the extract (3% and 1% respectively) contributed 1.5% and 13 % to this inhibition.

11.2.1.17 Egieyeh, EO

Inter-professional Collaboration between General Practitioners and Community Pharmacists: General Practitioners' Perspective

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Background:

There is a global movement towards enhancing inter-professional collaboration in the management of patient drug therapies. In light of the increasing potency and complexity of drug regimens, particularly in the chronically ill where poly-pharmacy is rife, collaborative patient management by general practitioners (GPs) and community pharmacists (CPs), in particular, has the potential to enhance patient therapeutic outcomes at all levels of healthcare. There is a dearth of published studies addressing this important issue in South Africa and little is known about the perceptions of pharmacists or general practitioners toward collaboration in practice.

Objectives:

To assess general practitioners' perceptions of the professional roles of CPs, attitudes and barriers to inter-professional collaboration, current level collaboration and future prospects of collaboration with CPs. To determine whether a correlation exist between GPs perceptions of the roles of CPs and current level of collaboration.

Method:

This was a descriptive, cross-sectional, face-to-face survey of GPs in the Cape Metropole region of the Western Cape. A random sample of GPs in private practice was selected from a list obtained from the South Africa Medical Association. The questionnaire contained a range of statements with Likert scale response options. Medians were used to summarise descriptive data and Spearman's correlation coefficient was used for bivariate analysis. Ethical approval was granted by the Senate Research and International Relations Committee, University of the Western Cape (Ethical Clearance Number: 10/4/29).

Results:

The results indicate a higher level of agreement to the traditionally technical roles (e.g. dispensing) compared to a lower level of agreement to more clinically inclined roles (e.g. monitoring patient adherence). GPs generally displayed a strong willingness to collaborate with CPs although this was not observed in current practice. There was a statistically significant correlation between perceptions of the professional roles of CPs and the current level of collaboration (Spearman's $\rho=0.358$, $p=0.008$).

Most GPs were concerned that patients may get conflicting information regarding medicine use from CPs, citing this as a potential barrier to collaboration. They were of the opinion that If they are to entrust their patients to CPs, they would need to be more "professional". Most GPs agreed that joint continuing professional education organized by pharmaceutical companies or other groups will increase interaction and enhance collaboration.

Conclusion:

Inter-profession collaboration between GPs and CPs is essential to complement primary health care delivery in the public sector. A high level of perception of the professional roles of CPs is not sufficient to facilitate high level of collaboration.

11.2.1.18 Egieyeh, S

Effect of Alginate, Gum Arabic and Polyvinyl pyrrolidone on the hygroscopicity of freeze dried aqueous extract of *Artemisia afra*

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Introduction:

There is a rising trend globally in the use of plant-based drugs (herbal medicines) and formulations in health care purportedly due to better cultural acceptability, better compatibility with the human body and lesser side effects. Traditionally, herbal medicines are mostly used as aqueous decoctions which are not suitable dosage forms for the evaluation of these herbal medicines in clinical trials. Freeze drying has been used to convert aqueous extracts of herbal medicines into dry powders that may be formulated into tablets or capsules. However freeze dried aqueous extracts of most herbal medicines is hygroscopic (prone to moisture sorption when exposed to environments with even moderate humidity).

This study evaluated the effectiveness of three polymers; Alginate, Gum Arabic, and Poly vinyl pyrrolidone (PVP) in reducing the hygroscopicity of freeze dried aqueous herbal extracts of *Artemisia afra* (FDEA). Level of hygroscopicity was assessed by moisture sorption kinetics.

Method:

Each polymer was dissolved in aqueous extract of *Artemisia afra*, frozen and freeze dried. Freeze dried aqueous herbal extracts of *Artemisia afra* (FDEA), without polymer, was used as control. Moisture sorption was determined gravimetrically in a desiccator containing 0.5% NaCl in distilled water to simulate the desired relative humidity (RH) of 100%. The data obtained was then fitted into a two parameter non-exponential empirical model proposed by Peleg (Peleg, 1988).

Results:

The polymer-FDEA powders and FDEA powder were in the size range of 0.600 μm -0.740 μm (equivalent diameter) and have circularity in the range 0.60-0.75 (circularity of 1 for a perfect sphere). Flowability of Gum Arabic-FDEA powder and PVP- FDEA were good, while Alginate-FDEA powder and FDEA powder showed poor flowability.

Moisture sorption profile showed that Alginate-FDEA exhibited higher moisture sorption ability (hygroscopicity) than FDEA and the two other polymers, Gum Arabic-FDEA and PVP-FDAE. Moisture sorption profile data showed very good fit ($R^2 > 0.9$) to Peleg's model. According to Peleg's model, lower K_1 and K_2 values implies higher initial rate of moisture sorption and moisture sorption capacity respectively. Gum Arabic-FDEA showed the lowest rate of moisture sorption (highest K_1 value of 0.9043 ± 0.1236) and moisture sorption capacity (highest K_2 value of 0.7638 ± 0.3350). Rate of moisture sorption and moisture sorption capacity of PVP-FDEA ($K_1 = 0.6014 \pm 0.1063$) and Alginate-FDEA ($K_1 = 0.6781 \pm 0.1075$) were higher than that of FDEA ($K_1 = 0.7940 \pm 0.1309$). This indicates their ineffectiveness to reduce the hygroscopicity of FDEA.

Conclusion:

The results therefore showed that Gum Arabic was the only polymer, amongst the polymers studied, that effectively reduces the initial rate of moisture sorption and moisture sorption capacity (i.e. hygroscopicity) of FDEA.

11.2.1.19 Fadare, JO

Survey of Geriatric Prescriptions at the Outpatients Department of a Tertiary Health Facility in Rural South-West Nigeria

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Background:

Drug use in the elderly is fraught with a lot of problems mainly due to the physiologic changes of ageing, potential drug-drug and drug-disease interactions because of attendant comorbidities. Polypharmacy and inappropriate use of medicines in the elderly have been identified as major types of non-rational prescribing in the elderly. The main objective of this study is to investigate the prescriptions written for elderly patients (above 65 years) attending the outpatients department of the Federal Medical Centre, Ido-Ekiti, South-West Nigeria.

Methods:

Study Setting: The study was conducted at the general outpatients department (GOPD) of the Federal Medical Centre, Ido-Ekiti, a tertiary health care facility in rural South-West Nigeria. Ethical clearance was obtained from the hospital Ethics and Research Committee (ERC/2011/05/01) before the commencement of the study. The study was a retrospective cross sectional study involving the records of elderly patients (65 years and above) attending the GOPD. The information retrieved from the case notes included the bio-data (age, sex), diagnosis and the list of prescribed drugs. The following drug use indicators was assessed using the WHO guidelines on investigation of drug use in health care facilities: average number of drugs per prescription, percentage of encounters with antibiotics, analgesics, antihypertensives, oral hypoglycaemic agents and sedatives. Data generated from the case notes were recorded on spread sheet and analysed using SPSS statistical version 12 software. Results are expressed as means, frequencies and percentages.

Results:

Two hundred and twenty patient records were included and analysed. There were one hundred and twenty-eight (128) patients accounting for 58.2% of the study population. The average age of the patients was 72.8 ± 7.2 yrs (range 65-100 yrs). A total of eight hundred and thirty-seven medications were prescribed for patients with a mean of 3.8 ± 1.3 (Range 1-8). Sixty-five (29.5%) patients had five drugs or more prescribed for them while one hundred and fourteen (51.8%) had 3-4 drugs. Prescribing by generic name was found in only 43% of the cases. Antihypertensive drugs accounted for 30.6% of the prescriptions followed by analgesics (10.8%), antibiotics (7.8%), oral hypoglycaemic agents (7.5%) and sedatives (2.6%).

Conclusion:

The study has shown clearly that polypharmacy is common among prescribers in Nigeria. There is a need to improve on prescribing for elderly patients in our health care institutions.

11.2.1.20 Fakee, J

Isolation and Characterization of a C₁₅ acetogenin from *Laurencia natalensis*

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Purpose:

The marine algal genus *Laurencia* has built a reputation to produce a wide array of secondary metabolites which display favourable bioactivity. Examples of such molecules isolated include the analgesic Neorogioltriol from *Laurencia glandulifera*, and the antiviral Venustatriol from *Laurencia venusta*. There is on-going research into the bioactivity of these secondary metabolites and as a result pursuits to isolate such molecules from the various *Laurencia* species has increased. In this study, an attempt to isolate pure secondary metabolites from *Laurencia natalensis* was made.

Method:

The frozen seaweed was extracted with MeOH and a 2:1 MeOH CH₂Cl₂ solvent system to produce a crude extract. The crude was further fractionated to produce fraction B3 using silica gel step gradient column chromatography. Structure elucidation was carried out by analysing 1D and 2D NMR data which included ¹H, ¹³C, DEPT 135 – (Distortionless enhancement by polarization), COSY – (Homonuclear correlation spectroscopy), HSQC – (Heteronuclear Single Quantum Coherence) and HMBC – (Heteronuclear Multiple Bond Coherence) spectra.

Results and conclusion:

The molecule isolated in fraction B3 was a 15 Carbon chain containing 4 cis double bonds and 1 terminal triple bond. The molecule was later identified from the literature as Laurencenyne, a proposed precursor to a complex metabolite namely Bromofucin which has previously been isolated from *L.flexuosa*. It can thus be concluded that there is a possibility to find molecules such as Bromofucin in *L. natalensis*.

11.2.1.21 Hassan, S

Introducing a Clinic-Based Service Learning Programme for Final Year Pharmacy Students at the University of the Western Cape

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Background:

The University of the Western Cape (UWC) School of Pharmacy's Service Learning in Pharmacy (SLIP) programme forms part of the current undergraduate curriculum. Service learning is defined as an approach to learning whereby theoretical knowledge is linked with service provision to the community (Eyler, 1999). One of the aims in service learning is to enable students to determine the relevance and applicability of pharmacotherapeutic concepts. The objective is to develop students' competency skills in dispensing at designated public sector healthcare facilities. Due to high patient load and gross understaffing (Frenk et al., 2010), student-patient contact time is minimal, and the opportunity to offer patient-centred care is non-existent. Berger (2009) challenges training institutions to uphold patient-centred care where pharmacotherapeutic concepts can be tested. Since health services globally, are underpinning primary healthcare initiatives (Peterson, 2001), public sector community healthcare centres (CHCs) afford an ideal opportunity to engage final year students in a structured clinic-based programme.

Method:

In designing a clinic-based programme, pharmacy students could engage in a clinic with ambulatory patients who are diagnosed with chronic conditions to: understand patient barriers to treatment adherence, interpret clinical data, check appropriateness of drug therapy and encourage preventive and follow-up care. A participatory action research approach with health service staff and patients would enable students to identify and address adherence issues on chronic disease management, promote patient-centred care and strengthen the service learning partnerships. A pocket reference guide adapted from the Essential Drug List (2008), along with medication identification visual aids, could be developed to assist students in interpreting clinic data and checking appropriateness of therapy. Training students on the use of the pocket reference guide will be given before clinic placement at the designated CHC. Such a programme could contextualise learning.

Analysis:

Multi-method analysis will be conducted. The clinic-based service learning programme's effectiveness will be assessed by survey responses from clinic staff and patients regarding students' competence in clinical skills. These findings will be compared with those from an independent evaluator.

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11.2.2 Session Two

11.2.2.1 Hwengwere, E

Use of Foreign Reference Products in the Registration of Generic Medicines in South Africa

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Background:

When a generic or multisource interchangeable medicine is to be registered, studies that prove /show that the generic is equivalent to the Innovator product (IP) are used. The Medicines Control Council (MCC) ensures that generic medicines meet the requirements of quality, safety and efficacy. Generics may be registered using bioequivalence data obtained from comparison with a domestic reference product (usually the local innovator product) or in certain cases, a foreign reference product (FRP).

Objectives:

To note the changes in the MCC guidelines regarding the use of reference products and the tests required to show equivalence over the years. . To report on preliminary enquiries using the Promotion of Access to Information Act (PAIA) about the use of FRPs.

Methods:

The relevant MCC guidelines were consulted. Circulars distributed by the Registrar of the MCC since 1989, regarding generic medicines registration were also consulted. Parliamentary minutes of questions about registration of medicines including the use of FRPs were also consulted.

Results:

In 1989, MCC Circular 14/89 stated that the only standard against which tests would be done was the Innovator product (IP). In 1995, Circular 14/95 informed applicants that the reference could be the IP or another reference product if a justification for the choice was offered. In the MCC's Pharmaceutical and Analytical guidelines of 2007 four options are given the last of which is the use of a FRP with sufficient justification. The Minister of Health said that "The MCC does not use generic products for comparative purposes" when asked in parliament about the use of FRPs.

Discussion:

Over the years the guidelines have changed and seem to have become more flexible regarding use of reference products and tests required for registration of generics. A series of enquiries have been made to the MCC and certain generic medicine registration certificate holders (9 Pharmaceutical companies) to shed light on what comparators were used as reference products for a selected sample of generic products. To date, one outright refusal citing "trade secrets" has been received, Another has responded also citing trade secrets but indicating that providing the information would depend on a confidentiality agreement. One positive response has been received for the use of a FRP. The question posed about FRPs to the Minister of Health in Parliament has been posed again to the Registrar of the MCC as the question was not answered.

11.2.2.2 Jones, E

Development of an Assay Method for Didanosine Quantification in Buccal Polymeric Films

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Introduction and Objectives:

Didanosine (ddI) is only available for the oral route of administration. Its controlled transbuccal administration may improve bioavailability by avoiding hepatic first-pass metabolism and gastrointestinal degradation. Poor drug content for controlled release buccal polymeric films containing ddI has been reported previously (Jones *et al.*, 2008). In order to comply with compendial specifications, oral dosage forms of ddI should contain between 90% and 110% of the stated amount of ddI (Ph.Int. 4th ed.) This study aimed to develop a suitable assay method for quantification of ddI in buccal polymeric films.

Method:

Multipolymeric films containing ddI were prepared using Eudragit® RS100 (EUD) and Hydroxypropyl methylcellulose (HPMC) in a ratio of 1:0.5:10 (ddI:HPMC:EUD) by the homogenization/solvent casting/evaporation method. The films were prepared in a silicone-moulded tray and dried at 37°C. Films were assayed for ddI content using an initial method consisting of a 5% water + 95% ethanol mixture. Individual films were cut into pieces, crushed in the solvent and then agitated in a heated shaking water-bath (40°C) for 24 hours before quantification via UV spectroscopy at 250nm. This method was then optimized by altering the ratio between the organic and aqueous phase of the solvent system and by changing the individual solvent components in a stepwise manner. The final optimized assay method entailed soaking the polymeric films in 20mL methanol before the addition of 80mL phosphate buffered saline (PBS) pH 7.4. After periodic manual agitation for 3 hours, complete dissolution of the films was observed, ddI was then quantified using UV spectroscopy.

Results and Discussion:

The initial assay method resulted in 82.9±1.7% ddI content. By alteration of the method to consist of 20% ethanol + 80% water, we were able to produce assay values of 82.4±1.6%. Further modification of the method to 20% methanol + 80% distilled water yielded 87.0±1.2% ddI. As a final modification, the distilled water was changed to PBS pH 7.4. This resulted in 100.1±2.8% drug content, which is well within compendial specifications. The newly developed assay method holds additional benefits as it allows for complete dissolution of the polymeric film in the solvent system without the need for cutting and crushing of individual films. The need for a heated shaking water-bath was eliminated, the duration of the process was reduced from 24 hours to 3 hours and less organic solvent is required, making this optimized assay method cost-effective with a reduced environmental impact.

Conclusion:

An assay method with the potential for effective quantification of active pharmaceutical ingredients, such as ddI, in polymeric buccal films was developed. A solvent system comprising of methanol and PBS pH 7.4 was found to be optimal for this purpose.

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11.2.2.3 Joubert, JP

Synthesis of Artemisinin-Acridine Hybrids for Antimalarial Activity

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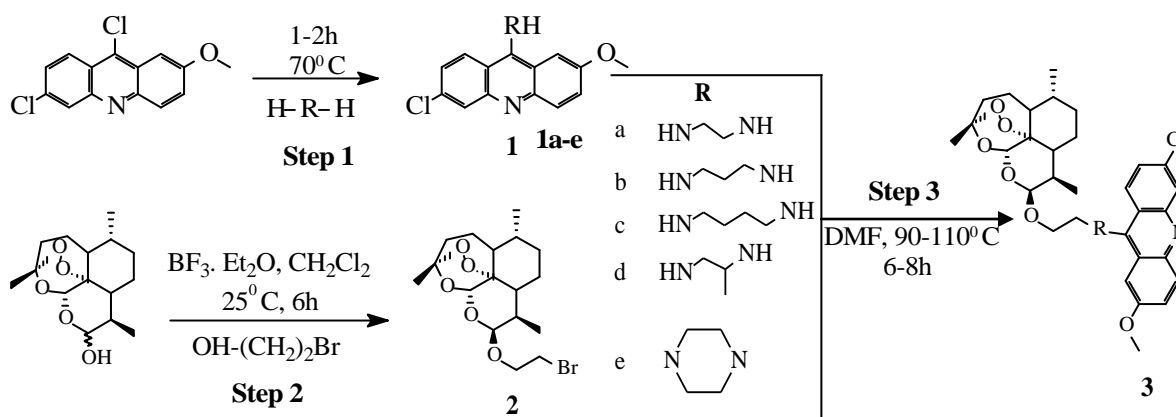
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Background:

Malaria, caused by a protozoan parasite of the genus *Plasmodium*, was responsible for 225 million cases and 781 000 deaths in 2009 as reported by the WHO. The phenomenon of drug resistant malaria has shown a dramatic increase in malaria related deaths in the last two decades of the twentieth century. *P. falciparum*'s resistance toward antimalarial drugs has become increasingly distressing as the Thai-Cambodian border already detected a slow parasite clearance time subsequent to artemisinin treatment. This indicates the beginning of resistance development.

Purpose:

The aim of this study is the synthesis and *in-vitro* antimalarial evaluation of artemisinin-acridine hybrids.

Methods:**Results:**

The intermediates 1a-e were synthesized in 80-95% yield and their structures were confirmed by NMR techniques.

11.2.2.4 Kellermann, T

Quantification of Linezolid Using LCMS and Liquid-liquid Extraction from Human Plasma

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Purpose:

Multi-drug resistant tuberculosis (MDR-TB) and extensively drug-resistant TB (XDR-TB) are associated with high mortality rates world-wide. Linezolid is an oxazolidinone drug with relatively broad-spectrum *in vitro* activity and is used in the clinical treatment of infections caused by aerobic Gram-positive bacteria. This drug has reportedly been used in the treatment of MDR-TB and XDR-TB in the absence of randomized clinical trials to determine its clinical efficacy for the treatment of these diseases. Furthermore, co-infection with HIV could possibly affect the pharmacokinetics of linezolid, as has been seen with other anti-tuberculosis drugs. A study was designed to evaluate the pharmacokinetic and pharmacodynamic effects of low dose, limited duration linezolid treatment in patients enrolled in a phase I/II pilot randomized clinical trial as part of multi-drug treatment of MDR-TB and XDR-TB. The study was conducted among a population with a high prevalence of HIV infection in KwaZulu-Natal, South Africa (University of Cape Town ethics number 241/2008). A previous method using a step-gradient system proved to be highly susceptible to matrix effects, resulting in the enhancement of linezolid levels. A sensitive, robust, validated method was developed for the detection of linezolid from 25µl of human plasma by means of LCMS.

Methods:

A volume of 25µl of sample with lithium-heparin anticoagulant was extracted using a liquid-liquid system consisting of a pH9 universal Britton-Robinson buffer and ethyl acetate containing locostatin as internal standard. The organic layer was further concentrated by drying under nitrogen after which samples were resuspended in mobile phase and 10µl was injected onto a Phenomenex Luna PFP(2) 100A (50x20mm) 5µm column. A gradient LC system at a flow rate of 0.7ml/min starting at a high concentration of 0.1% formic acid increasing to 98% ACN over 3.5 minutes was utilized for the elution of linezolid and internal standard. The method had a total run time of 8 minutes.

Results:

Linezolid and locostatin eluted at 2.17 and 3.38 minutes, respectively, with neither analyte nor internal standard subjected to matrix effects. The within-day precisions for linezolid were 104 and 94.6% for 0.117 and 30µg/ml, respectively. The between-day precisions for linezolid were 103 and 98% for 0.117 and 30µg/ml, respectively. Overall assay accuracy and precision for 18 replicates tested over three days was 102, 99.9, 94.2 and 95.8% at 0.117, 0.234, 15 and 30µg/ml, respectively. Linezolid produced a linear calibration curve across a range of 39ng/ml (lower limit of quantification) to 40µg/ml. It was shown to be stable after three freeze-thaw cycles and displayed on-bench stability in plasma. Furthermore, the presence of 1 and 2% haemolysed blood did not affect the sensitivity of the assay.

11.2.2.5 Killian, C

The Antioxidant Activity and Cytotoxicity of Natural Sesquiterpenes

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Purpose:

Researchers use natural products to find new pharmaceutical lead compounds. The interest in the antioxidant activity of natural compounds has grown due to the fact that free radicals are related to some neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases. Free radicals play an important role in oxidative stress which is a contributing factor in these neurodegenerative diseases. Antioxidants reduce oxidative damage to biomolecules by modulating the effect of reactive oxidants.

In a previous study on the antioxidant activity of *Gymnosporia buxifolia* an active compound with a sesquiterpene core structure was isolated. There are a considerable number of sesquiterpenes that have been isolated from plants, but only a few have been investigated to any extent. This is a large group and biological activity depends more on the characteristics of the specific compound than that of the group. The sesquiterpenes used in this study are: farnesol, nerolidol, α -humulene, guaiazulene, (+)-cedrol, (-)-thujopsene and (-)-epiglobulol.

Methods:

The nitro blue tetrazolium assay is a simple reliable technique for assaying superoxide anion and other free radicals. The assay is based on the ability of free radicals to reduce nitroblue tetrazolium to the insoluble nitroblue diformazan. When the superoxide reacts with the nitro blue tetrazolium a colour change occur from light yellow to dark purple. This colour product is extracted with glacial acetic acid and is measured by spectrophotometer at 560 nm.

Lipid peroxidation was measured using the thiobarbituric acid (TBA) assay. This assay is based on the reaction of malondialdehyde (MDA) equivalents with TBA this form a pink coloured complex which can be extracted with butanol and read at 530 nm.

All assays were done under supervision of the North-West University ethics committee, ethics clearance number 05D05.

The metabolic activity/annexin V/ dead cell apoptosis kit with C12 resazurin, APC annexin V, and SYTOX® green for flow cytometry was used to determine the cytotoxicity of the most promising compounds. The stained cells are analysed by flow cytometry, measuring the fluorescence emission at 530 nm and 575 nm using 488 nm excitation and at 660 nm using 633 nm excitation. Live cells, apoptotic cells and dead cells can be quantified.

Results:

In the nitroblue tetrazolium assay thujopsene (1 mM), nerolidol (1 mM), (-)-epiglobulol (1 mM) and α -humulene (1mM, 0.5mM) were able to significantly reduce superoxide generation compared to the toxin (KCN).

With the thiobarbituric assay guaiazulene (1 mM, 0.5 mM, 0.25 mM), α -humulene and farnesol (1 mM) significantly lowered the lipid peroxidation compared to the toxin (H_2O_2 + Vitamin C + $FeCl_3$).

α -Humulene and guaiazulene were regarded as non-toxic.

11.2.2.6 Lourens, ACU

Structure-based Virtual Design and Synthesis of Novel Heterocycles as Potential Adenosine A_{2A} Receptor Ligands

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Purpose:

Parkinson's disease (PD) is characterised by symptoms such as bradykinesia, rigidity and tremor caused by the degeneration of dopaminergic nigrostriatal neurons. It is the second most common neurodegenerative disorder and with the worldwide increase in life expectancy, due to improvements in preventative, diagnostic and therapeutic interventions for several disease states, an increase in age-related neurodegenerative disorders such as PD is expected. As the use of current therapies is often associated with negative effects such as dyskinesias, there is need for newer and more effective agents. Owing to the unique central nervous system distribution of the A_{2A} receptor and promising symptomatic, potential neuroprotective and antidyskinetic effects obtained by blocking these receptors, antagonists of the A_{2A} receptor are promising non-dopaminergic targets for the treatment of Parkinson's disease. The purpose of this study is to design and synthesise novel heterocyclic compounds as antagonists of the A_{2A} receptor.

Methods:

Ligand design: Heterocyclic scaffolds were selected based on A_{2A} antagonistic activity reported in literature. Aminopyrimidines, as well as triazolotriazines, were designed using available pharmacophore models and then docked into the A_{2A} receptor active site using FRED (Openeye) and Discovery Studio (Accelrys®). Docked scores and visual inspection of the binding poses were used as guidelines in the selection of compounds for synthesis. Compounds were also screened for drug-likeness using MOE (Chemical Computing Group).

Synthesis: Aminopyrimidines were synthesised in three steps. Selected formylbenzoic acids were condensed with different aldehydes to yield chalcones, which were coupled with amines, using 1,1'-carbonyldiimidazole to obtain desired amides. The last step involved cyclisation with guanidine hydrochloride to yield the final aminopyrimidine products. A five to six step synthetic approach is envisaged for the synthesis of the triazolotriazines.

Results:

Molecular modelling results indicated that all proposed structures had drug-like properties (Lipinski and Operea). Both the aminopyrimidines and triazolotriazines showed promise in the docking studies. Aminopyrimidines were successfully synthesised, using the above mentioned method, while the synthesis of the triazolotriazines is still in progress.

11.2.2.7 Lubbe, MS

Prescription Patterns of Dispensing Doctors and Other Medicine Providers

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Objective:

The main objective of this study was to analyse the prescribing patterns of dispensing doctors and other medicine providers in a section of the private health care sector of South Africa for 2005 to 2008 by using a medicine claims database.

Method:

A retrospective drug utilisation study was performed on medicine claims data of a pharmacy benefit management company in South Africa during 1 Jan 2005 until 31 Dec 2008. Dispensing doctors were classified as doctors who prescribed medicines and provided them. Other health care providers included non-dispensing doctors and specialists who only prescribed medicines (fulfilling the role of prescriber); and pharmacists who only dispensed medicines (fulfilling the role of provider) or provided OTC and claimed it through the patient's medical scheme. Ethical consent for this study was given by the medicine claims database company, as well as the North-West University (Ethical application number: NWU-0046-08-S5).

Results:

The results revealed that dispensing doctors had a lower cost per prescription compared to other health care providers (R112.44 vs. R256.77) and also had a lower cost per medicine item (R39.48 vs. R112.00) for the entire study period from 2005 to 2008. Dispensing doctors provided more items per prescription compared to other health care providers (2.85 items vs. 2.29 items) but other health care providers claimed more prescriptions per patient per year (7.30 prescriptions vs. 3.30 prescriptions). A higher percentage of generic medicine items were provided to patients visiting dispensing doctors. Both dispensing doctors and other health care providers issued a majority of medicine items to treat acute conditions. The results also revealed that dispensing doctors provided relatively inexpensive medicine items, including generic and innovator items.

Conclusion:

From 2005 to 2008 dispensing doctors in South Africa issued more medicine items per prescription compared to other health care providers, but did so at a lower cost.

11.2.2.8 Maharaj, B

The Prevention of Infective Endocarditis in Black Patients

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Introduction:

Infective endocarditis is associated with significant morbidity and mortality. Prevention is, therefore, very important. In developing countries, this disease occurs most frequently on valves damaged by rheumatic heart disease (RHD).

Study 1:

Many patients with RHD are unaware of the presence of their underlying heart disease and are, therefore, not eligible to receive prophylaxis against infective endocarditis. Black primary school children (n=4408) were examined to determine if screening would be a useful means of detecting undiagnosed RHD. Rheumatic heart disease was diagnosed in 4 school children, 3 for the first time. We concluded that screening programmes would be effective.

Study 2:

Antimicrobial prophylaxis is recommended prior to dental extraction in order to prevent post-extraction bacteraemia and the subsequent development of infective endocarditis. Antibiotics have been shown to reduce, but not prevent, post-extraction bacteraemia. One possible explanation is that the state of oral health was not considered in these studies. We studied 108 black patients and found that transient bacteraemia occurred in 29.6% of patients. There was no relationship between the state of oral health and the incidence of post-extraction bacteraemia. In another 2 groups of black patients we found that the frequency of bacteraemia after tooth brushing and mastication was 10.8% and 0% respectively. One patient had a positive blood culture prior to dental extraction; his oral health status was poor.

Study 3:

We evaluated some of the regimens used for antimicrobial prophylaxis prior to dental extraction in 160 black patients. The proportion of patients who had a positive blood culture after dental extraction in the amoxicillin, clindamycin, chlorhexidine and control groups was 7.5%, 20%, 40% and 35% respectively. None of the regimens prevented post-extraction bacteraemia.

Study 4:

The maintenance of good oral health is another important aspect of the prevention of infective endocarditis. We studied 44 black patients with severe rheumatic heart disease to determine if adequate attention is paid to this aspect of prophylaxis. The plaque index and gingival index was classified as good in only 13.6% and 15.9% of patients respectively. Furthermore, abnormalities were detected on the panoramic radiographs of 84.1% of patients.

Conclusion:

The prevention of infective endocarditis has been neglected in the past. It is hoped that the care of patients at risk of developing infective endocarditis will be improved. The first step in the prevention of infective endocarditis in developing countries would be to reduce the pool of patients who are susceptible to this infection. The second step would be the early identification of at risk patients and prompt referral to oral health specialists for comprehensive evaluation and treatment. The third step would be to integrate infective endocarditis prophylaxis into rheumatic fever/rheumatic heart disease prevention programmes and provide a more holistic care of patients with rheumatic heart disease.

11.2.2.9 Makhathini, KB

To Investigate the Neurochemical Effects of Tat Protein on the Hippocampal Neurons

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Purpose:

Human immunodeficiency virus type 1 (HIV-1) is one of the problematic diseases in South Africa. Studies have shown that in 50-60% of the people infected with HIV, the virus can enter into the central nervous system (CNS) and cause a progressive disorder like HIV associated neurocognitive dementia (HAND). Tat and gp120 are two of the proteins that are released by the infected HIV macrophages and microglial cells in the brain. Tat is a transactivator of TAR (Tat associated region), it contains 72-104 amino acids in its sequence. The primary aim of the present study is to investigate whether tat protein can induce neurocognitive deficits in animals.

Methods:

Male Sprague-Dawley rat (250-300g) was used in this study. The ethical clearance was obtained from the animal ethics sub-committee of University of KwaZulu-Natal (067/11). Tat protein (5µg/100µl) was injected bilaterally into the dorsal hippocampus of the animal using stereotaxic techniques. Control group received an injection of saline/tat buffer (100µl) and the experimental group received an injection of tat protein (5µg/100µl) into the hippocampus. Two behavioral tests, the Morris water maze and the light/dark box were performed to investigate the effects of these injections on learning and memory. The dorsal and ventral hippocampus were dissected from each rat, snap frozen in liquid nitrogen and stored at -80° C. Total RNA will be extracted from the tissue using RNeasy Kit protocol. The purity of RNA will be checked by using nano-drop machine, before it converted to cDNA. The iScriptTM cDNA Synthesis kit will be used to convert RNA to cDNA. The real time polymerase chain reaction (RT-PCR) will be used to quantify the expression or up regulation of CASP3 gene (caspase 3). The Western Blot will be used to quantify the up regulation of the caspase 3 protein.

Result:

Two behavioural tests were performed light dark box and Morris water maze. Our behavioural results show that tat protein exacerbates the loss of learning and memory in the experimental group. Also the animal on the control group show the high significant of learning and memory. In addition to these behavioural tests we will also investigate the role of apoptosis in tat protein-induced toxicity. We intend to focus on the upregulation of caspase 3 gene using Real-time PCR.

11.2.2.10 Malan, MM

Modulation of Rhodamine 123 Efflux Across Excised Rat Intestinal Epithelial Tissue by Hydroxy- and Methoxy-flavonoids

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Purpose:

Different flavonoids have been shown to exhibit P-gp related modulatory effects and because inhibition of efflux transporters can have a significant effect on drug bioavailability it is important to investigate the effect of compounds on drug absorption by this mechanism. The purpose of this study was to determine the effect of selected flavonoids on the *in vitro* transport of the P-glycoprotein substrate, Rhodamine 123 (Rho123).

Methods:

The transport of Rho 123 was measured in the apical to basolateral (AP-BL) and basolateral to apical (BL-AP) direction across excised rat jejunum segments in a Sweetana-Grass diffusion apparatus in the absence (control) and presence of the flavonoids (10 μ M and 20 μ M). The North-West University Ethics Committee approved the research project (NWU-0018-09-A5). Samples were analysed by a validated HPLC method and the rate of transport was expressed as the apparent permeability coefficient (P_{app}) and the extent of active transport as the efflux ratio (ER).

Results:

From the P_{app} values of the test compounds (i.e. selected flavonoids) it is clear that all had an enhancing effect on the permeability of Rho 123 in both directions across the rat intestinal tissue compared to the control group. All the test compounds reduced the Rho 123 transport in the BL-AP direction in comparison to its respective transport in the AP-BL direction. This clearly indicates inhibition of Rhodamine 123 efflux, which may explain the increased transport in the AP-BL direction. Statistically significant ($p < 0.05$) inhibition of the efflux of Rho 123, as expressed by ER values of the experimental groups compared to the control group, was obtained for morin, galangin, 3-methoxyflavone and 7-methoxyflavone at both concentrations, while kaempferol only showed a statistically significant effect at 20 μ M. Although *in vitro* pharmacokinetic interactions do not always result in clinically significant effects *in vivo*, it is important to obtain information regarding potentially unwanted pharmacokinetic interactions.

11.2.2.11 McCartney, J

Cardiovascular Risk Management by Community Pharmacists in the Nelson Mandela Metropole

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In South Africa, the community pharmacist is a readily accessible provider of healthcare. Pharmacists' involvement in the provision of point-of-care cardiovascular risk screening and monitoring services as well as disease-related counselling, can play an essential role in the cardiovascular disease management process.

Purpose:

The aim of this study was to describe the nature of the services provided by the community pharmacist with respect to cardiovascular disease management. The objectives of the study were (i) to describe the nature of cardiovascular risk screening, monitoring and management services by community pharmacists in the Nelson Mandela Metropole; (ii) to determine the extent to which community pharmacists assess cardiovascular risk profile.

Methods:

Ethical approval (reference H06Hp-031) was granted by NMMU's Research Ethics Committee (Human) prior to commencement of the research. A cross-sectional, questionnaire-based survey was conducted, targeting all community pharmacies in the Nelson Mandela Metropole, South Africa. A response rate of 87.2% (41, n=47) was achieved.

Results and Discussion:

All participating pharmacies (100%, n=41) provided blood pressure and glucose screening services and 87.8% (37, n=41) provided blood cholesterol screening services. Pharmacists were found to be actively involved in the provision of point-of-care testing services. However, after the initial referral, pharmacist involvement was sporadic and incomplete. Little evidence was found of cardiovascular risk assessment although 12.8% of pharmacists (6; n=47) were found to use risk identification questionnaires. None of the pharmacists were involved in setting individualized treatment goals or on-going disease monitoring. Barriers were identified which prevented pharmacists from participating in cardiovascular disease management practices.

11.2.2.12 Meissner, O

Complementary/alternative Medicine – Proof of Efficacy

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This review started out from an article by Steinman and Jobson entitled ‘Multiple organ failure – death of consumer protection?’¹ The next startling statement I read declared CAM to be an ‘inconvenient reality in today’s medical practice.’²

CAM is indeed a highly complex topic. Definitions and boundaries are not fixed and vary from time and time and from country to country. Many observers criticize the lack of scientific evidence for treatment efficacy, that is the lack of randomized controlled trials (RCTs). At the same time it has been contended that the RCT creates an environment foreign to the cultural and philosophical belief context within which many CAM therapies are most effective, when the benefit is based on a sense of hope, positive expectations and an activation of a self-healing process, when the endpoint is a feeling of emotional and spiritual well-being. Therapies might be highly effective within the proper cultural and belief context, but totally ineffective within the foreign environment created by the conduct of an RCT. Thus, dogmatic adherence to the RCT as the as the exclusive method for legitimizing CAM may be inappropriate for many CAM therapies when philosophies and mechanism of action are not scientifically interpretable. Anthropological research methods have been suggested for the assessment of some CAM modalities which highlight the philosophical rather than the pathophysiological aspects. Likewise, however, anthropological research methods on their own have their limitations by de-emphasizing the significance of scientific validation.

No single research methodology yields all the knowledge necessary with respect to safety and efficacy of CAM treatments. A pluralistic approach is necessary that retains the value of Western science yet respects the diversity of radically different concepts about health, disease and illness.

11.2.2.13 Mhaka, FA

Development of a RP-HPLC Method with Diode Array Detection for the Analysis of Captopril in Pharmaceutical Dosage Forms

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Purpose:

Published methods for the quantitative analysis of Captopril (CPT) are limited by the scope and performance of conventional single channel ultraviolet-visible detectors. The use of photodiode array (PDA) detection would ensure fast scan speeds, high signal to noise ratio and wavelength precision as compared to the single channel ultraviolet detection. The objective of this research is to develop a simple, accurate and sensitive RP-HPLC method using ultraviolet (UV) detection for the quantitative analysis of CPT.

Method:

The RP-HPLC system used consisted of a Waters[®] Alliance 2695 Separations module (Waters[®], Milford, MA, USA) equipped with a Waters[®] Alliance 2695 solvent delivery module (Waters[®], Milford, MA, USA), Waters[®] Alliance 2695 online degasser (Waters[®], Milford, MA, USA) and a Waters[®] 2996 photodiode array ultraviolet detector (Waters[®], Milford, MA, USA). Data were collected using Empower Pro data acquisition software (Waters[®], Milford, MA, USA). Isocratic chromatographic separation was achieved using a 5 μ m Phenomenex[®] Luna C₁₈ (2) (150 x 4.6mm i.d.) column as the stationary phase. The mobile phase consisted of methanol: water in a ratio of 47:53 v/v adjusted to pH 3 using 85% v/v ortho-phosphoric acid. Aliquots of 10 μ l were injected onto the column at a flow rate of 1.0 ml/min. The column temperature was maintained at 25°C and UV detection was used at a wavelength of 200nm and bandwidth of 1.2nm. Standard stock solutions of CPT (100 μ g/ml) and salicylic acid (SCY) (100 μ g/ml) were prepared separately. The stock solutions were diluted to prepare solutions of CPT ranging in concentration from 2 μ g/ml to 60 μ g/ml with the SCY used at a concentration of 10 μ g/ml. The effect of mobile phase composition on the retention time of the compounds was evaluated and the tailing, asymmetry and resolution factors were calculated.

Results:

An increase in the percentage of methanol in the mobile phase decreased the retention time of CPT and SCY. The method was linear over the range 2 μ g/ml - 60 μ g/ml with a resultant R² value of 0.9997. The equation for the line was $y = 0.0273x + 0.0226$. The peaks were sharp, wide and symmetrical.

Conclusion:

A RP-HPLC method was successfully developed and validated using the ICH guidelines. The validation included the assessment of accuracy, precision and stress conditions on the method performance.

Acknowledgements:

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11.2.2.14 Mibei, M

Determination of Compatibility of Herbal Active Ingredients Using Differential Scanning Calorimetry

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Introduction:

Bioadhesive herbal buccal tablet containing *Salvia officinalis* Aloe vera gel and *Glycyrrhiza gabra* was formulated. These plant extracts contain rosmarinic acid and caffeic acid from *Salvia officinalis* and glycyrrhizinic acid from *Glycyrrhiza gabra* as active ingredients. The aim of this study was to investigate compatibility of these herbal active ingredients with each other using differential scanning calorimetry (DSC). This buccal tablet is intended for the treatment of aphthous ulcer which is a mucosal disorder.

Method:

A DSC Q20 (TA instrument) was used to obtain thermograms of the selected compounds. Closed aluminium pans were heated at 10°C per minute from 25°C to 500°C under a nitrogen flow of 50 ml/min. Samples weighing 2-5mg were studied alone and in binary mixtures with a 1:1 ratio (Balestrieri *et al.*, 1996:337; Lira *et al.*, 2007:1).

In this study, a change in peak temperature of less than 10°C was not considered as an incompatibility. A temperature shift of more than 10°C was accepted as an indicator of a possible interaction between two materials. Temperature changes greater than 15 °C were accepted as an indicator of a likely interaction. Analysis of the thermograms was performed using the DSC universal analysis software.

Results:

Binary mixtures of rosmarinic acid and caffeic acid showed signs of incompatibility, this is because the first peak formed on the binary mixture had shifted from 53°C to 58°C, the second peak has shifted from 165°C to 226°C and the third peak has shifted from 173°C to 232°C.

Binary mixtures of rosmarinic acid and glycyrrhizinic acid also showed signs of incompatibility because the first peak had shifted from 72°C to 101°C the second peak shifted from 168°C to 204°C and the third peak has shifted from 194°C to 219°C.

Conclusion:

Based on the results, it was therefore concluded that rosmarinic acid is not compatible with either caffeic acid or glycyrrhizinic acid.

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11.2.2.15 Moller, M

Development and Validation of a Single Analytical Method for the Determination of Tryptophan, and Five of its Metabolites in Rat Plasma

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Purpose:

Tryptophan metabolism via the kynurenine pathway has an important role in several fundamental biological processes, including neuronal excitability, antioxidant status, cell growth and cell division in various cell types. The kynurenine pathway also mediates interactions between immunological and neuronal functions, and this interrelationship has been implicated in a wide range of (patho) physiological disorders, such as HIV-infection, Huntington's disease, malaria, major depression and schizophrenia. In order to study the clinical importance of tryptophan and its predominant metabolites (kynurenines), it is essential to monitor the activity of as many metabolites in the kynurenine pathway as possible, in a large series of samples with high accuracy and reliability in any single experimental protocol. This study therefore developed and validated an analytical method for the measurement of tryptophan, kynurenine, kynurenic acid, 3-hydroxyanthranilic acid (3OH anthranilic acid), anthranilic acid and quinolinic acid in rat plasma with solid-phase extraction-liquid chromatography-electrospray ionization tandem mass spectrometry.

Methods:

Plasma samples were obtained from drug naïve, healthy rats (ethical clearance number: NWU-0035-08-s5), and pre-purified by solid-phase extraction (SPE) using a polymeric water-wettable reversed-phase sorbent (Oasis HLB 3cc, Waters, Milford, MA part no. WAT094226). Plasma (800 µl) was treated with 1% formic acid (in water v/v) (1 ml) and loaded on an extraction column, which were conditioned with methanol (2 ml) and then 1% formic acid (2 ml). The column was then washed with 1% formic acid (2 ml) and dried for 10 min and subsequently eluted with 1% NH₄OH: 50% methanol (50:50 v/v) (2 ml). The eluted samples were evaporated under nitrogen, dissolved in 1% NH₄OH: 50% methanol (150 µl), and injected on the LCMS. Chromatographic separation of the analytes occurred by C18 reversed phase chromatography (Ultra Aqueous C18, 5 µm, 150 x 2.1 mm, Restek, Bellefonte, PA). Mass spectrometric detection was performed using a mass spectrometer in positive and negative electrospray ionization, with a flow rate of 0.2 ml/min and an injection volume of 10 µl. Total run time including sample clean-up was 15 min.

Results:

The limit of quantification (LOQ) for each analyte were as follows: tryptophan 0.0176 µM, kynurenine 0.0173 µM, kynurenic acid 0.0175 µM, anthranilic acid 0.047 µM, 3-OH anthranilic acid 0.084 µM, and quinolinic acid 0.096 µM. Linearity was excellent ($R > 0.95$) in the following calibration range: tryptophan (0.0703 – 1.1262 µM), kynurenine (0.069 – 1.1047 µM), kynurenic acid (0.07 – 1.12 µM), anthranilic acid (0.093 – 1.5 µM), 3-OH anthranilic acid (0.084 – 1.3452 µM) and quinolinic acid (0.09 – 1.5 µM). Recovery for all analytes were $> 94\%$ and percentage stability for all analytes were $>88.98\%$ after 3 hours, $>83.53\%$ after 6 hours, 78.08% after 9 hours, 70.33% after 12 hours and 52.49 after 24 hours. This study describes how plasma tryptophan, kynurenine, kynurenic acid, anthranilic acid, 3-OH anthranilic acid and quinolinic acid can be measured accurately and precisely by LCMS, opening a new window on pharmacological and diagnostic research.

11.2.2.16 Moloi, TW

Lipopolysaccharides and Kianic Acid-induced Febrile Seizures are More Prone to Prenatally Stressed Rats, *Rhus chirindensis* Prevents Reoccurrence of these Seizures

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Purpose:

Febrile seizures (FS) are convulsions brought on by fever in infants and children, and commonly affect 3-5% of children between the ages of 3 months and 5 years. Symptomatic alleviation of FS is achieved by drugs like valproate and benzodiazepam since there is no cure. It is possible to create an animal model with FS by using lipopolysaccharides and kianic acid. Studies have shown that prenatally stressed offspring are more prone to neurological insults. For the purpose of this study Lipopolysaccharides (LPS) and Kianic acid (KA) was used in creating models on prenatally stressed pups so that we understand the effects of prenatal stress on febrile seizures by analysing the expression of interleukin 1 beta. The plant extract, *Rhus chirindensis* was used as a therapy drug to investigate its anti-convulsant activity.

Methods:

The study was approved by the Animal Ethics Research Committee of UKZN research office. The ethics number is 063/11/Animal.

Prenatal stress: immobilizing stress, rodent strainer was considered for stressing the dams. Three stressing sessions a day were employed with each sessions lasting for 45 minutes. The first session was at 8:00 am, second session at 12:00, and the last session at 15:00. Stressing ran from prenatal day 14 to 20.

Seizure induction: lipopolysaccharides were used to induce fever while kianic acid was used to generate seizures. On postnatal day 28 doses of 200µg/kg of LPS and 1.75µg/kg of KA were intraperitoneally injected per animal. The dose of 1000µg/kg of *Rhus chirindensis* was injected an hour later after LPS and KA injections. For reoccurrence on PND 38 doses were reduced by half.

Behavioural assessments: light and dark box was applied to study learning and memory of the pups. Each pup was trained in light dark box and 6 days later each pup was given a minute to reach the dark side of the box. The quicker the pup to find the dark side shows good learning and memory.

Neurochemistry: halothane was used as anaesthetic prior to decapitation on PND 38. Hippocampus and plasma was collected. Elisa kit was applied for analysis of interleukin 1 beta.

Results:

Results suggest that prenatal stress affect learning and memory, prenatally stressed pups took 49 seconds to reach the dark side while prenatally non stressed pups took 38 seconds at begin of training. But at the end of training stressed pups demonstrated slight change to 37 seconds, non-stressed took 19 seconds. Injections did not affect learning and memory of the pups. 80% of stressed pups responded to seizures while only 62% of non-stressed pups responded. Animals treated with *Rhus chirindensis*, only 10% of stressed pups reached late stages of febrile seizures while neither of non-stressed pups.

11.2.2.17 Motau, HT

The Antimalarial Activity and Selectivity of Novel Nucleoside Derivatives for *Plasmodium falciparum*

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Purpose:

Plasmodium falciparum infections are responsible for over 80% of the clinical malaria cases that result in high mortality rates especially amongst children and pregnant women in sub-Saharan Africa. Unlike mammalian cells, *Plasmodium* parasites lack the ability to synthesise the purine ring *de novo* and are reliant upon salvage of purines from the human host. A transport protein, PfENT1 expressed on the parasite plasma membrane enables purine uptake during the trophozoite stage of *P. falciparum*. Many cytotoxic nucleoside derivatives have demonstrated potent activity against *P. falciparum* by interfering with the biosynthesis of nucleic acids. In this study, the inhibitory effect of novel nucleoside derivatives on the *in vitro* growth of erythrocytic stages of *P. falciparum* was investigated.

Methods:

Compound synthesis: A number of novel nucleoside derivatives based on guanosine, inosine, uridine and cytosine were synthesized according to established procedures. These compounds were fully characterized by spectroscopic methods and 12 of the derivatives were further investigated in this study.

Parasite culture: A synchronized, chloroquine-sensitive strain of *P. falciparum* (3D7) was aseptically maintained in culture at 5-10% human haematocrit and 5% parasitaemia.

[³H]-Hypoxanthine incorporation assay: In order to evaluate antimalarial activity, parasites were incubated at 37°C with test compounds for 48 hrs (single-cycle) and the amount of [³H]-hypoxanthine incorporated into parasitic DNA quantified. Active compounds that inhibited *in vitro* growth of parasites by ≥80% at 100µM were tested for toxicity.

Red blood cell (RBC) toxicity: The haemolytic activity of active compounds on uninfected RBCs was determined spectrophotometrically at 412 nm following 48 hrs of incubation.

Mammalian cell toxicity assay: The toxicity of the active compounds on transformed human embryonic kidney epithelial (HEK293 or Graham) cells was investigated using the [³H]-thymidine incorporation assay after 72 hours exposure.

Results:

The uridine derived JLP118.1 (IC₅₀ = 1.79±0.124 µM), demonstrated the most inhibitory activity against parasite growth compared to inosine (IC₅₀ = 2.06±0.176 µM), JLP093 (IC₅₀ = 2.38±0.106 µM) and guanosine (IC₅₀ = 10.16±2.16 µM). These compounds were all significantly less active (*p* < 0.0001) compared to quinine (IC₅₀ = 103.9±8.29 nM). Although the compounds did not induce significant haemolysis (< 1% comparable to quinine), their selectivity for parasites, relative to the epithelial cells was poor with safety indices < 10. The study provides a basis for further structure-activity relationship analysis of the nucleoside analogues to enhance selectivity for the *Plasmodium* parasite, thereby reducing cytotoxicity.

11.2.2.18 Mpofana, T

Effects of Postnatal Stress on Mitochondrial Function in an Animal Model of Parkinson's Disease

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Purpose:

Stressful early life experiences have been shown to have a detrimental effect on the development of the brain. Disruptions in the mother-infant relationship has been shown to affect the hypothalamic pituitary adrenal (HPA) axis leading to increased secretion of the plasma stress hormones ACTH and cortisol during the stress hyporesponsive period. Increased glucocorticoid secretion has been shown to negatively affect the developing neurocircuitry and is therefore linked to neurodegenerative diseases such as Parkinson's disease. In this study we investigated the effects of postnatal stress on the adult brain later in life.

Methods:

Ethical clearance was obtained from the Animal ethics Sub-committee of the University Research and Ethics Committee for all our animal work (039/11). 40 male Sprague Dawley rats were used for this study. Maternal separation was used as a model of postnatal stress on the behaviour of a 6-hydroxydopamine (6-OHDA) treated rat. We also investigated the effects of caffeine (20mg/kg i.p.) in alleviating motor deficits in Parkinson's disease. The step and cylinder tests were used to assess the behaviour of the rat before and after 6-OHDA lesion.

Results:

Following 6-OHDA, Maternally separated rats had a longer step length than the non-stressed rats suggesting an impaired ability to initiate movement. Caffeine treated rats showed improved motor activity compared to the non-caffeine rats. These results suggest that events occurring early in life make dopamine neurons more susceptible to insults and thus degeneration in old age, but caffeine attenuates the effects of 6-OHDA on dopamine neurons.

11.2.2.19 Mubare, DK

NMR-based Chemical Profiling of *Pelargonium sidoides* and *Pelargonium reniforme*

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Purpose:

Ethanollic extracts of *Pelargonium sidoides* and *Pelargonium reniforme* are currently being marketed around the world as herbal remedy for upper and lower respiratory tract infections. Bioactivity studies carried out on 11% ethanollic-aqueous root extract of *P. sidoides* have shown some activity against Gram negative and Gram positive microbes, however, activity against Mycobacteria species could not be established. The effect that the extracts have on Tuberculosis has therefore been attributed to the immuno-modulatory effects of the extracts. ^[1] The purpose of this research was to develop a rapid NMR-based method for the chemical profiling and differentiation of *P. sidoides* and *P. reniforme*.

Method:

Cultivated *P. sidoides* and *P. reniforme* were obtained from the Botany Department, Rhodes University. The crushed and dried *P. sidoides* and *P. reniforme* leaves and roots (500 mg) were extracted with different solvents (chloroform/water and 10% EtOH). The solvents were dried and the extracts reconstituted in suitable deuterated solvents (CDCl₃, D₂O or DMSO-d₆). ¹H NMR spectra were obtained on a Bruker Avance 400 MHz NMR spectrometer using standard pulse sequences.

Results:

The ¹H-NMR of the chloroform-water extract from the leaves of *P. sidoides* and *P. reniforme* were distinctly different the former showed presence of eugenol methyl ether whilst this compound was not detected in the leaves of *P. reniforme*. Furthermore the spectra of the ethanollic extracts of *P. sidoides* and *P. reniforme* were also distinctly different.

Conclusion:

¹H NMR spectroscopy provides quick, simple and convenient methods for the comparison of *P. sidoides* and *P. reniforme* metabolite profile. ^[2]

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11.2.2.20 Mufamadi, MS

Application of Ligand-Functionalize Nanoliposomes to Reduce the Neurotoxicity Associated with B-Amyloid Aggregation in Alzheimer's Disease

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Purpose:

The purpose of this study was to provide an innovative solution to the neurotoxicity induced by A β (1-42) aggregates employing nanoliposomes (NLPs) surface-modified with chelating ligands such as zinc acetate (ZnAc), histidine, and ethylenediaminetetraacetic acid (EDTA).

Methods:

Preparation of the nanoliposomes: Nanoliposomes (NLPs) formulated with DSPC, cholesterol and DSPE-mPEG2000 using an adapted reverse phase evaporation technique. Chelating ligands were coupled within the surface NLPs consist of DSPE-mPEG-COOH conjugate.

Determination of size distribution and zeta potential: Determination of average particle size and zeta potential of modified nanoliposomes were analysed by a Zetasizer NanoZS instrument.

In vitro A β aggregation: A β aggregation was induced by reacting 5mM of A β (1-42) with 20mM of copper chloride (CuCl₂) or zinc chloride (ZnCl₂) incubation in 20mM Tris/150mM NaCl buffer (pH 7.4) at 37°C. Soluble A β (1-42) was quantified by a NanoPhotometer™ spectrophotometer.

Ex vivo neurotoxicity: Neurotoxicity was established by exposing neuronal cell line (PC12 cells) to ZnA β (1-42) or CuA β (1-42), thereafter treated with modified NLPs in order to enhance neuronal cells protections. Neurotoxicity for this study was assayed using CytoTox-Glo™ Cytotoxicity kit. Dead and live cell signals were measured by luminometer Victor™X3 Perkin Elmer 2030.

Results:

The results showed that NLPs produced have a nanometer size range 125-178nm and zeta potential range -35 to -37mV. *In vitro* studies showed that 30-40% soluble A β (1-42) attained when ZnA β (1-42) or CuA β (1-42) formed. However, when modified NLPs were incorporated 70-80% soluble A β (1-42) was recovered. *Ex vivo* studies showed about 60-80% cell viability after treatment with modified NLPs.

Conclusion:

High cellular survival postulated to be influenced by post surface-modified NLPs with EDTA, Histidine and ZnAc hampered ZnA β (1-42) or CuA β (1-42) aggregate formation while promoting high quantity of non-toxic soluble A β (1-42).

11.2.3 Session Three

11.2.3.1 Müller, AC

Process Validation of a Low-Dose, Low-Solubility Drug During Pharmaceutical Development of a Fixed-Dose Combination Capsule

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Purpose:

In the manufacture of solid oral dosage forms such as capsules, content uniformity is a critical quality attribute especially when one or more of the active pharmaceutical ingredients (APIs) constitutes a low percentage (w/w) of each dosage unit. Dry mixing, post-granulation blending (extra-granular and lubrication) and capsule filling may be considered critical process parameters in the manufacture of such dosage forms. Pharmaceutical development of these types of products should thus involve robust blending and transfer processes which will not lead to segregation of the mixture. Process validation is required as part of process design to ensure that each unit operation of a manufacturing process is controlled in order for the finished product to meet all quality attributes, including content uniformity. The purpose of this study was to conduct process validation of a fixed-dose combination analgesic capsule containing meloxicam at a concentration of 0.625% (w/w) per capsule, in order to assess blend homogeneity during the critical processes and unit dose sampling during capsule filling.

Methods:

A capsule formulation containing 0.0625 % (w/w) meloxicam and two other active pharmaceutical ingredients per capsule was developed. During process development, common unit operations were dry mixing, wet granulation, drying, blending (extra-granular and lubrication) and capsule filling. Appropriate blend sampling techniques and procedures were also developed, including consideration of sampling devices and sample size. During process validation, blend sample analysis was conducted on two batches of the formulation by extensively sampling at three locations in the blender (n=10) during extra-granular and lubrication stages. In addition, stratified sampling of the dosage units (capsules) was performed taking samples at defined intervals and locations (n=60) throughout the capsule filling process. Comparisons between the blend and dosage unit data were made to investigate any discrepancies observed between the two sets of data. A validated Ultra Performance Liquid Chromatography method was used for analysis of blend samples and dosage units.

Results:

All individual samples were within the mean +/- 10 % absolute (n=10) with an RSD < 5,0% for the blends and each location mean (n=20) was within 90,0 % - 110,0 % of target potency. Moreover, all dosage units were within 75,0 % - 125,0 % of target potency with an RSD < 6,0 %.

Conclusion:

The results of the study confirm the homogeneity of the blend and content uniformity of the dosage units and provide sufficient assurance that the process design used is robust enough to ensure uniformity of meloxicam content when this drug is 0.0625% (w/w) of the formulation in this fixed-dose combination capsule.

11.2.3.2 Mupfumira, R

Four Grahamstown Community Pharmacists' Opinion on Traditional, Complementary and Alternative Medicines in Pregnancy: A Case Report

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Introduction:

The use of Traditional, Complementary and Alternative medicines (TCAMs) and is a rapidly growing healthcare system and is of economic importance.[1, 2] Many health practitioners discourage the use of herbal medicines in pregnancy due to the lack of knowledge or documented data about the adverse effects and interactions of herbal products.[3]

Methods:

This case report is a sub-study within a larger study about TCAMs and pregnancy. Four community pharmacists were asked to fill in a questionnaire containing both open-ended and closed questions. The questionnaire was developed for the study and piloted using pharmacy colleagues in the Faculty of Pharmacy at Rhodes University.

Results:

General: All four pharmacists said that CAMs are “somewhat effective” and sold them at their pharmacies although none of them were aware of whether they were registered with the MCC or not.

Knowledge: None of the pharmacists appeared to have an in-depth knowledge of TCAMs.

Attitudes: All four pharmacists said that it is important to have a basic understanding of TCAMs before using them, although they did not agree on the reasons for this. Three pharmacists “agreed” that it is important for patients to consult a healthcare professional before using TCAMs, but one felt that a patient should have obtained sufficient information from sources such as the internet. All of them felt that a pharmacist has a professional responsibility to provide information on TCAMs (especially herbal preparations) and two felt that it is part of a medical doctors' responsibility.

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11.2.3.3 Mwila, C

Development of a Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) Method for the Simultaneous Determination of Lopinavir and Ritonavir in Pharmaceutical Dosage Forms

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Introduction:

Lopinavir and ritonavir are antiretroviral drugs which belong to the class of protease inhibitors. The fixed combination of these drugs constitutes a cornerstone of second line antiretroviral therapy. It is also used as first line therapy in children under 3 years and in Post-Exposure prophylaxis (PEP). About 10% of patients on first line antiretroviral therapy will require second line therapy per annum, hence the need to research more on these drugs in order to improve adherence.

Purpose:

To develop a simple, rapid and accurate HPLC method for the simultaneous determination and quantitation of lopinavir and ritonavir in dosage forms. Various HPLC methods have been developed for the analysis of lopinavir and ritonavir and involve the use of isocratic separations with a ternary mobile phase or gradient elution using a binary mobile phase. These analytical methods are relatively complex and hence there is a need to develop a simple reversed phase HPLC method.

Method:

Reversed phase HPLC using UV detection was selected for use and the separation was achieved on a Phenomenex[®] C₁₈ (2), 5µm, 250 x 4.6mm I.D stationary phase. Diazepam was selected for use as the internal standard and detection was performed at a wavelength of 215nm. The mobile phase consisted of acetonitrile and water and the optimum conditions for the separation of the two compounds and the internal standard were achieved by altering the composition of the mobile phase. A mobile phase of acetonitrile and water in the ratio of 56: 44 v/v was used for the separation and the flow rate selected for use was 1.0ml/min. samples were introduced onto the system using a 20 µl fixed loop Spectra Series AS100 autosampler and the analytical run time was 13 minutes.

Results:

The retention times were 8.20, 9.70 and 11.35 minutes for diazepam, ritonavir and lopinavir respectively. The assay was linear over the range 8.2 - 260 µg/ml for lopinavir with the calibration curve producing a linear regression coefficient of 0.9996 with the equation $y = 0.0055x - 0.0039$. For ritonavir assay, the calibration curve linear regression coefficient was 0.9995 with the equation $y = 0.0043x - 0.0012$ over the concentration range of 2.05 - 65 µg/ml. The method was validated using ICH guidelines and the parameters monitored included precision, accuracy and the limits of quantitation and detection.

Conclusion:

A simple and rapid HPLC method for the simultaneous determination and quantitation of lopinavir and ritonavir using diazepam as an internal standard has been developed and applied to the analysis of commercially available dosage forms.

Acknowledgement:

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11.2.3.5 Nkomozepe, P

Ki-67 and DCX Immunohistochemistry on Free-Floating Immersion Fixed Human Hippocampal Sections

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Background:

Doublecortin (DCX) and Ki-67 immunohistochemistry techniques have been used extensively in adult neurogenesis studies to detect proliferating cells and immature neurons respectively. DCX is a microtubule associated protein expressed in immature neurons and Ki-67 is a chromosomal associated protein expressed in proliferating cells. Most of these studies however were carried out in rodents, less in non-human primates and least in humans. Studies in humans used paraffin embedded sections. Following those protocols pose a challenge when using immersion- fixed free- floating sections. Experience of such challenges has necessitated the development of alternative protocols. This paper presents an improved protocol on the immunohistochemical staining of proliferating cells and immature neurons in the human hippocampus.

Methods:

Samples: Hippocampal specimens were obtained from two male individuals (23 and 30 years) from the Forensic Pathological Services, Johannesburg with permission from the University Witwatersrand Human Ethics Committee (clearance number M091151) with postmortem delays of 18 and 10 hours respectively.

Tissue preparation: Hippocampi were removed from the temporal lobes, divided into three blocks and immersion-fixed in 4% paraformaldehyde in 0.1M phosphate buffered saline solution (pH 7.6) for 10-14days before transferring into cryoprotectant. 50 µm sections were cut using a freezing microtome.

Immunohistochemistry: Immunohistochemical staining was done following standard protocols except for variations in primary antibody dilutions (for both Ki-67 and DCX) and different antigen retrieval methods for Ki-67.

Primary antibody dilutions: The following dilutions in Tris buffered saline with Triton (TBST) were used for DCX: 1:500; 1:300; 1:250; 1:200 and Ki-67: 1:2000; 1:1000; 1:800.

Antigen retrieval for Ki-67: The following methods were used in sodium citrate buffer pH 6: No Antigen retrieval; boiling at 94°C for 10; 20; 40 minutes, and microwaving at 750W for 30 seconds; 10 minutes.

Sections were assessed for quality of tissue and staining following each method.

Results:

DCX: No positive staining was obtained with 1:500 and 1:300 dilutions while staining was demonstrated with both 1:250 and 1:200. The 1:200 sections however had increased background staining.

Ki-67: No staining was found for all the dilutions without antigen retrieval. With antigen retrieval however, staining was found at all dilutions with 1:1000 giving the best quality. Boiling or microwaving for more than 10 minutes produced tearing or wrinkled tissue sections.

Conclusion:

DCX dilution of 1:250 and Ki-67 dilution of 1:1000 with antigen retrieval by microwaving for 10 minutes at 750W produced a high quality staining of the human hippocampi.

11.2.3.6 Nyambe, MN

An Investigation of the Antioxidant (Radical Scavenging) Activity of Selected South African Marine Algae

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Purpose:

Marine algae have emerged as a reliable source for compounds with great medicinal value. Examples of such include algal-derived cytotoxics, antibiotics, antivirals, anticoagulants and antioxidants. In continuation of our search for biologically active natural products from marine algae, we have investigated the antioxidant activity of *Styopodium multipartitum*, *Dictyopterus ligulata*, *cystosphora fibriosa* *Bifurcariopsis capensis* and *Sargassum* sp.

Methods:

The algae were pulverized and extracted with methanol-dichloromethane (1:2) under sonication. The crude extracts were dried *in vacuo* and analysed by ¹H NMR spectroscopy and thin layer chromatography using a methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) as spray reagent. Step gradient fractionation (*n*-hexane/ethyl acetate) of the *Styopodium multipartitum* crude extract yielded nine fractions which were further purified by column chromatography and normal phase HPLC.

Results:

Styopodium multipartitum, *Cystosphora fibriosa* and *Bifurcariopsis capensis* gave a positive DPPH anti-oxidant test. *Dictyopterus ligulata* and *Sargassum* sp showed little colour change. ¹H NMR and TLC analysis of these extracts revealed different chemical profiles. Fractionation and purification of the *S. multipartitum* extract yielded a tetraprenylquinone and a carotenoid metabolite which are, in part, responsible for the radical scavenging activity of the crude extract.

Conclusion:

These studies show that *Styopodium multipartitum*, *Cystosphora fibriosa* and *Bifurcariopsis capensis* possess some anti-oxidant/radical scavenging activity. Further work on the isolation and characterization of additional antioxidant molecules present in these algae are in progress.

11.2.3.7 Ojewole, E

Effect of Aloe Vera Gel on the Buccal Polymeric Films of Didanosine

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Purpose:

The buccal route can improve the bioavailability of drugs such as didanosine (ddI) which are susceptible to degradation in the gastrointestinal tract (GIT). However, the lining epithelium of the buccal mucosa constitutes the main cellular barrier to drug permeability, hence the need to include a permeation enhancer in buccal delivery systems. Aloe vera gel (Avgel) has the potential to enhance the buccal permeability of ddI (Ojewole E et al 2009). Moreover, Avgel can act as a suitable excipient in pharmaceutical modified-release formulations (Jani et al 2007). A buccal delivery system which can incorporate Avgel as a potential permeation enhancer could be an attractive modified-release system for systemic delivery of ddI. Therefore, this study aimed to determine the formulating effects of Avgel and its concentration on the buccal polymeric films containing didanosine.

Methods:

ddI-loaded films containing ddI, hydroxypropyl methylcellulose (HPMC) and Eudragit[®] RS-100 (EUD) in a fixed ratio of 1:0.5:10 (ddI films) were formulated using silicone moulded tray (SMT) with teflon coated perspex inserts by solvent casting / evaporation method. ddI/Avgel films comprising ddI:HPMC:EUD (1:0.5:10) and Avgel in varying ratios of 0.25, 0.5 and 0.75 were formulated by solvent casting / evaporation method. Routine evaluations of both ddI and ddI/Avgel films were performed in terms of appearance (Samsung digital camera, Japan), thickness (Digital micrometer, Mitutoyo), and weight (Mettler Toledo AB204-S). Drug assay and *in vitro* drug release using UV Spectrophotometer, and morphology using scanning electron microscope, LEO, Germany as well as surface pH (Hannah pH meter 211, Portugal) were assessed.

Results and discussion:

ddI films were homogenous, smooth and flexible with thickness and weight of 0.33 ± 0.02 mm; 305.55 ± 3.83 mg respectively. ddI/Avgel 0.5 films were thicker (0.55 ± 0.02 mm, $p = 0.210$), heavier (313.78 ± 5.33 mg, $p = 0.09$), and stickier with patchy surfaces compared to ddI films. Drug assay and *in vitro* drug release for ddI/Avgel 0.5 films, 88.93 ± 11.29 % (assay) and 45.13 ± 2.25 % (drug release) were lower as compared to those of ddI films, 94.58 ± 9.89 % (assay) and 62.39 ± 6.11 % (drug release). Assay values of the films formulated in this study were generally low, and this could be attributed to inadequate solvent extraction of the drug. However, the decrease in drug release over eight (8) hours could be credited to the modifying effects of Avgel. Surface morphology post dissolution showed pores and voids that indicated that ddI was released by gradual diffusion from the films. Surface pH remains similar to the salivary pH (6.75) with no remarkable significant changes over the test period.

Conclusions:

Results showed that Avgel can be incorporated as an excipient in the buccal polymeric films of ddI. DdI/Avgel-containing buccal films displayed controlled-release profiles. Based on the results in this study, the assay of the ddI buccal films require further investigation in order to obtain values that will comply with compendia specifications.

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11.2.3.8 Oosthuizen, F

Treatment Adherence of Psychiatric Patients in an Outpatient Setting

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Purpose:

Treatment adherence is the degree to which patients follow health professionals' recommendations regarding a prescribed treatment regimen and maintain the indicated treatment regimen. Medication non-adherence in psychiatric patients is associated with poor clinical outcomes and high resource utilization. Treatment non-adherence is a very critical issue in South Africa as it poses both a considerable financial burden as well as deleterious effects to health of the individual. Apart from treatment failures, non-adherence may result in relapse, re-hospitalizations, functional impairment, increased suicide attempts, and a huge socio-economic burden.

The aim of this study was therefore to assess the level of treatment adherence in psychiatric outpatients and determine the factors that influence treatment adherence amongst these patients. Assessing treatment adherence would lead to a better understanding of non-adherence in these patients and lay the groundwork for interventions aimed at increasing treatment adherence.

Methods:

This study was conducted amongst adult (≥ 18 years) psychiatric outpatients. Ethical approval was obtained from relevant regulatory bodies; written informed consent was obtained from all the participants subsequent to the aims and objectives of the study being described to them (FEHSC 005/10). Patients were assessed for treatment adherence using the Morisky Medication Adherence Questionnaire. The impact of various factors on treatment adherence was determined, including socio-demographic factors (type of illness, gender, age, race, marital status, history of illness, residential status, educational level and employment status) as well as treatment-related factors. Data was analysed using Statistical package for Social Sciences (SPSS) V15.0. Results were calculated as frequency (%) and median (p). Group comparisons were done by the Kruskal-Wallis and Mann-Whitney tests. Significance was calculated at $p < 0.05$.

Results:

A total of 95 outpatients participated in the study. This study found high adherence levels amongst 12.6%, moderate adherence levels amongst 50.8% and low adherence levels amongst 37% of participants. Statistically significant predictors of adherence to psychiatric treatment were age ($p=0.04$) and race ($p=0.05$). Socio-demographic variables such as the type of condition, employment status and educational level were found to be statistically insignificant in contributing to patient adherence.

Adherence levels amongst psychiatric outpatients were found to be low to moderate, with race and age significant predictors of treatment adherence. Patients' illness beliefs should be addressed since these may lead to changes in adherence behaviour. Medication adherence in psychiatric outpatients in general and particularly amongst the younger age group and the black population could be enhanced and improved by adequate intervention. This could be achieved through the elimination of stigma, extensive patient education, rigid support systems and education involving methods and techniques which assist patients in their compliance. This should be directed toward the younger patients and the black population since they could be viewed as high risk groups for non-adherence.

11.2.3.9 Parker, MB

Theory vs. Practice: An Investigation into the Reality of Primary Health Care Provision in Community Pharmacies in the Western Cape Metropole Region

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Purpose:

Community retail pharmacies are often the first and only port of call for patients seeking medical assistance and are thus noteworthy providers of Primary Health Care (PHC) services in the private health sector. In order to optimise these services and attain desirable therapeutic outcomes, pharmacists must adopt patient-centred practices and prioritise their interaction with patients. Worldwide, schools of pharmacy incorporate patient-centred philosophies into their curricula. A common instrument used to teach patient interaction is the Objective Structured Clinical Evaluation (OSCE). However, literature suggests that a discord often exists between what is taught by healthcare instructors and what actually happens in practice settings. Pharmacists too struggle to apply theoretical aspects into practice, creating a problem which may lead to suboptimal patient counselling. The objective of this study was to present evidence around the quality of PHC provision by pharmacists in community-retail pharmacies in the Western Cape, by comparing that which is taught in pharmacy curricula to actual practice patterns and behaviours in pharmacies.

Method:

Both qualitative and quantitative methodologies were employed. The qualitative component of the study comprised a focus group held with University of the Western Cape (UWC) undergraduate pharmacy students. Focus group responses were coded, kept confidential and classified as positive or negative, thereby allowing the researchers to identify significant themes that emerged from the analysed data. Quantitative data was obtained by way of a modified OSCE targeted toward practising pharmacists. The OSCE was used as a research tool to evaluate the interaction between pharmacists and trained mock-patients. The analysed OSCE data was made up of frequency scores obtained from the OSCE checklist observations.

Results:

Qualitative results suggest that many pharmacists exhibit sub-optimal patient counselling techniques. The following themes emerged from qualitative data: (a) Lack of adequate counselling due to infrastructural barriers, (b) Appropriate counselling is time consuming and (c) Varying individual counselling techniques. Quantitative data reflects that majority of pharmacists portray inadequate patient counselling skills as they often failed to ascertain vital pieces of information. Only 17% of pharmacists asked if patients had allergies to any medication, 47% directed the patient on the usage of their medication and 39% of pharmacists were able to exhibit adequate clinical reasoning and decision making skills.

Conclusion:

The quantitative aspect of this study suggests that a discord exists between what is taught in the pharmacy curriculum and what is practiced by pharmacists in community retail pharmacies. Further efforts are required to ensure community retail pharmacists provide an acceptable quality of patient-interaction true to the philosophy of pharmaceutical care.

11.2.3.10 Qulu, L

Exposure to Early Life Stressors Enhances the Prevalence of Febrile Seizures in Young Rats

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Purpose:

A febrile seizure (FS) is a neurological disorder that occurs following an infection that results in a rapid rise in body temperature. This leads to the release of Interleukin-1beta (IL-1 β) a cytokine used as a marker for FS. This disorder commonly affects children between the ages of 3 months and 5 years. Existing evidence suggests that neurological disorders can be exacerbated in offspring exposed to stress prenatally. Currently there is no cure for febrile seizures. *Rhus chirindensis* (*Rhus*) is a traditional plant used for many illnesses as well as dementia. In our study we investigated whether febrile seizures are exacerbated in the offspring of rats that were prenatally stressed and whether *Rhus* can prevent the recurrence of FS.

Method:

On gestational day 14 (GND 14) the rats were divided into two groups viz, the non-stressed and the stressed rats (ethical clearance numbers: 076/10 and 041/11/animal). The non-stressed rats were left untouched and receive food and water *ad libitum*. The stressed rats were taken to a separate room and placed in rodent restrainers for 45 min three times a day at 3h intervals. On Postnatal day 14 a seizure was induced by injecting 200 μ g/kg of LPS intraperitoneally (i.p.) and two and a half hrs later the animals were injected with kainic acid (1.75 μ g /kg i.p.). After kainic acid and LPS injection the rats received the *Rhus* (1000mg/kg) plant extract. *Rhus chirindensis* aqueous stem-bark extract was prepared and administered to the groups receiving *Rhus*. Behaviour was assessed using a light dark box which measures anxiety like behaviour and an ELISA kit was used to detect the levels of IL-1 β in hippocampal tissue.

Results:

Our results have shown that 1). Stress exacerbates anxiety like behaviour 2). Stress prolongs the duration of the seizure and 3). *Rhus* seems to have antagonistic effects on lipopolysaccharide and Kainic acid. Our results have also shown that stress increases the levels of IL-1 β and this increase is reduced by *Rhus* during seizures. According to our results we have also found that triterpenes are the active compound in *Rhus* which induces an anti-inflammatory response during a seizure.

11.2.3.11 Rambharose, S

Investigating the Potential of Polyethylene Glycol (PEG) for Enhancing the Buccal Permeability of Tenofovir (TNF) and Didanosine (DDI)

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Background:

Buccal drug permeation is limited by low membrane permeability. Permeation enhancers have been shown to overcome these limitations. Pharmaceutical excipients such as Polyvinyl pyrrolidone (PVP) and Polyvinyl alcohol (PAA) have mucoadhesive and controlled-release properties and could also act as permeation enhancers (Moralis and McCornville, 2011, Patel and Poddar, 2010). PEG has been reported to have mucoadhesive and controlled-release properties (Patel et al., 2011, Shojaei et al., 1998). The aim of this study was therefore to determine the potential of PEG as a buccal permeability enhancer for TNF and DDI.

Methodology:

Ethical Clearance was obtained from UKZN (24/11/Animal). *In vitro* buccal permeation of TNF and DDI (20 mg/ml) was studied in the absence of PEG as well as in the presence of various concentrations of PEG (0.25%, 0.5%, 1%, 2%, 4%, 6% (w/v)) using porcine buccal mucosa and Franz diffusion cells. TNF and DDI were quantified by UV Spectroscopy. Permeability parameters were determined by linear regression analyses.

Results:

The cumulative amount of both TNF and DDI permeation was enhanced in the presence of PEG. TNF in the presence of 4% PEG displayed the greatest steady state flux, which increased from 102.103 ± 19.8094 to $217.49 \pm 18.88 \mu\text{g} / \text{cm}^2.\text{hr}$ (ER = 2.13). However DDI in the presence of 0.5% PEG displayed the greatest steady state flux, which increased from 181.62 ± 23.62 to $295.94 \pm 15.24 \mu\text{g} / \text{cm}^2.\text{hr}$ (ER = 1.63). The increase in these flux values were statistically significant ($p < 0.05$).

Conclusion:

PEG at 4% and 0.5% shows potential as a permeation enhancer for the buccal permeability of TNF and DDI respectively.

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11.2.3.12 Rosenkranz, B

Pharmaceutical Medicine – a New Postgraduate Diploma Course in South Africa

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Background:

The development and maintenance of medicines requires specialist knowledge, comprehensive skills and competencies of the responsible scientists in pharmaceutical industry, commercial contract research organizations, or regulatory agencies. As a consequence, the discovery, development, evaluation, licensing and monitoring of medicines, and the medical aspects of their marketing have become a highly specialized scientific area for which adequate training and experience are mandatory. Dedicated training courses in pharmaceutical medicine are offered in various countries, including Belgium, Brazil, Germany, Ireland, Mexico, Serbia, Spain, Sweden, Switzerland, UK, Argentina, Australia, Austria, France, Italy, and USA. Most of these courses are open to medical and non-medical postgraduate students and lead to a Master of Sciences degree or a Postgraduate Diploma. A standard syllabus has been developed by the International Federation of Associations of Pharmaceutical Physicians (IFAPP, www.ifapp.org), which has been adopted by the UK Faculty of Pharmaceutical Medicine of the Royal Colleges of Physicians (www.fpm.org.uk). A European harmonised training programme is being developed by the EU IMI initiative PharmaTrain (www.pharmatrain.eu).

For medical doctors, Pharmaceutical Medicine has been formally recognized as a medical specialty in Switzerland (1999), UK (2002) and Ireland (2005). The UK Faculty of Pharmaceutical Medicine of the Royal Colleges of Physicians (www.fpm.org.uk) is currently pursuing the broader international recognition of this field.

South African Situation:

South Africa is well recognised for its experience in performing clinical trials which could be confirmed in a recent survey including local CROs and pharmaceutical industry. Feedback obtained on origin and timelines of industry sponsored studies will be presented. Formal training of local experts in pharmaceutical medicine will be essential in order to meet the increasing demands of academic trial centres, industry and regulatory agencies.

Conclusions:

The experience with a postgraduate diploma course in Pharmaceutical Medicine offered by the Division of Pharmacology at Stellenbosch University together with Tiervlei Trial Centre will be presented. This course has been accredited by the Department of Education in February 2011.

11.2.3.13 Sarjoo, M

The Influence of Suspending Agents on the Release Profile of Ibuprofen in a Formulated Suspension

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Purpose:

Pharmaceutical suspensions are liquid dosage forms that require the addition of suspending agents in order to stabilise their system. An oral suspension of ibuprofen was formulated, using Xanthan Gum and Light Kaolin as suspending agents. The USP states that not less than 80% of the labelled amount of Ibuprofen must be dissolved in 60 minutes. In order to optimise the formulation to meet this requirement, the influence of the suspending agents was investigated to understand their effect on the drug release profile of Ibuprofen.

Methods:

The individual effect of Xanthan Gum and Light Kaolin on the release profile of ibuprofen was investigated by formulating suspensions containing different concentrations of each suspending agent. *In vitro* dissolutions studies were performed on a total of six suspensions, *viz.* three prepared with a fixed concentration of Light Kaolin and concentrations of Xanthan Gum of 0.36%^{m/v}, 0.48%^{m/v} and 0.6%^{m/v}, as well as three suspensions containing a fixed concentration of Xanthan Gum with varying concentrations of Light Kaolin of 0.35%^{m/v}, 0.7%^{m/v} and 1.0%^{m/v}, respectively. *In vitro* dissolutions studies were conducted using a USP rotating paddle method (apparatus 2) at 50 r.p.m. in phosphate buffer pH 7.2. and the analysis was performed by HPLC.

Results:

A decrease in the concentration of Xanthan Gum as well as Light Kaolin showed an increase in the % release of ibuprofen in 60 minutes.

Conclusion:

The findings of this study suggest that an optimised formulation, which meets USP requirements, may be obtained by decrease in the concentration of both the suspending agents in combination and not independently of one another.

11.2.3.14 Shabalala, TB

An Immunocytochemical Investigation into the Effects of Tat Protein on Hippocampal Neurons

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Purpose:

HIV-associated dementia (HAD) is a neurological disorder that affects up to 60% of people infected with HIV. HAD is caused by a viral protein, transactivating protein (Tat), which is synthesized during viral replication. Tat protein enters the brain mainly by penetrating through the blood-brain barrier. This integrates the integrity of the blood-brain barrier thus allowing other neurotoxins to reach the brain and cause other HIV-associated neurological disorders. In the brain Tat induces HAD dysfunctions by altering the calcium homeostasis, interfering with glutamate neurotransmission, promoting apoptosis and inducing oxidative stress. The effects of Tat protein are currently irreversible. In this study we wish to examine whether Tat protein induces damage to neurons, glial cells and neurons alike within the hippocampus.

Methods:

The ethical clearance for the use of animals was obtained and the ethical clearance number is 067/11. 40 male Sprague-Dawley rats (250-300g) will be used. These rats will be divided into two groups (n=20), the experimental group and the control group. The experimental group will receive 5µg/µl of Tat protein injected bilaterally into the dorsal hippocampus using the stereotaxic procedure. The control group will receive 1µl of saline also injected into the dorsal hippocampus. Two behavioural tests (Morris water maze and light/dark box) will be performed to test for learning and memory. Immunocytochemistry will be performed on brain tissue using glial fibrillary acidic protein (GFAP) antibodies for glial cells, synaptophysin antibodies for synaptic vesicles and microtubule-associated protein 2 antibodies for neurons.

Results:

In the behavioural tests we anticipate that there will be an increase in learning and memory of the control group while the experimental group is expected to show a decrease or no improvement in learning and memory. We also anticipate that the quantity of glial cells, neurons and synaptic vesicles in the hippocampi will be decreased in Tat injected animals when compared to saline injected animals. We also anticipate that the amount of neuronal damage will be the same for the neurons, glial cells and synaptic vesicles. These results may confirm the proposed neurodegenerative activity of Tat protein.

11.2.3.15 Sibiya, SG

Evaluating a New Drug Agent to Combat Alzheimer's Disease

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Purpose:

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that affects mostly the limbic system and the neocortical areas of the brain (Sipos *et al.* 2007) and it is the most prevalent form of dementia affecting the elderly population (Klein *et al.* 2001). It has 2 hallmark lesions namely the extracellular deposition of β -amyloid protein fibrillar plaques (A β) and intraneuronal neurofibrillary tangles (NFTs) composed of abnormally hyperphosphorylated microtubule-associated tau protein (Fen *et al.* 2010). Current therapies of (AD) are merely palliative and treatments that address underlying pathologic mechanisms are completely lacking. In a recent study a novel compound Poly-*N*-methylated Amyloid Beta (A β) - Peptide C - Terminal Fragments (MEPTIDES) has been shown to reduce A β toxicity *in vitro* and in *Drosophila melanogaster* (Bose *et al.* 2009). In this study we will be investigating the effects of Poly-*N*-methylated A β -Peptide C-terminal Fragments (MEPTIDES), in the reduction of neurotoxicity of β -Amyloid on the rat brain thus suggesting it as a possible (AD) treatment drug.

Methods:

Ethical clearance was obtained from the University of KwaZulu-Natal Ethics Committee. The reference number is 078/11/Animal. 40 fully grown female Sprague-Dawley rats weighing between 250-300g (8/9 weeks of age) will be used, they will randomly be assigned into 4 groups (n= 10/group), 2 control groups and 2 experimental groups (A β and saline). All the groups will receive an intracerebral injection of either saline or A β . Learning and memory tests will be performed using the Morris water maze and the light dark box. The drug, (MEPTIDES), or saline will be administered i.p. at a point of greatest cognitive performance in the learning and memory tasks. All efforts will be made to minimize animal suffering and to reduce the number of animals used.

Results:

We hope to yield results that will show improved learning and memory in the animals treated with the drug, (MEPTIDES), suggesting it as a potential treatment for disease progression. This will have substantial medical, social and financial benefit to the patient, family members and the entire health care fraternity.

11.2.3.16 Tarirai, C

Effects of Dietary Fruits, Vegetables and a Beverage on the *In Vitro* Transport of Cimetidine: Comparing Caco-2 Cells with Pig Jejunum Tissue

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Background:

Dietary botanicals are often consumed together with allopathic medicines, which may give rise to pharmacokinetic interactions such as altered drug absorption. *In vitro* intestinal models are useful to identify botanical-drug interactions, but they may exhibit different expressions of transporters or enzymes. This study compared the effects of selected dietary botanical extracts on cimetidine transport across two *in vitro* intestinal models.

Methods:

Preparation of plant materials: Extracts of different materials of natural origin, including fruits, bulbs and aerial parts of plants were obtained by various extraction techniques such as blending of the fruit pulp and preparation of infusions, which were then freeze-dried and packaged in opaque and moisture-free containers.

In vitro drug transport experiments: Bi-directional transport of cimetidine was measured across Caco-2 cell monolayers and excised porcine jejunum tissue in the absence (negative control) as well as the presence of verapamil (positive control) and selected plant extracts (experimental groups).

The study was approved by the Ethics Committee of Tshwane University of Technology (Ref#2006/04/002/TariraiC), which took note that no ethical consideration applied since pig jejunum tissue was obtained from pigs slaughtered elsewhere for food purposes (not for research) and no humans or living animals were used in the study.

Results:

Sclerocarya birrea Hochst. (Anacardiaceae) (marula) and *Psidium guajava* L. (Myrtaceae) (guava) crude extracts significantly ($p < 0.05$) decreased cimetidine efflux in both *in vitro* models resulting in increased absorptive transport of the drug. On the other hand, *Dovyalis caffra* Sim. (Flacourtiaceae) (Kei apple), *Prunus persica* (L.) Batsch (Rosaceae) (peach), *Aspalathus linearis* (Burm. f.) R. Dahlgren (Rabaceae) (rooibos tea), *Daucus carota* L. (Apiaceae) (carrot), *Prunus domestica* A. Sav. (Rosaceae) (plum), *Beta vulgaris* L. (Chenopodiaceae) (beetroot) and *Fragaria x ananassa* (Weston) Duchesne ex Rozier. (Rosaceae) (strawberry) crude extracts exhibited different effects on cimetidine transport between the two models.

Conclusion:

Caco-2 cells were more sensitive to changes in cimetidine transport by the plant extracts and therefore may overestimate the effects of co-administered plant extracts on drug transport compared to the excised pig tissue model, which is congruent with findings from previous studies. The excised porcine jejunum model seemed to provide a more realistic estimation of botanical-drug pharmacokinetic interactions than the Caco-2 cell model.

11.2.3.17 Van Eyk, AD

The Effect of 5 Artificial Sweeteners on Caco-2, HT-29 and HEK-293 Cells

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Purpose:

Some artificial sweeteners have been associated with the possible development of tumours in animals and humans. For this purpose, the effects of 5 artificial sweeteners were studied on the following cell lines: Caco-2, HT-29 and HEK-293 using various techniques.

Methods:

Morphological changes: Cells were exposed to various concentrations of 5 artificial sweeteners (0 – 100mM sodium cyclamate, sodium saccharin, sucralose and acesulfame-K and 0 – 35mM aspartame) over 24, 48 and 72h. Results were presented photographically.

MTT cell viability assays: Cells were exposed to different concentrations of the same artificial sweeteners as above over 24, 48 and 72h. The blank consisted of DMEM medium without any cells and the controls were: DMSO, DMEM medium and cells (either Caco-2, HT-29 or HEK-293) grown without any artificial sweeteners as well as positive controls consisting of 1% (v/v) Triton X-100 as well as camptothecin (100µM). Results were presented graphically as % viability versus artificial sweetener concentration.

Trypan blue cell viability studies: Cells were exposed to the artificial sweeteners as mentioned above. After each time period of incubation, the cells in the wells were washed with PBS, treated with a trypsin/EDTA solution to lift the cells and complete media added. Trypan blue (0.25% w/v) were then added in a 1:1 ratio and the cells counted in a haemocytometer. The ratio of % viable: % dead cells were then determined and presented graphically.

DPPH antioxidant assays: The possible free radical scavenging activity and effect on ascorbic acid (100µM) antioxidant activity by artificial sweeteners (0-50mM), were studied. The blank consisted of methanol and the controls consisted of DMSO and DPPH and a positive control consisting of ascorbic acid (100µM).

Alkaline Comet assays: Possible DNA damage induced by artificial sweeteners (10mM, treated for 24h) was studied. The appearance of ‘comets’ were labelled from no damage to severe damage (0-4). Untreated cells (PBS) and cells treated with H₂O₂ (100µM/4°C/1h in the dark) were used as negative and positive controls, respectively.

Results:

Morphological changes in all the cells were noted at concentrations of artificial sweeteners above 20mM. The cells became flatter, less well defined and granular at higher concentrations. At the lowest concentrations of artificial sweeteners (0.1 – 0.5mM), a slight increase in cell number was noted especially in the HT-29 and Caco-2 cell lines. At higher concentrations (1 – 50mM), decreased cell viability was noted with increasing concentration for all cell lines tested. Decreasing cell viability was noted with increasing time of incubation in artificial sweeteners (% cell viability: 24H>48h>72H). Little if any antioxidant activity was found for all the artificial sweeteners and when combined with 100µM ascorbic acid, the antioxidant activity of ascorbic acid decreased in all of the cases. In general, the DNA of the HEK-293 cells seemed to be effected to a lesser degree than the two other cell lines when exposed to artificial sweeteners. Sucralose and sodium saccharin seemed to elicit the greatest degree of single strand DNA breaks of all the sweeteners tested in all the cell lines used.

11.2.3.18 Van Heerden, M

The Antioxidant Properties and Cytotoxicity of Sesquiterpene Lactones

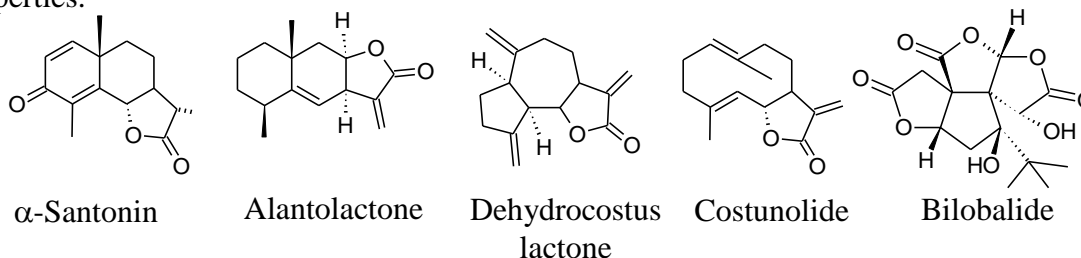
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Purpose:

The interest in antioxidants is to combat oxidative stress by targeting free radicals and stopping the chain reaction that contributes to neurodegenerative diseases. Antioxidants are ubiquitous in plants and plants are therefore a rich source for potential compounds. However, research has shown that many plants used in traditional medicine are potentially toxic and thus this study will also take cytotoxicity in consideration. Since the flavonoids are already known for their antioxidant properties, other plant constituents will be researched. Plants containing sesquiterpene lactones are utilized as phytomedicines based on ancient knowledge and certainly of importance to examine, both for their beneficial and toxic effects. For this study, the sesquiterpene lactones; α -santonin, alantolactone, dehydrocostus lactone, costunolide and bilobalide were chosen for research into their antioxidant and cytotoxic properties.



Methods:

The *in vitro* antioxidant assays; NBT –and TBA were used to assess the compounds antioxidant properties in rat brain homogenate. The TBA-assay measured lipid peroxidation, *via* hydroxyl anions (OH^{\bullet}) scavenging, on the bases of the complex formation between malondialdehyde, an end product of lipid peroxidation, and thiobarbituric acid (TBA), generating a pink colour to be measured spectrophotometrically at 530 nm. The NBT-assay relied on the reduction of nitro-blue tetrazolium (NBT) to nitro-blue diformazan in the presence of the superoxide anions ($\text{O}_2^{\bullet-}$) and the spectrophotometrical measurements thereof at 650 nm. The North-West University Ethics Committee approved the experimental protocol (05D05). The cytotoxicity assay measured the percentage of live, dead and apoptotic neuroblastoma cells after exposure to compounds.

Results:

α -Santonin (concentrations 0.5 and 1 mM) and costunolide (1 mM) showed slight significant OH^{\bullet} scavenging and lipid peroxidation attenuation compared to the toxin combination (H_2O_2 , FeCl_3 and ascorbic acid). α -Santonin (0.25, 0.5 and 1 mM) and alantolactone (0.5 and 1 mM) were able to scavenge $\text{O}_2^{\bullet-}$ generated by the toxin, potassium cyanide. Costunolide (50 μM) was eliminated due to its toxicity towards neuroblastoma cells indicating an apoptotic nature. Based on all the results obtained, α -santonin was identified as the most promising compound for further studies on its antioxidant profile.

11.2.3.19 Van Zyl, J

Thermodynamic Comparison of Lung Surfactant Replacements

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Purpose:

Pulmonary surfactant is a complex mixture of phospholipids, neutral lipids and proteins. Under normal conditions, it lowers the alveolar surface tension in the lungs to nearly zero on exhalation, thereby markedly reducing the work of breathing. However, a variety of compounds including lipases, proteases, cellular degradation products, haemoglobin and blood-derived lipids and proteins have been shown to result in a loss of its function. To strengthen the comprehensive analysis of data generated during animal studies with a synthetic surfactant (Synsurf[®]), we investigated the *in vitro* dynamic surface tension lowering ability and resistance to inactivation by albumin and cholesterol of Synsurf[®] in comparison to the mammalian-derived surfactants Survanta[®] and Curosurf[®].

Methods:

Thermodynamic experiments to investigate normal surfactant activity of Curosurf[®], Survanta[®] and Synsurf[®] as well as the inactivation studies with albumin and cholesterol were done at 23°C in a Langmuir trough (surface area 242 cm²). For these studies, surfactants were diluted 1:30 in 0.9% NaCl solution. To maintain the ionic strength in the trough, the subphase (120 ml) contained 150 mM NaCl, 2 mM CaCl₂ and 0.2 mM NaHCO₃ buffered to pH 7. Surfactant inactivation by albumin was studied with a final albumin concentration of 2 mg/ml. In order to mimic supraphysiological cholesterol concentration at the alveolar wall, surfactant inactivation was studied with increasing cholesterol concentrations to a maximum ratio of 0.12 mg cholesterol/mg surfactant.

Results:

Both Curosurf[®] and Survanta[®] reached surface pressures of about 40 mN/m while that of Synsurf[®] was about 30 mN/m. Considering the kinetics; Curosurf[®] adsorbed the fastest followed by Survanta[®] and Synsurf[®]. Compared to a normal breathing cycle, the adsorptions of all three surfactants are very slow. In the inhibition studies with albumin, adsorption behaviour of all three surfactants were very similar and surface pressure was lowered to about 51 mN/m at the highest cholesterol concentration. This result showed that all three surfactants behaved similarly by concentrating albumin at the interface without replacing it.

Conclusion:

The outcomes of these studies showed that equilibrium surface pressures, as an indication of adsorption behaviour, were similarly dependent on concentration and temperature for all three surfactants. It was demonstrated that albumin interfered with the re-spreading mechanism of surfactant molecules, inhibiting the activity of all three preparations. Furthermore, cholesterol, which is elevated in conditions such as ARDS, stabilised the maximum surface pressures at ~51 mN/m for all three surfactants.

11.2.3.20 Zindove, C

Development and Validation of an HPLC Method for the Analysis of Stavudine in Pharmaceutical Dosage Forms

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Purpose:

To develop a simple, rapid, precise and accurate RP-HPLC method for the analysis of stavudine (D4T) in pharmaceutical dosage forms. Zidovudine (AZT) was used as the internal standard. A number of HPLC methods have been developed for the analysis of stavudine that involve the use of acidic buffer systems and organic modifiers, such as acetonitrile that are expensive. Furthermore the retention times reported are longer than 10 minutes and other complex procedures are required for analysis. Therefore there is a need to develop a rapid, simple, cheap, accurate and precise RP-HPLC method for formulation development and quality control purposes.

Method:

An isocratic RP-HPLC system was used for method development and consisted of a SpectraSERIES[®] P100 solvent delivery module (San Jose, USA), a Waters Associates WISP[®] 710B autosampler (Milford, MA), a Linear[®] UVIS 200 detector (Reno, Nevada, USA) and a Perkin-Elmer[®] 561 strip chart recorder (Tokyo, Japan). A Phenomenex[®] 5 μ m ODS C₁₈ (150mm x 4.6mm x) column was used as the stationary phase and the mobile phase composition was methanol: water in a ratio of 30:70 v/v delivered at a flow rate of 1ml/min. The eluant was monitored at a wavelength of 265nm and the injection volume was 10 μ l. A calibration curve was constructed over a range of 1-60mg/ml by serial dilution of the stock solution and then plotting the peak height ratios of D4T to AZT versus concentration. The concentration of AZT was constant throughout the experiment. The effect of mobile phase composition on retention times of the compounds was also assessed and the method was validated according to ICH guidelines.

Results:

The retention times of both D4T and AZT were 4 minutes and 8 minutes respectively with an overall run time of 10 minutes. The method was found to be linear over the range 1-60 μ g/ml with a resultant R² value of 0.9991 and an equation for the line of $y=0.0268x + 0.001$. The method was also found to be precise and accurate. D4T was found to be unstable in acid, alkali, light, hydrogen peroxide and at a high temperature.

Conclusion:

A simple, rapid, linear, precise and accurate RP-HPLC method has been developed for the analysis of stavudine and has been applied to the analysis of commercially available stavudine containing dosage forms.

Acknowledgements:

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11.2.3.21 Zulu, SS

HIV Protein Tat Induces Neurocognitive Impairments

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Purpose:

HIV- induced cognitive impairment is among the most common neurological disorders associated with AIDS. Despite the introduction of HAART, continuous neuronal cell death remains a problem found in AIDS patients. Although the use of HAART increases survival time of HIV-positive individuals, more concerning is the escalation in the prevalence of HIV-associated cognitive impairments. Since HIV cannot infect neurons directly it seems that an indirect mechanism has to be involved in mediating the neuron death that occurs in HIV. In our study we wish to investigate whether HIV protein tat (transactivator) can induce oxidative stress and apoptosis in hippocampal neurons and thereby lead to the observed cognitive impairments.

Methods:

Male n= 46 Sprague Dawley rats (250g-300g) were used, ethical clearance was obtained from the University of KwaZulu-Natal ethics no.067/11. In the study animal were randomly divided into two groups with n=23 each group. Animals were bilateral injected stereotaxical into hippocampus with 5µg/µl tat protein for experimental group and 5µl saline for the control group. Behavioural analyses were performed on the Morris water maze and light/box tests. After behavioural test animals were decapitated and brain tissue collected for neurochemistry analysis. Flow cytometry will be used for neurochemistry to detect oxidative stress, while caspase-3 activity will be measured as an indicator of cell undergoing apoptosis.

Results:

Behavioural tests both Morris water maze and light/dark box showed that tat protein induce neurocognitive impairments and the results showed significant. Animals injected with tat protein experimental group showed memory and learning deficit and the saline group did not show any memory or learning deficit. Neurochemistry results are still to be analysed.

12. Delegate List

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