Proudly hosted by

The Department of Pharmacy and Pharmacology,
University of Witwatersrand,
Johannesburg,
South Africa.

CONTENTS

WELCOME – ORGANISING COMMITTEE

A warm welcome to Gauteng and the 4th International Conference on Pharmaceutical and Pharmacological Sciences (ICPPS). We hope you will enjoy your stay and that you will take the opportunity to participate in the academic programme and then relax in our tranquil surroundings on the banks of the Vaal River.

We are privileged to host a number of eminent national and international scientists at the meeting, and trust that you will benefit from their expertise. We extend a warm acknowledgement to all of our plenary speakers for making time in their demanding schedules to travel all the way to Vanderbijlpark to share their knowledge with us. The speakers are supported by a strong scientific programme and we extend a particular welcome to an enthusiastic cohort of Young Scientists who will present their work in this forum.

In addition to the scientific programme, we encourage you to participate in the delights of our relaxing venue. On Wednesday night, you are cordially invited to mingle with fellow delegates at the cocktail party. Thursday night promises to be an entertaining interactive experience at the informal dinner and on Friday night the highlight of the social programme will be the gala dinner.

Our special thanks to all of the sponsors who made this conference possible. (Please consult the list included in the programme).

Warm wishes for a wonderful conference experience,

The Organizing Committee.

WELCOME – APSSA

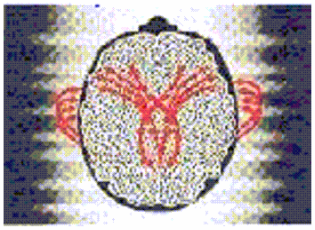
It is with great pleasure that the Executive Committee of the Academy welcomes all delegates to the joint Academy and Pharmacological Society Conference. The conference this year has the theme "*Excellence in Research*" and is being hosted by the University of the Witwatersrand. This conference will provide an opportunity for *all researchers and practitioners* involved in the field of pharmaceutical sciences to share information with their colleagues in both a formal (*podium or poster presentation*) and/or informal manner (interaction on an individual basis during and after sessions). We all need to make use of the valuable opportunities for networking that arise at conference.

The Academy Conference also provides an opportunity for *recognition of excellence* in the pharmaceutical sciences. As part of the proceedings prizes will be awarded for: the Best Publication in each of the fields of Pharmacology, Pharmacy Practice, Pharmaceutics and Pharmaceutical Chemistry; the Pharmacy Teacher of the Year and; the Young Scientist (judged from presentations at conference).

We look forward to a stimulating two and a half days of academic interaction.

Shirley-Anne Boschmans
Chairman: Academy of Pharmaceutical Sciences

WELCOME – SANS



On behalf of the Southern African Neuroscientists Society, I would like to welcome you to what promises to be a very exciting few days. I hope that you will have an enriching experience, make new friends and establish fruitful collaborations.

Looking forward to meeting you…

Prof. Willie Daniels
(Chair)

WELCOME - SAPS

Dear Colleagues,

Welcome, all participants at the 4th International Conference on Pharmaceutical and Pharmacological Sciences (ICPPS), with a most relevant theme **'Excellence in Research'** of medicinal drugs. This is the 4th ICPPS conference in the series, following the successes of the 1st ICPPS meeting held in Midrand, the 2nd ICPPS in Cape Town and the 3rd ICPPS in Boksburg. These ICPPS Conferences are based on the successful joint venture between The Academy of Pharmaceutical Sciences and The South African Pharmacology Society. The South African Neurosciences Society has also joined this 4th ICPPS conference, complementing the synergy.

The South African Pharmacology Society wishes to express our sincere thanks to our hosts, The Department of Pharmacy and Pharmacology, WITS, in particular to our Congress Convenors, Professors Paul Danckwerts, Ivan Havlik and their staff, for the tremendous effort and hard work to uphold an enviable tradition. A special word of thanks also to each and every one of our sponsors for their loyal and generous support of this Conference and all the scientific activities associated therewith.

On behalf of our Society, we extend a hearty welcome to our overseas participants. We value our contact and friendship with you very highly and we look forward to your scientific contributions as well as at a more personal level. We appreciate your acceptance of our invitations and feel honoured to have you as guests.

We wish you all as participants, as well as accompanying persons, a very pleasant and memorable 4th ICPPS conference 2006 at the Riverside Hotel, Vanderbiljpark, Gauteng, South Africa.

Prof. Douglas W. Oliver

President: S.A. Pharmacology Society

CONGRESS PROGRAMME

Wednesday 20 September 2006	
16.00 -18.00	**Registration and Checking in**
18:00-18:30	**Meeting for EXCO for SAPS, APPSA, SANS, WITS staff, Thebe (Venue: Meyerton)**
18:30 for 19.00	**Cocktail Function**

Thursday 21 September 2006

8:00-8:30	**Registration**
8:30-8:45	**Welcome note: Prof I Havlik**
	Opening address

Plenary Session I

Venue: Meyerton

Chair: Mrs S Moch

8:45-9:45	**Prof B Leonard** New approaches to the development of antidepressants

Academy for Pharmaceutical Sciences		SA Pharmacology Society / Neurosciences Society	
Venue: Vanderbijl Park A		**Venue: Meyerton**	
Chair: Prof T Govender		**Chair: Prof I Havlik**	
9:50-10:50	**Prof V Pillay** Biomaterials science and applications: Where to from here?	9:50-10:50	**Prof R Edwards** Adverse Drug Reactions

10:50-11:30	**Tea and Poster session (Venue: Vanderbijl Park B)**

Parallel Sessions A & B (Free talks/Young Scientist Competition I)

Academy for Pharmaceutical Sciences		SA Pharmacology Society / Neurosciences Society	
Venue: Vanderbijl Park A		**Venue: Meyerton**	
Chair: Prof S Malan		**Chair: Prof W du Plooy**	
11:30-11:45	**Ian Oberholzer** Peroral and nasal delivery of Insulin with pheroid technology	11:30-12:00	**Willie Daniels** How much stress is bad for you?
11:45-12:00	**Pieter van der Bijl** Permeability of human and porcine intestinal mucosa to lipophilic and hydrophilic molecules	12:00-12:15	**Ilse Pienaar** A gender-specific dose-related response shown towards 6-OHDA in the developing rat CNS
12:00-12:20	**Tjaart Coetsee (YS)** Investigating the potential neuroprotective effects of statins on DNA damage in mice striatum	12:15-12:30	**Keneuoe Thinyane** Transplantation of pre-differentiated mouse embryonic stem (ES) cells in 6-OHDA-lesioned rats.
12:20-12:40	**Clemence Tarirai (YS)** Cross-linked chitosan matrix systems for sustained drug release	12:30-12:45	**Jacqueline Faure** The effects of trauma in previously maternally separated rats
12:40-13:00	**Neha Singh (YS)** A novel multi-polymeric crosslinked glycosidic platform for site-specific delivery of nicotine	12:45-13:00	**Leonie Harmse** *Plasmodium falciparum* kinases as drug targets
13:00-14:00	**Lunch and Poster session (Venue: Vanderbijl Park B)**		

Parallel Sessions A & B (Young Scientist Competition II)			
Academy for Pharmaceutical Sciences		**SA Pharmacology Society / Neurosciences Society**	
Venue: Vanderbijl Park A		**Venue: Meyerton**	
Chair: Prof J Hamman		**Chair: Prof D Oliver**	
14:00-14:20	**Armand de Vries** Polycyclic indole derivatives as novel structures for neuroprotection	14:00-14:20	**Akash Ramjeeth** The evaluation of low-density lipoprotein cholesterol goals achieved in patients with established cardiovascular disease and/or hyperlipidaemia receiving lipid-lowering therapy (Preliminary results)
14:20-14:40	**Estee-Marie Holmes** The synthesis of stavudine derivatives for transdermal delivery	14:20-14:40	**Charise Joubert** The antioxidant, N-acetyl cysteine (NAC), increases vacuous chewing movements in rats: Evidence for striatal oxidative stress
14:40-15:00	**Deidre van den Berg** Inhibition of monoamine oxidase B by substituted benzimidazole analogues	14:40-15:00	**Hannes Clapton** Muscarinic cholinergic inhibition unmasks antidepressant-like properties of sildenafil: behavioural and neuroreceptor evidence
15:00-15:20	**Krishnaveni Naicker** The introduction of process analytical technology, using near infrared analysis, to a pharmaceutical blending process	15:00-15:20	**Ilse Groenewald** Cortical/hippocampal M1 receptor changes and acoustic startle response in an animal model of posttraumatic stress disorder
15:20-15:40	**Liezl Badenhorst** The dissolution analysis of sulfadoxine/pyrimethamine combination tablets	15:20-15:40	**Nico Liebenberg** The α_2-lytic properties of mirtazapine is not important for a putative earlier onset of antidepressant action
15:40-16:00	**Susan Neethling** The antioxidant properties of 4-hydroxyquinolines	15:40-16:00	**Sizwe Mjiqisa** Pulmonary effects of traditionally prepared *Artemisia afra* steam inhalation, nebulized aqueous extract and the possible involvement of flavonoid luteolin

16:00-16:30	**Tea and Poster session (Venue: Vanderbijl Park B)**

	Venue: Vanderbijl Park A	Venue: Meyerton
16:30-18:30	**AGM of the Academy for Pharmaceutical Sciences**	**AGM of the South African Pharmacology Society**

18:30 for 19:00	**Braai**

Friday 22 September 2006

08:00-08:30	**Registration**

Plenary Session I

Venue: Vanderbijl Park A		Venue: Meyerton		Venue: New Orleans
Chair: Prof V Pillay			Chair: Dr N Butkow	Chair: Mrs S Moch
08:30-09:30	**Prof S Iyuke** Nanotechnology in Pharmacy and Pharmacology: Carbon nanotubes and polymer based nanocapsules for drug delivery	08:30-09:30	**Mr F Hoffmann** "Generics – Quo Vadis?"	**Prof T Brink** Current trends in the use of technology for the teaching of health sciences in developed and developing countries.

Academy for Pharmaceutical Sciences (Young Scientist Competition III)		Neurosciences Society / SA Pharmacology Society (Young Scientist Competition III)		SA Pharmacology Society (Education)
Venue: Vanderbijl Park A		Venue: Meyerton		Venue: New Orleans
Chair: Dr G Enslin			Chair: Prof W Daniels	Chair: Prof T Brink
09:30-09:50	**Kevin Zoellner** The preparation and evaluation of xanthine analogues as inhibitors of Monoamine Oxidase B	09:30-09:50	**Yusuf Rasool** Anti-oxidant effects of novel gold compounds	**Shirra Moch** Exploring student attitudes to learning pharmacology: A qualitative analysis
09:50-10:10	**Miao-Juei Huang** *In vitro* percutaneous and Caco-2 cell monolayer transport of Rooibos (*Aspalathus linearis*) Aspalathin	09:50-10:10	**Adrienne Muller** Mechanisms by which acyclovir reduces the oxidative neurotoxicity and biosynthesis of quinolinic acid	**Linda Mabope** Quantity and quality in pharmacy education can be maintained with a problem-based learning approach
10:10-10:30	**Andra Kruger** The role of polyunsatured fatty acids in refractory epilepsy	10:10-10:30	**Layla Cassim** Melatonin counteracts the 5-fluorouracil-induced decreases in brain neurotransmitter levels but does no alter the hepatic metabolism of 5-fluorouracil by cytochrome P450	**Hannelie Meyer** Experiential learning - A practice-based learning approach in the UL (Medunsa Campus)/TUT B.Pharm programme
11:00-11:20	**Louis Prins** Pentacyclo[5.4.0^{2,6}.0^{3,10}.0^{5,9}]undecane as carrier molecule for neuroprotective NSAIDS	11:00-11:20	**Admire Dube** Standardization and preparation of *Artemisia afra* herb in tea bag dosage form for use in clinical trials	**Boitumelo Khudaga** Using a popular autobiography as a teaching tool; medical students' perceptions
11:20-11:40	**Thabiso Lepheana** Formulation of a non-alcoholic mouthwash for the treatment of halitosis	11:20-11:40	**Mark Scheepers** L-Dopa administration enhances 6-hydroxydopamine generation	**Eugene Olivier** Teaching the next generation of pharmacists

11:40 - 12:00	**Tea and Poster session (Venue: Vanderbijl Park B)**

Plenary Session II			
Academy for Pharmaceutical Sciences		**SA Pharmacology Society / Neurosciences Society**	
Venue: Vanderbijl Park A		**Venue: Meyerton**	
Chair: Prof D Oliver		**Chair: Mrs S Moch**	
12:00-13:00	**Prof J Gasteiger** The role of chemoinformatics in drug design	12:00-13:00	**Prof B Leonard** Depression as a neurodegenerative disease: the end of the amine hypothesis?
13:00-14:00	**Lunch and Poster session (Venue: Vanderbijl Park B)**		

Parallel Sessions A & B			
Academy for Pharmaceutical Sciences		**SA Pharmacology Society / Neurosciences Society**	
Venue: Vanderbijl Park A			**Venue: Meyerton**
Chair: Prof H Leng			**Chair: Prof I Havlik**
14:00-14:15	**Armorel van Eyk** *In vitro* diffusion of the immunosuppressant tacrolimus through human and rabbit corneas	14:00-15:00	**Prof G Norton** Mechanisms of Adrenergic-Induced Cardiac Dysfunction
14:15-14:30	**Lisa du Toit** Evaluation of novelly-formed entero-spheres for targeted delivery of an anti-tuberculosis drug	15:00-15:15	**Johannes Bodenstein** N-terminal genetic variations of regulator of G protein signalling 2 (RGS2) in hypertensive patients: decreased expression and function of the mutant RGS2-Q2L
14:30-14:45	**Johann Van Zyl** Surfactant treatment for respiratory distress syndrome: A study in preterm lambs	15:15-15:30	**Sandy van Vuuren** Exploring the antimicrobial activity of medicinal aromatic plants used in traditional healing practices
14:45-15:00	**Lezanne Moll** Comparative *in vitro* permeability of cyclosporin A and tacrolimus across human vaginal mucosa	15:30-15:45	**Ortrun Meissner** From traditional medicines to traditional rituals: the good, the bad and the ugly. Traditional male circumcision in the Eastern Cape
15:00-15:15	**Yahya Choonara** A novel controlled release PLGA alginate-pectinate polyspheric drug delivery system	15:45 - 16:00	**Ashenafi Assefa Bahita** Bronchodilator and anti-inflammatory activities of *Adhato schimperiana*, Acanthaceae
15:15-15:30	**Heiner Seifart** HPLC/MS/MS technology for analysing substances of abuse		
15:30-16:00	**Roche** Inhibiting VEGF. A new approach to colorectal cancer		
16.00 -16:30	**Tea and Poster session (Venue: Vanderbijl Park B)**		

Venue: New Orleans	
16.30 -18.30	**AGM of the Southern African Neurosciences Society**

18:30 for 19.00-…	**Gala Dinner**

Saturday 23 September 2006

08.00-08.30	**Registration**

Plenary Session I

	Venue: Vanderbijl Park A	Venue: Meyerton
	Chair: Prof D Oliver	**Chair: Prof I Havlik**
08.30 - 09.30	**Prof Vasilevskij** Research of biological active compounds in series of unsaturated pyrazoles	**Prof A Viljoen** Pharmacognosy – the pitfalls and challenges of an unique research opportunity in South African

Parallel Sessions A & B: 1

	Academy for Pharmaceutical Sciences	SA Pharmacology Society
	Venue: Vanderbijl Park A	**Venue: Meyerton**
	Chair: Ms S-A Boschmans	**Chair: Prof A Viljoen**
09:30-09:45	**Shirona Naidoo** Development of a symptom focused HRQOL tool for patients on highly active antiretroviral therapy	**Maria Paraskeva** The antibacterial and anti-oxidant activity of South African indigenous *Commiphora* species and the isolated compounds from *C. glandulosa*
09:45-10:00	**Karine Wabo Ruud** Antiretroviral treatment programme in Grahamstown's public health sector	**Arina Lourens** Isolation of selected bio-active lactones from South African *Helichrysum* species
10:00-10:15	**Jeanette Lotter** The design of patient information leaflets for the unique South African situation: Suggestions from a documant design perspective	**Guy Kamatou Poungoue** The antimalarial activity and cytotoxic effects of solvent extracts of South African *Salvia* species and isolated compounds from *S. radula*
10:15-10:30	**Magdalene van Jaarsveld** Effect of temperature and pH on valproic acid and acyclovir *in vitro*	**Mmamosheledi Mothibe** The acetone extract of *Combretum woodii* leaf inhibits the respiratory burst of isolated human neutrophils
10.30-11.00	**Tea and Poster session (Venue: Vanderbijl Park B)**	

Parallel Sessions A & B: 2

	Academy for Pharmaceutical Sciences	SA Pharmacology Society	
	Venue: Vanderbijl Park A	**Venue: Meyerton**	
	Chair: Mr Y Choonara	**Chair: Prof B Harvey**	
10:30-10:45	**Ruan Botha** Manufacture and optimization of tubular nickel membrane supports	10:30-11:00	**Brian Harvey** Aripiprazole: Role of partial agonism and its relevance in psychiatry
10:45-11:00	**Sibongile Sibambo** The effects of polymeric cross-linking on the *in vitro* dissolution of amitryptiline from a PLGA-based monolithic system	11:00-11:15	**Vivienne Russel** Prenatal stress decreases the beneficial effects of exercise on motor function in rats given intracerebral injection of 6-OHDA
11:00-11:15	**Dewald Kapp** Manufacturing and development of a zeolite X coated membranes	11:15-11:30	**Susan Van Rensburg** Mending the myelin in Multiple Sclerosis: The role of 5,10 methylene-tetrahydrofolate reductase (MTHFR)
11:15-11:30	**Jeanette Lotter** A novel and scalable biocatalytic process for the production of chiral pharmaceutical intermediates	11:30-11:45	**Schaun Korff** Modification of cAMP levels and PDE4 activity through SSRI and NRI treatment in a putative animal model of OCD

11:45-12:00	**Closing of Congress (Venue: Meyerton)**
12.00-14.00	**Lunch**

POSTER PRESENTATION PROGRAMME

Thursday 21 September -- 10:50-11:30		
Presenting Author	**Poster Title**	**Poster Board No.**
Afolayan, A	Isolation and structure elucidation of halogenated monoterpenes from *Plocamium cornutum*	A 03
Bawa, Y	Triamterene crystals obtained from DMF and DMF/water	A 11
Chadawar, V	Studies on ionotropic gelation based controlled release spherical matrices	A 13
Choonara, Y	An investigation into the physicomechanical and erosion dynamics of a novel implantable polymeric matrix	A 17
de Bruyn, T	Nasal delivery of insulin with pheroid technology	B 01
du Toit, L	Approaches to fabricating anti-TB nanosystems embodying a salted-out and cross-linked architecture	B 04
Joshi, S	The effectiveness of insulin sensitizers in achieving improved glycaemic control in Type 2 Diabetics	B 09
Kolawole, O	Development of a novel gastroretentive drug delivery system for rate-controlled drug delivery	B 15
Murphy, C	Assessment of various approaches for nanoparticle formulation	C 04
Patnala, S	Isolation and semi-synthesis of sceletium alkaloids	C 14
Pillay, S	Synthesis, characterization and preliminary evaluation of hydrophilic polymeric nanoparticles and scaffolds for controlled drug delivery	C 16
Pillay, V	A multi-layered double-disk polymeric device for phase-controlled drug delivery	C 17
Pillay, V	A novel multi-unit gastrofloatable device for delivery of "Narrow Absorption Window" (NAW) bioactives	C 18
Pretorius, J	Metallothionein expression in tissues of rotenone-treated rats	D 02
Steyn, R	Investigation into the prescribing patterns and cost of antidiabetic medicine in South Africa	D 09
van der Watt, H	The applicability of sphericaly agglomerated chitosan formulations	D 16

Thursday 21 September --- 16:00-16:30		
Presenting Author	**Poster Title**	**Poster Board No.**
Au, W	Evaluation of pilot dose duration-response methodology for a topical corticosteroid cream containing clobetasol 17-propionate	A 06
Dairam, A	Non-steroidal anti-inflammatory agents, tolmetin and sulindac attenuate quinolinic acid (QA)-induced oxidative stress in primary hippocampal neurons and reduce QA-induced spatial memory deficits in male Wistar rats	A 18
Davids, H	Antileukemic activity of the resins of the *Commiphora* Sp.	A 19
Gerber, M	Synthesis of selected lamivudine derivatives for transdermal penetration	B 05
Joone, G	An in vitro investigation of the anticancer potential of *Sutherlandia frutescens*	B 08
Katende-Kyenda, N	Prevalence of Drug-Drug Interactions (DDIs) of Antiretroviral Agents (ARVs) in the Private Health Care Sector in South Africa	B 11
Lubbe, M	Estimating the need for the involvement of the community pharmacy sector in the ARV roll-out programme in South Africa	B 16
Marx, C	Solubilisation and kinetics of degradation of nevirapine	B 19
Naidoo, S	Development of a symptom focused HRQOL tool for patients on highly active antiretroviral therapy	C 05
Nell, M	Anti-tumour properties of novel gold compounds	C 06
Ogwal, S	Development and optimization of an analytical method for the analysis of ibuprofen in dosage forms	C 08
Panagiotopolous, H	The cytotoxic effects of novel synthetic biaryls and aromatic esters	C 13
Rasool, Y	Anti-oxidant activity of novel gold compounds	D 03
Scholtz, D	Prescribing patterns of antiretroviral drugs in a section of the private health care sector in South Africa	D 04
Strydom, S	Preparation and thermal stability of polymorphic forms of stavudine	D 10
Tetty-Amlalo, R	Percutaneous diffusion of ketoprofen across human skin by dermal microdialysis	D 12
Truter, I	Prescribing patterns of non-steroidal anti-inflammatory drugs	D 14
van Rensburg, S	Zinc and Vitamins A and D in Ulcerative Colitis	D 18
Zheve, G	Investigation of neuroprotective effects of Nevirapine and Efavirenz	E 04

Friday 22 September -- 11:40-12:00		
Presenting Author	**Poster Title**	**Poster Board No.**
Aucamp, M	Formulation of a veterinary pour-on containing clonsantel sodium	A 07
Chaibva, F	The development of a dissolution test for oxytocin release from in situ gel forming parenteral formulations manufactured with Pluronic®F127	A 15
Chaibva, F	The characterisation of Pluronic®F127 as a potential vehicle for the parenteral delivery of oxytocin	A 14
Dube, A	Design and evaluation of placebo material for Artemisia afra leaves.	B 02
Mandimika, N	Development of a quantitative HPLC method using a minibore column for the analysis of azithromycin	B 17
Mann, M	Detection of halogenated monoterpene aldehydes by chemical derivatisation and mass spectrometry	B 18
Olivier, E	Influence of chemical modification on the efficacy of a sulphadiazine cream	C 09
Otto, D	Power law size scaling of copolymer particles employing GPC-MALLS	C 12
Perumal, V	Development of a silicone moulded compartmentalised tray: An approach to enhance drug uniformity in preparing polymeric films	C 15
Singh, N	The influence of barium and calcium on the hydrational and physicomechanical properties of nicotine-loaded Alginate-hydroxyethylcellulose gelispheres	D 07
Syce, J	The formulation, manufacture and evaluation of capsules containing freeze-dried aqueous extracts of *Leonotis leonorus* or *Mentha longifolia*	D 11
van der Walt, J-S	Bioavailability of an L-carnitine-magnesium fixed dose combination product in healthy male volunteers	D 15
Wa Kasongo, K	Development and validation of an in vitro test method for the assessment of clobetasol 17-propionate release from topical formulations	E 03

Friday 22 September -- 16:00-16:30		
Presenting Author	**Poster Title**	**Poster Board No.**
Assefa, A	Spasmolitic activity of the aqueous extract *Solanum incanum*, Solanaceae	A 05
Baaisi, T	The impact of pharmaceutical care interventions on cardiovascular patients at Dr George Mukhari Hospital	A 08
Blignault, S	Reflexology in the management of tension headaches	A 12
Gous, A	What is it like to live with Epilepsy?	B 06
Harilall, S	Preliminary investigation into the synthesis of polymeric nanoparticles and scaffolds intended for implantation into the brain	B 07
Joubert, J	Fluorescent structures for assay of neuroprotective properties	B 10
Khamanga, S	Influence of different grades of methocel on verapamil release from mini matrix tablets	B 12
Killian, C	Antioxidant properties of *Gymnosporia buxifolia* szyszyl	B 14
Mugabo, P	Effect of Leonotis leonorus aqueous extract on the isolated perfused rat heart	C 02
Ogunrombi, M	Structure - Activity Relationship and Neurotoxicity Studies with the MAO-B Substrate, 1-methyl-3-phenyl-3-pyrroline.	C 07
Oosthuizen, F	Evaluating anticholinergic/antipsychotic prescribing patterns at different levels in the South African healthcare system	C 10
Osuch, E	Elastic properties of the aorta and endothelial function in ACE Inhibitor treatment in hypertensive patients	C 11
Pretorius, A	The role of acetyl-L-carnitine on the long-chain fatty acid metabolism in MPTP treated rats	C 19
Pretorius, C	Antioxidant Properties of *Lippia Javanica* (Burm.f) Spreng	D 01
Shiri, C	Impact of Therapeutic Education on Hypertensive Patients' levels of Adherence to Therapy	D 05
Sibambo, S	In vitro dissolution and optimization of melatonin-loaded PLGA implants for use in neurodegenerative disorders	D 06
Truter, I	Prescribing patterns of anti-migraine drugs in a primary care patient population	D 13
van Heerden, M	Antioxidant properties of *Plumbago auriculata*	D 17

Saturday 23 September -- 10:30-11:00		
Presenting Author	**Poster Title**	**Poster Board No.**
Adsetts, J	Standard of pharmaceutical services provided by institutional pharmacies: public vs. private sector	A 01
Adsetts, J	Standard of pharmaceutical services provided by community pharmacists in South Africa	A 02
Assefa, A	Preliminary *in vivo* investigation of nine anti-malarial plants traditionally used in and around Dollo Mena, Ethiopia.	A 04
Basson, W	The availability of private vs. public pharmaceutical services in different geographical areas in South Africa	A 10
Basson, W	Patient pharmacy distribution systems : RSA experience 2003-2005	A 09
Chen, C-T	*In vitro* antiplasmodial activity of corrinoid derivatives	A 16
du Plooy, W	The Brine Shrimp Test, Really!	B 03
Kilian, G	The biological activity of selected histidine-containing cyclic dipeptides	B 13
Milne, P	Synthesis and evaluation of selected glycine-containing diketopiperazines	C 01
Mukinda, J	Preliminary acute toxicity study of the aqueous extract of *Artemisia afra* in mice	C 03
Srinivas, C	Basline studies to initiate pharmacy and therapeutics committees in twelve primary health care centers in Karnataka, India	D 08
van Vuuren, S	The pharmacological interaction of commercial essential oils in combination with conventional antimicrobials	D 19
Van Zyl, P	The effect of multiple doses of tribulus terrestris on serum lh and testosterone levels in healthy males	E 01
Van Zyl, R	Antimalarial activity of indigenous South African Medicinal plants	E 02

INVITED SPEAKERS

Prof. Christiaan Beyers Brink

Christiaan Beyers Brink (Tiaan) was born on 26 October 1967 in Stellenbosch (Western Cape, South Africa) and currently resides in Potchefstroom (North-West Province, South Africa). He is married to Corné, a clinical psychologist, and they have three children, Anchenique (11), Theowald (8) and Henco (6).

Tiaan matriculated in 1985 at Wolmaransstad Secondary School, obtained a degree in Pharmacy (B. Pharm. - *cum laude*) in 1989, a Master's degree in Pharmacology (M.Sc.) in 1991 and a doctoral degree in molecular Pharmacology (Ph.D.) in 1998 at the former Potchefstroom University for Christian Higher Education (Potchefstroom, North-West Province, South Africa). During 1998/9 he completed postdoctoral studies in signal-transduction pharmacology at the University of Michigan (Ann Arbor, Michigan, U.S.A.) under Prof Dr Richard R Neubig.

Tiaan is currently an Associate Professor in Pharmacology at the Potchefstroom Campus of the North-West University (Potchefstroom, North-West Province, South Africa). He is also the manager of a distance-learning, Web-based, postgraduate degree and CPD programme in pharmacology that receives international recognition and the manager of a central Laboratory for Applied Molecular Biology. He is currently the secretary of the Teaching Section of the International Union of Pharmacology (IUPHAR) and of the South African Pharmacology Society. Tiaan is a registered pharmacist at the South African Pharmacy Board since 1992 and is a member of the Academy of Pharmaceutical Sciences of the Pharmaceutical Society of South Africa. He recently co-steered a successful bid by the South African Pharmacology Society in 2006 to host the IUPHAR World Congress in Basic and Clinical Pharmacology 2014 in Cape Town, South Africa. He also acts as co-manager/facilitator for the 'Pharmacology for Africa Initiative' to organise pharmacology in Africa, involving key role players in pharmacology in Africa and which is supported by the South African Government, the International Union of Pharmacology (IUPHAR) and the International Council for Sciences (ICSU) Regional Office for Africa.

Tiaan's interest and expertise in ***education*** include technology-based learning in health sciences, as well as the general pharmacology of cardiovascular drugs. He received the VERKA Educational Learning Merit Award of the North-West University for extraordinary contributions to tertiary education in 2003.

His interest and expertise in ***research*** include the field of molecular and signal transductional pharmacology, with specific application to the investigation of the biomolecular basis of the action of drugs used for the treatment of anxiety and stress disorders. He was author and co-author of 9 articles in international journals (including *Trends in Pharmacological Sciences* and *Journal of Pharmacology and Experimental Therapeutics*) and contributed 36 presentations (invited, podium and poster) at international and national conferences. Tiaan currently holds Y-rating from the National Research Foundation of South Africa. He received best publication awards (including the Janssen-Cilag Award), supervised and co-supervised 14 postgraduate students and his students received Young Scientist Awards at national conferences. Tiaan serves on the Editorial Boards of *Current Clinical Pharmacology* and *Recent Patent Reviews on CNS Drug Discovery* (Bentham Sciences) since 2005 and of *Health SA Gesondheid* since 2006. He peer reviewed several manuscripts for several international journals, including *Trends in Pharmacological Sciences*, *Molecular Pharmacology*, *Journal of Pharmacology and Experimental Therapeutics*, *Life Sciences* and *Neuropharmacology*.

Tiaan is also a Biographee in *Marquis Who's Who in Medicine and Health Care* (2006-2007 – 6th ed) and in M*arquis Who's Who in the World* (2006 - 23rd ed).

Current Trends in the Use of Technology for the Teaching of Health Sciences in Developed and Developing Countries

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Background:

Technology-based learning revolutionized teaching and learning during the past two decades in several ways, also improving accessibility and quality in many, but not all cases. Distance learning benefited from improved and faster communication, access to electronic databases and information on the Web and virtual/electronic student support. Computer software that enhance and facilitate self-directed, asynchonous learning have some value for the distant learner. In this regard the widening knowledge gap and digital divide between the developing and developed world is of great concern. However, technology-based teaching and learning also impact on traditional teaching styles and current approaches to learning for so-called "contact students". Some emerging trends and current thinking, concepts and terminology related to technology-based learning in Health Sciences will be discussed.

Technology-based learning for Distance Learning:

There are examples of the successful development and implementation of technology-based learning programmes in Health Sciences. These are several ways to enhance the learner support and learning process, also for the distant learner. An example of such an application of technology to enhance learning will be demonstrated and the results from an investigation to determine student perception of the quality of learning in a virtual learning environment will be discussed. New guidelines/criteria for ensuring the quality of *e*-learning in South African higher education has recently been derived from case-studies and may eventually be adopted by the Council for Higher Education of South Africa as regulatory framework and measuring instument.

Africa and Technology-Based Learning:

In the African context technology-based learning, including the utilization of the internet, offers solutions to many of the educational challenges, as expressed also in several reports. There is an urgent need for electronic infrastructure and *e*-learning expertise, while some regions/institutions in Africa have already implemented advanced tecnology-based learning successfully. The results from an investigation into the the utilisation of technology in the developing world will be discussed. Africa is known to take leaps in the utilization of technology and wireless technology in particular has become popular and widely available. Future predictions are that this trend will continue and many fast-growing developments in communication technology are ongoing all over the African continent. Future *e*-learning software for targeting Africa should include 3G-satellite hybrid wireless technology.

Conclusion:

The use of technology for learning has become specialized, but also an essential part of education. The emergence of new technologies creates new opportunities and horizons that will greatly shape learning in the years to come. However, the didactic principles of learning remain the same and the inclusion of technology does not necessarily constitute added value or good learning, so that appropriate application and integration of technology in the total learning package is vital. Principles for the utilization of technology to enhance learning in the African context will be discussed. Teachers need to take up the challenge to utilise technology to optimize learning.

Prof. Ivor Ralph Edwards

Ivor Ralph Edwards is currently the Professor and Director of the World Health Organization (WHO) Collaborating Centre for International Drug Monitoring (the Uppsala Monitoring Centre). He attained this position having served for 5 years as the Chairperson of the Advisory Group, WHO Collaborating Centre for International Drug Monitoring in Sweden (1985-1990).

He originally obtained his MBChB. degree in Birmingham then, in a career spanning 4 continents, he went on to achieve his MRCS. LRCP (London), MRCP. (UK), FRCP (London) and FRACP. Ralph has held clinical posts as a consultant physician in the United Sheffield Hospitals, UK, Groby Road Hospital, Leicester, UK, Parirenyatwa Hospital in Zimbabwe and Dunedin Hospital in New Zealand.

His academic posts include being the Sub-Dean, Sheffield University Medical School (1972-1975); Senior Lecturer in Pharmacology and Therapeutics-University of Leicester (1975-1978); Senior Tutor, Leicester University Medical School (1975-1978); Professor of Medicine, University of Zimbabwe (1978-1981); Director of the National Toxicology Group & Associate Professor in Clinical Pharmacology, Otago University and Medical Research Council (1981-1990). In addition he was a clinical research adviser at Leo Laboratories (1975-1978) and the Leprosy Officer at South Island, Ministry of Health New Zealand (1983-1990).

Ralph's extensive expertise in pharmacology and drug monitoring is expressed in a multitude of publications covering adverse health effects associated with herbal medicines, data mining and adverse drug reactions, signal detection, drug specific adverse drug reactions and communication in the pharmacovigilance arena.

Adverse Drug Reactions

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Drug safety scares are well known from the media and cause crises of confidence in health professionals, the pharmaceutical industry, government, and health care providers.

This presentation looks at the current processes in pharmacovigilance (drug safety) and gives some examples of regulatory decisions regarding drugs. The strengths and weaknesses of the current situation in pharmacovigilance will be discussed and suggestions made for improvements.

A key area of improvement will be better use of individual case safety reports to understand the broader area of patient safety whilst using drugs, and not focussing on the drugs themselves

Prof. Dr. Johann Gasteiger

Prof. Dr. Johann Gasteiger studied chemistry and has had positions at the University of Munich, University of California, Berkeley, USA, and Technical University of Munich. In 1994 he moved to the University of Erlangen-Nuremberg where he co-founded the "Computer-Chemie-Centrum". He is one of the initiators of Chemoinformatics in Germany and has produced more than 250 scientific publications in this field. Prof. Gasteiger has obtained a variety of awards: in 1991 the Gmelin-Beilstein Medal of the German Chemical Society for Achievements in Computer Chemistry, in 1997 the Herman-Skolnik-Award of the Division of Chemical Information of the American Chemical Society, in 2005 the Mike Lynch Award of the Chemical Structure Association Trust, and in 2006 the American Chemical Society Award for Computers in Chemical and Pharmaceutical Research.

He has been consultant to Beilstein Institute, FIZ CHEMIE, MSI, and various pharmaceutical companies. In 1997 he founded Molecular Networks GmbH, a company developing and distributing software for chemical applications.

The Role of Chemoinformatics in Drug Design

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The development of a new drug is an expensive and time-consuming process. Reasons are the complex nature of the relationships between chemical structure and biological activity and the huge amount of data to be processed.

At the interface of chemistry and computer science a new field, chemoinformatics [1], has developed that can assist in the drug design process. Methods have been developed for the discovery of new lead structures, for the optimization of lead structures, and for finding optimum pharmacokinetic profiles (ADME = adsorption, distribution, metabolism, excretion).

In this process, the representation of chemical structures is of critical importance. A hierarchy of descriptors for chemical structures has been developed [2]: From the constitution through 3D structures [3] to molecular surface properties.

The relationships between the structure of a molecule and its biological activity are too complex to be calculated directly by theoretical methods. In such situations, the modeling of the relationships between structure and properties by inductive learning methods such as statistical analyses, pattern recognition methods, or neural networks [4] offers the only amenable solution.

Application of these methods to the separation of molecules with different biological activity, to finding new lead structures, to the definition of the diversity of a library of compounds, and to the analysis of high-throughput screening data will be given.

[1] *Chemoinformatics – A Textbook* , J. Gasteiger, T. Engel (Editors), Wiley-VCH, Weinheim, 2003.

[2] *Physicochemical Effects in the Representation of Molecular Structures for Drug Designing*, J. Gasteiger, *Mini Rev. Med. Chem*., 2003, *3,* 789-796.

[3] http://www2.chemie.uni-erlangen.de/software/corina and http://www.mol-net.de

[4] J. Zupan, J. Gasteiger, *Neural Networks in Chemistry and Drug Design*, VCH, Weinheim, 1999

Mr Francois Hoffmann

Francois Hoffmann qualified as a Medical Microbiologist at the SA Institute of Medical Research and has been in the Pharmaceutical market for over 25 years. He holds Diplomas in marketing from the IMM and completed his Postgraduate Diploma in Business from UCT Business School.

Francois has worked for companies such as Merck (MSD), Johnson and Johnson in Marketing, well as being the MD for Grey Healthcare Communications for 10 years. He is currently the Marketing Executive for the Pharmaceuticals division of Adcock Ingram Healthcare and has predominantly been involved in the marketing of Prescription drugs, as well as the launch of the Adco Generics business unit for Adcock Ingram.

Generics – Quo Vadis?

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What does the future hold – is it one of new groundbreaking technologies and products or is there a significant growth expected in Generics? What will be the added value of new drugs, at what cost? And where will Generics end up? Why is there still a negative perception around generics and what does the future hold in terms of Biotechnology, Bio -Similar, Ever greening, ARV's, Oncology. Therapeutic substitution – is it a reality or a pipe dream? Managed healthcare and Chronic Care – are generics the saving grace in an ever cost increasing pharma market. What about Bio - Equivalence, IP protection, Hatch Waxman Act and TRIPS – where to now?

Prof. Sunny E. Iyuke

Professor Sunny E. Iyuke joined the School of Chemical and Metallurgical Engineering, University of the Witwatersrand at the beginning of 2005, upon his return to the mother continent from a 10-year teaching and research experience in Malaysia. Iyuke's internationally recognised research works are in Nanotechnology and Proton Exchange Membrane (PEM) Fuel Cell Technology, to which he has several years of experience on carbon nanotechnology and nanobiomaterials for drug delivery. He holds a BSc Honours degree in Chemistry, B Eng equivalent and MSc in Chemical Engineering from Ahmadu Bello University, Nigeria. In 1999 he received his PhD also in Chemical Engineering from the National University of Malaysia, where he researched on hydrogen purification for PEM Fuel Cell use. He is a Chartered Engineer with the Engineering Council, United Kingdom, and member of the Institution of Chemical Engineers IChemE, International Mesostructured Material Association, The Association for Environmental Health and Sciences, and American Association for the Advancement of Science.

Professor Iyuke has published more than 36 journal articles, two chapters in books, over 50 conference papers, and boasts a number of outstanding research leadership grants won at University Putra Malaysia and the University of the Witwatersrand. Iyuke has won numerous awards and prizes on R & D in Fuel Cell, Palm Oil research and Carbon Nanotube Technology. Amongst them is the "Rysoil-high performance synthetic lubricants from palm oil" award he jointly won with co-workers from Ideen-Erfindungen, Germany in 2004, and carbon nanotube synthesis. He has made several Invited/Keynote/Plenary Talks in many countries, such as USA, Malaysia, Nigeria and South Africa. Professor Iyuke consecutively won Excellence in Service Awards four times at Universiti Putra Malaysia from 2001-2004.

Nanotechnology in Pharmacy and Pharmacology: Carbon Nanotubes and Polymer based Nanocapsules for Drug Delivery

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Research into new and efficient drug delivery systems is of fundamental importance to improve the pharmacological profiles of many classes of therapeutic molecules, due to their inability to penetrate difficult and remote sites, such as the Brain Blood Barrier (BBB), poor solubility, etc. Ostwald-Freundlich and Noyes-Whitney equations recommend that application of a drug in a reduced particle size is a promising and effective way to improving drug bioavailability of poorly-soluble substances due to the increased saturation solubility and dissolution rate of nano-sized substances. Thus devoid of viral genome sequences, protein-transducing nanoparticles (PTNs) enabled transient and dose-dependent delivery of therapeutic proteins at functional quantities into a variety of mammalian cells in the absence of host chromosomes modifications. It has been reported that PTNs delivering Cassava (*Manihot esculenta*) linamarase into rodent or human tumour cell lines and spheroids mediated hydrolysis of the innocuous natural prodrug linamarin to cyanide and resulted in efficient cell killing. Upon injection of linamarin into nude mice, linamarase-transducing nanoparticles impacted solid tumour development through the bystander effect of cyanide (Link *et al*., 2006).

Biodegradable nanoparticles formulated from biodegradable polymers such as poly(D, L-lactide-co-glycolide), PLGA, poly ethylene glycol (PEG), etc have been extensively used for sustained and targeted delivery of different drugs, including plasmid DNA, proteins and peptides and low molecular weights analogues. Numerous researchers have shown that both tissue and cell distribution profiles of anticancer drugs can be controlled by their entrainment in nanoparticles in order to increase anti-tumour efficacy, while reducing systemic side-effects. Carbon nanotubes (CNTs) have also emerged as a new alternative and efficient tool for transporting and translocating therapeutic molecules. Unlike the popular biodegradable materials, CNTs when functionalised display low toxicity, not immunogenic, and can be excreted via urine and faeces. Such systems hold great potential in the field of nanobiotechnology and nanomedicine. Thus CNTs can be functionalised with bioactive peptides, proteins, nucleic acids and drugs, and used to deliver their content to cells and organs. Discernibly, all of these nanoparticles have been shown to penetrate all organs including the brain.

Prof. Brian E. Leonard

Brian Leonard commenced his university education with a BSc (Hons) degree in Medical Biochemistry and Pharmacology, at the University of Birmingham, UK, in 1959, and went on to complete a PhD in Pharmacology at the same university in 1962. He obtained a DSc in Neuropharmacology for research publications, from the National University of Ireland in 1976.

From 1962-1968 he held the position of Lecturer in Pharmacology at the University of Nottingham, UK, and then moved into the industry, becoming the Technical Officer for ICI Ltd, Pharmaceutical Research Laboratories, UK. In 1972 he transferred to Organon International, Holland, where he was the Head of the Neurochemistry Laboratory.

In 1974 he was invited to occupy the Chair of Pharmacology at the National University of Ireland, Galway, a position which he held with verve and innovation for 25 years. During this time he produced over 400 publications in neuro- and psycho-pharmacology international journals and his novel research findings have made an enormous contribution to current psychopharmacological and psychoneuroimmunological thinking. He is the author of the seminal book "Fundamentals of Psychopharmacology" published by John Wiley and Sons, UK, which is now in its 3rd edition (2004). In 2000 he produced "Fundamentals of Psychoneuroimmunology" (with Cai Song), also published by John Wiley and Sons. He is on the editorial board of 5 international psychopharmacology journals and, until 2000, was the Editor-in-Chief of the Journal of Human Psychopharmacology.

Brian is currently the Emeritus Professor of Pharmacology at the National University of Ireland, a Visiting Professor and Faculty member in the Brain and Behaviour Research Institute at the University of Maastricht, (Netherlands) and an Honorary Professor in the Department of Psychiatry at the University of Hong Kong. In addition he is an honorary member of the British Association of Psychopharmacology, the South African Association of Psychiatrists, and the Egyptian Psychiatric Association, as well as being a member of the Royal Irish Academy and an Associate Member of the Royal College of Psychiatrists, UK.

Brian has excelled in international leadership positions, bringing commitment and energy to the Presidency of the British Association of Psychopharmacology from1986-1988 and the Society for the Investigation of Stress from1998-2000. In July this year, Brian completed his 2 year term of office as the President of the Collegium Internationale Neuropsychopharmacologicum (CINP) and now occupies the similarly demanding role of Past President until 2008. His previous CINP responsibilities include being a Member of Council from 1998-2000, Treasurer from 1992-1996, and Vice President from 2000-2002.

In addition to all his current responsibilities, Brian still finds time for consulting on research as the Deputy Chairman of the Lundbeck Institute for Neuropsychiatric Research.

Depression as a neurodegenerative disease:
The end of the amine hypothesis?

Brian E. Leonard

Pharmacology Department, National University of Ireland, Galway and

Brain and Behaviour Research Institute, University of Maastricht, The Netherlands.

Over the past decade, evidence has emerged that the concentration of pro-inflammatory cytokines are increased in the blood and CSF of patients with major depression. The possible causal link with the symptoms of depression is provided by the observation that, in non-depressed patients being treated with interferon alpha for the treatment of hepatitis, depression is a frequent side effect.

In addition to these pro-inflammatory cytokines, the concentration of prostaglandin E2 is also increased in the blood and CSF.

The hypersecretion of cortisol has long been identified as a feature of major depression. The chronic increase in the activity of the HPA axis is attributable not only to the stress induced activation by corticotrophin releasing factor but also due to the stimulation by interleukin-1. As a consequence of the desensitization of the central glucocorticoid receptors due to the chronic rise in cortisol, the negative feed-back control of cortisol secretion from the adrenals is reduced. Thus in major depression there is an increase in the proinflammatory cytokines together with cortisol; these apparently contradictory changes are probably due to the desensitisation of the glucocorticoid receptors on some immune cells (such as the monocytes and macrophages, the major source of proinflammatory cytokines).

The proinflammatory cytokines, that are also secreted by the astrocytes and microglia in addition to the peripheral macrophages, together with PGE2, are responsible for neuronal damage. The normal repair mechanisms that involve the secretion of neurotrophic factors such as brain derived neurotrophic factor, are inhibited by the high cortisol concentration in the brain. This probably accounts for the neuropathological damage seen in patients with major depression, namely the hippocampal shrinking, cortical atrophy and the predisposition to dementia in later life.

Recent research has provided further support for this hypothesis by the discovery that the concentration of 3 hydroxykynurenine and quinolinic acid are increased in the serum of patients with major depression. These potent neurotoxins are the end products of the kynurenine pathway formed from tryptophan following the induction of indoleamine-dioxygenase by the proinflammatory cytokines.

In conclusion, there is growing evidence to support the view that central inflammatory mechanisms play a vital role in the long-term neurodegenerative changes in depression. These changes are linked to the increase in apoptosis in those brain regions that are responsible for many of the key symptoms of major depression.

New Approaches to the Development of Antidepressants

Brian E. Leonard

Pharmacology Department, National University of Ireland, Galway and Brain and Behaviour Research Institute, University of Maastricht, The Netherlands.

It is generally assumed that all effective antidepressants enhance monoaminergic function in some way. Despite this belief, the mode of action of antidepressants remains an enigma.

The disparity between the acute effects of antidepressants and the delay in their therapeutic action has long been recognised. The observation that the adaptive changes in post synaptic monoaminergic receptors approximately coincides with the onset of their therapeutic action has led to a change in emphasis from the pre-synaptic to the post-synaptic intracellular changes. This has resulted in the molecular hypothesis of antidepressant action that postulates that adverse environmental conditions, acting on genetic vulnerability, cause maladaptive changes in neuronal networks. Effective antidepressant treatment normalises the functioning of these networks, possibly by increasing neurotrophic factor synthesis and enhancing neurogenesis.

This new hypothesis, linking depression to malfunctioning neural networks, has stimulated novel approaches to antidepressant development. For example, the S100 beta peptide is known to be important in neurogenesis. It is linked to the 5HT 1B receptor via the p11 peptide. The p11 peptide has been shown to be reduced in post mortem brains from depressed patients; chronic antidepressant treatments, and ECT, increase the synthesis of this peptide and reverses depressive-like behaviour in rodent models of depression. This could provide a starting point for the development of as new class of antidepressants.

A disruption if the circadian rhythm is a characteristic feature of depression. This has stimulated the development of agomelatin, a melatonin-1 receptor agonist and a 5HT2C antagonist. While the effects on the circadian rhythm may be of importance, it would appear that the reduction in the 5HT2C receptor function may be the main explanation for its antidepressant action.

There are several non-monoaminergic approaches that are receiving attention. Several tachykine receptor antagonists have been developed. These drugs would appear to indirectly enhance serotonergic function. A more novel approach involves antagonists of the NMDA glutamate receptors. These drugs have been shown to have antidepressant activity in experimental models of depression, possibly by blocking the neurodegenerative changes in the hippocampus caused by environmental stress. The novel sigma receptor antagonist, igmesine, that exhibits antidepressant activity, may block these neurodegenerative changes by blocking the action of glycine on the NMDA receptor complex. Other amino acid receptor targets include antagonists of the G-protein coupled GABA –B receptors.

The HPA axis has become an important target for antidepressant action due to the key role that stress plays in the pathology of depression. Some success has been obtained in the development of glucocorticoid type 2 receptor antagonists such a mifepristone. In addition, centrally acting anti-inflammatory drugs such as celecoxib have been shown to enhance antidepressant response in depressed patients who fail to show an optimal response to a conventional antidepressant. As there is now substantial evidence to show that low grade inflammatory changes play an important role in the pathology of depression, it is postulated that centrally acting anti-inflammatory drugs may have antidepressant properties in their own right.

Other more molecular approaches that could lead to novel antidepressant targets include drugs that act on mitogen activated protein kinase (MAP-kinase). This enzyme stimulates the synthesis of brain derived neurotrophic factor, a key neurotrophic factor involved in the repair of damaged neurons.

Whether any of these approaches will result in new and more effective antidepressants only the future will tell!

Prof. Gavin Norton

Gavin Norton completed his MBBCh in 1987, his internship in 1988, and his PhD in 1993 at the University of the Witwatersrand. After a Postdoctoral Fellowship at the University of Massachusetts in 1997 he returned to the University of the Witwatersrand to establish the Cardiovascular Pathophysiology and Genomics Research Unit, a University recognized research unit, in the School of Physiology.

He is currently Director of this research unit and an Associate Professor in charge of Health Science teaching in the same School. Gavin has been awarded the Friedel Schellshop Research Award, the Vice Chancellors Research Award, and the Convocation Distinguished Teachers Award from the University of the Witwatersrand. He has published over 50 original research papers, many of which are in high impact cardiovascular journals, including five in the journal *Circulation*. Over the past 5 years his research papers have been cited over 600 times, 3 editorials have been generated from his publications and he has been cited in textbooks for key discoveries.

His current research interests include the role of genetic factors in the development or severity of hypertension, cardiac hypertrophy and idiopathic dilated cardiomyopathy; the environmental and phenotypic basis of hypertension; the determinants of and importance of cardiac hypertrophy, large artery dysfunction and renal target organ damage; the cellular and molecular mechanisms of ventricular dilatation, systolic dysfunction and diastolic dysfunction in heart failure; and the role of inflammatory changes in cardiovascular disease.

Mechanisms of Adrenergic-Induced Cardiac Dysfunction

Gavin R. Norton

Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, University of the Witwatersrand

Heart failure is associated with chronic sympathetic activation, and the degree of sympathetic activation correlates well with adverse outcomes. Based on these findings, large-scale clinical trials have unequivocally established the use of beta adrenoreceptor blockers in the management of chronic heart failure. These agents are indicated for use in all classes of heart failure, except in decompensated heart failure. However, the negative inotropic effect of beta adrenoreceptor blockers raises concerns about tolerability and safety. Surprisingly, the beneficial effect of this class of agents is in a disease that is fundamentally one of a reduced inotropic ability. For years this conundrum was explained by alterations in beta adrenoreceptor sensitivity, with chronic adrenergic activation resulting in down-regulation of adrenergic receptors, and beta adrenoreceptor blockade reversing this effect.

More recently, it has been proposed that chronic sympathetic activation promotes apoptosis and necrosis and hence that beta adrenoreceptor blockade would prevent progressive myocardial dysfunction. However, recent studies indicate that neither the sensitivity of beta adrenergic receptors to stimulation nor the degree of beta adrenoreceptor-mediated apoptosis and necrosis correspond well with myocardial function in animal models of chronic sympathetic stimulation. In contrast, as will be discussed, recent studies support the notion that chronic adrenergic activation primarily promotes pump dysfunction and heart failure through cardiac dilatation (adverse chamber remodelling). The potential mechanisms and impact of adverse chamber remodelling will be highlighted.

Prof. Viness Pillay

Viness Pillay, Ph.D., is currently an Associate Professor of Pharmacy and Head of Pharmaceutics at the University of the Witwatersrand, Department of Pharmacy and Pharmacology, South Africa. Prior to this Viness Pillay served as an Associate Professor of Pharmaceutics at the College of Pharmacy and Pharmaceutical Sciences, Florida A&M University in Tallahassee, United States (4^{th} largest Pharmacy School in the USA). Prof. Pillay, a Fulbright Scholar, obtained his Ph.D. from Temple University School of Pharmacy (Philadelphia, United States of America) under the advisory capacity of Professor Reza Fassihi, an internationally acclaimed scientist in the field of drug delivery research. The main focus of his doctoral research encompassed novel formulation approaches in the design strategy, development and evaluation of oral rate-modulated polymeric drug delivery systems. He embarked on this research path at the School of Pharmacy and Pharmacology, University of Durban-Westville, South Africa, by pursuing a Master of Pharmacy degree which was awarded *cum laude*.

Prof. Pillay's research findings have been extensively published in peer-reviewed journals and he has been awarded a patent on a monolithic tablet for controlled drug delivery in 2000. This patent has been licensed by an American Pharmaceutical company. Numerous other patents are currently being pursued via Wits Enterprise at the University of the Witwatersrand. Prof. Pillay has made numerous scientific presentations at both local and international conferences in the area of pharmaceutical technology, drug delivery and polymer science. His current research focus is in the field of polymer and materials science, with emphasis on *in situ* chemical/excipient interactions (crosslinking/salting-out) for modifying the physicochemical and physicomechanical characteristics of hydrophilic swellable materials which have potential in the design and development of specialized drug delivery devices for a host of bioactives using Experimental Design tools such as Factorial and Response Surface Designs, as well as Artificial Neural Networks.

Prof. Pillay has served on the Pharmaceutical and Analytical Committee of the Medicines Control Council (MCC) of South Africa. He is currently rated as a scientist with the National Research Foundation of South Africa for his work in Rate-Controlling Polymeric Complexes. He has also been awarded the Chancellor's Research Award at the University of Durban-Westville (South Africa) for his research excellence and most recently in 2005, Prof. Pillay was the recipient of the prestigious Friedel Sellschop Award at the University of the Witwatersrand, an award which recognises exceptional young researchers. Prof. Pillay has been selected by the National Research Foundation of South Africa as a member of their Research Advisory Committee to review grant applications in the Pharmaceutical Sciences, inspect research facilities, and interview potential grant-holders. He has also been appointed by the South African Pharmacy Council as a member of their Specialist Team monitoring the inspection of Pharmacy Schools in South Africa. Currently Prof. Pillay and other academics at the University of the Witwatersrand, and members of the CSIR are in the process of establishing a Biomaterials Centre of Excellence.

Biomaterials Science and Applications: Where to from here?

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Biomaterials are defined as and encompass materials used in medical devices in which contact with the tissues of the patient is an important and guiding feature of their use and performance. They include a range of metals and alloys, glasses and ceramics, natural synthetics, polymers, biomimetics, composites and natural or tissue-derived materials, including combinations of synthetic materials and living tissue components. Employed in many biomedical and pharmaceutical preparations, they play a central role in extracorporeal devices and are essential components of implants. There are many current biomaterials applications, found in about 8000 different kinds of medical devices, 2500 separate diagnostic products, and 40000 diverse pharmaceutical preparations.

Biomaterials, as a multidisciplinary field, contribute greatly to advancements in areas such as regenerative tissue engineering, drug delivery systems, molecular signaling devices and non-clinical applications (water purification and metal extraction). The requisite for new and improved polymer, ceramic and metal systems and methods for characterizing them still exists. Novel synthetic techniques are thus instituted to impart desirable chemical, physical, and biological properties to biomaterials. Advanced computer techniques allow researchers to follow the kinetics of formation of 3-D structures of these biomaterials.

In realising the challenges inherent in addressing present and future drug delivery concerns, we take a look forward at the role of ceramics, polymers and metals as biomaterials; biomaterials in drug, gene and protein delivery; regenerative tissue engineering; and nanotechnological principles applied to biomaterials for the synthesis of complex architectures possessing unprecedented properties. Technologies in the Division of Pharmaceutics in the Department of Pharmacy and Pharmacology, University of the Witwatersrand centre on the dynamic mechanisms of biomaterial modification with emphasis on salting-out and subsequent cross-linking of native biopolymers, and the synthesis of novel metal-polymer and ceramic-polymer composites possessing desirable physicochemical and physicomechanical attributes for diverse applications, having the ability to function as a platform for well-controlled functions from the nano- to the macro-level.

Prof Sergey F. Vasilevskiy

Prof. Sergey F. Vasilevskiy graduated from Irkutsk State University (honors diploma) in 1964. He obtained his PhD in 1971 at Institute of Chemical Kinetics and Combustion, Novosibirsk. Beginning from 1990, he is a full Professor (Novosibirsk State University). He is an Honorary Inventor of USSR. He was awarded, by Medal of International Biographic Center, Cambridge, the "2000 Outstanding Scientists of XX Century". Prof Vasilevskiy is a Member of the United Scientific Council by defense of the theses. He was a Member of Editorial Boards of Siberian Chemical Journal in 1989 – 1994.

At present time, Prof. Vasilevskiy is the Head of the Laboratory of Spin-labeled & Acetylenic Compounds of Institute of Chemical Kinetics and Combustion. Area of his scientific interests follows: The development of the methods of syntheses of aryl- and hetarylacetylenes with application of transition metal catalysis. The investigation of the heterocyclization of vicinal functionally substituted aryl- and hetarylacetylenes and study of biological activity of heterocyclic compounds.

He is author of about 150 papers, 5 reviews and 1 book - L.Brandsma, S.F.Vasilevsky, H.D.Verkruijsse "Application of Transition Metal Catalysts in Organic Synthesis" Springer-Verlag, Heidelberg, Berlin, New- York 1998, 335p.

Research of Biological Active Compounds in Series of Unsaturated Pyrazoles

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In last two decades the pyrazole derivatives attract attention of scientists as very promising class of heterocycles in searching of compounds with new types of therapeutic activities (antihypertensive, antiarrhythmic, anticancer activity etc). From the other hand, more then 3000 acetylenic derivatives are found in nature and possess high and diverse biological activity (antibacterial, fungicidal activity).

In this work are presented developing of methods of synthesis of such molecules, which combine both pharmacophoric groups.

Large systematic series of acetylenylpyrazoles was obtained. Role of nature, place and number of substituents in the pyrazole ring on biological activity is shown.

Results of screening *in vivo* of synthesized unsaturated pyrazoles are given. The structure-activity relationship (SAR) of new classes of pyrazoles with triple bonds is demonstrated. It is shown that pyrazoles bearing unsaturated substituents have less toxicity the correspondent saturated ones. Some pyrazolylacetylenes have remarkable antihypoxic activity.

This work is supported by Grant of Ministry of Education and Science of Russian Federation, "Integration" program grant SB RAS *№32*, The Chemical Service Centre of SB RAS.

References

1. S.F.Vasilevsky, E.V.Tretyakov. J.Elguero *Adv. Het. Chem*. **2002**, Vol.82, 1.
2. P.J.Connolly, S.K.Wetter, K.N.Beers *et al*. *Bioorg. Med. Chem. Lett*. **1999**, vol. 9, 979.

Prof. Alvaro Viljoen

Alvaro Viljoen was born in 1969, Pretoria South Africa. He matriculated with distinction in 1987 from Witbank High School and completed a BSc, BSc Hons. (cum laude) and MSc (cum laude) in Botany at Stellenbosch University. The latter was on the "Essential oil chemistry of indigenous Pelargonium species". In 1991 he was invited to CIRAD-IRAT on Reunion Island for training in essential oil analysis. He commenced with a PhD in 1994 on the chemotaxonomy of the genus Aloe. During this period he was awarded the South African Druggist Prize for the best PhD presentation at the annual post-graduate symposium at the Rand Afrikaans University in both 1994 and 1995 and received the Best Young Scientist Award in 1995 from The South African Association of Botanists. A total of 16 peer reviewed research papers emanated from his PhD study on Aloe, challenging various aspects of the infrageneric taxonomy of this commercially important genus. He completed his doctorate in 1999.

In 1999 he was appointed lecturer in Pharmaceutical Chemistry in the Department of Pharmacy, University of the Witwatersrand where he established a Pharmacognosy research group and supervised 31 postgraduate students in the group. Sixteen post-graduate students have graduated (9 with distinction) under his supervision since 2002. His research interest is the phytochemistry and biological activity of medicinal and aromatic plants. In recognition of his contribution to research excellence, The University of the Witwatersrand awarded him the Friedel Sellschop award as outstanding young scientist in 2000. In 2002 the University of the Witwatersrand promoted him to senior lecturer and in 2005 to Associate Professor.

In July 2005 he was appointed as a research professor in the School of Pharmacy, Tshwane University of Technology. He has authored / co-authored 66 (peer reviewed) papers in both national and international journals, mostly on the phytochemical exploration and biological activity of indigenous medicinal and aromatic plants. A total of 98 papers / posters have been presented at various scientific conferences. Many of these presentations have been credited with awards (e.g Best Ethnobotany Contribution at the Annual Conference of the South African Association of Botanists in 2001, 2003 and 2004).

Pharmacognosy – the pitfalls and challenges of an unique research opportunity in South African

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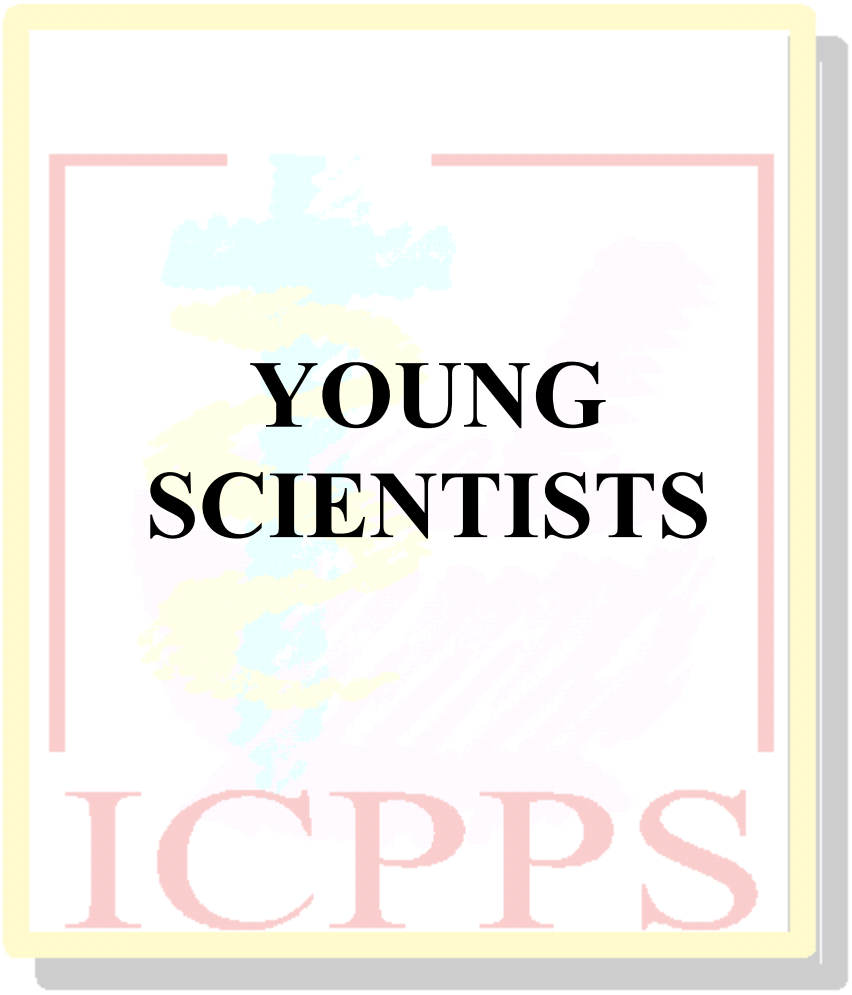
Ethnophamacognosy is a multi-disciplinary science in which local communities, botanists, traditional healers, chemists and pharmacologists interface their expertise to research the ethnobotany, chemistry and biological activity of plant extracts.

Given the immense botanical and cultural diversity in South Africa, it is surprising that natural product research remains a neglected science at Pharmacy Schools in South Africa. Ironically, ethnopharmacological research in South Africa is mostly carried out in Botany Departments which is very different from the international trend where Pharmacognosy forms an integral part of most undergraduate syllabi of Pharmacy students.

Several problems are often encountered when embarking on a project of this nature. Based on examples emanating from post-graduate research projects, the limitations and pitfalls of pharmacognostic studies will be discussed. Sourcing of plant material is often problematic because sampling protocols and plant identification are not properly documented. The new biodiversity bill and intellectual and indigenous property issues complicate research on natural resources. The method of extraction and the choice of assay to study biological activity is often not related to the traditional use of the plant. Furthermore, one has to be cognisant of the immense complexity and variation of plant extracts and the instability of isolated molecules which often obstruct the research process.

However, despite the numerous challenges, natural product research may be extremely rewarding. The WHO estimates that 80% of people in the third world rely exclusively on traditional medicinal plants for their health care needs. It is gratifying to search for the scientific rationale to validate the use of plants in healing rites. It is imperative that our indigenous resources are systematically studied with the ultimate ideal of transforming these latent botanical assets into consumer products to the benefit of South Africa, the local communities and abroad.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The Dissolution Analysis of Sulfadoxine/Pyrimethamine Combination Tablets

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Purpose:

Sulfadoxine/pyrimethamine in a combination product is used in the treatment of malaria and is widely used in Africa.

Many generic products are available on the market and nearly 90% of these products fail the requirements for the USP dissolution test with regard to the pyrimethamine. It is thus suspected that the dissolution medium as prescribed in the USP is not ideally suited for testing the dissolution of the combination product.

The aim of this study is thus to investigate alternative dissolution media for the dissolution studies taking into account the physical chemical properties of both entities. Secondly an alternative method of analysis by means of UV spectroscopy will be investigated in order to facilitate the analysis of these products in countries where high performance liquid chromatography (HPLC) technology is not available.

Methods:

The current USP method was used during method development to establish if the mobile phase was adequate for HPLC analysis. However, it was suggested to adjust the pH in order for the pyrimethamine peak to appear later in doing so preventing sulfadoxine impurities from interfering.

Results:

Preliminary studies indicated that certain media promote dissolution of pyrimethamine without compromising dissolution of sulfadoxine. Five generic products will be tested under the same criteria mentioned to determine whether the outcome correlates with that of the Fansidar® dissolutions.

Melatonin Counteracts the 5-Fluorouracil-Induced Decreases in Brain Neurotransmitter Levels but does not Alter the Hepatic Metabolism of 5-Fluorouracil by Cytochrome P450

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Purpose:

5-Fluorouracil (5-FU) is a potent antineoplastic agent, acts by disrupting DNA synthesis and is associated with numerous toxicities. Various clinical studies have shown that the co-administration of melatonin to cancer patients who are on 5-FU therapy significantly enhances their quality of life. This is due to melatonin reducing the toxicity and enhancing the efficacy of 5-FU, leading to a notable increase in patient survival time. We thus decided to investigate this potentially beneficial drug interaction between 5-FU and melatonin in more detail, by determining the effects of both drugs on liver tryptophan-2,3-dioxygenase (TDO) activity and subsequently on neurotransmitter levels in the brain. High performance liquid chromatography (HPLC) was furthermore used to determine whether an hepatic metabolic drug interaction occurs between 5-FU and melatonin in rat liver microsomes containing cytochrome P450, which metabolises both drugs.

Method:

TDO Activity: TDO is responsible for degrading tryptophan and its activity is thus inversely proportional to cerebral levels of the neurotransmitter serotonin, which is formed from tryptophan. Serotonin, in turn, influences the levels of other neurotransmitters such as norepinephrine and dopamine. The effects of 5-FU and melatonin on TDO activity were determined, and brain levels of serotonin, norepinephrine and dopamine were measured.

Microsomal Study: Microsomes containing cytochrome P450 were isolated, incubated with either 5-FU, melatonin or a combination of these, for 60 minutes and drug concentrations subsequently measured using HPLC. A sensitive and suitable HPLC method that allowed optimal separation and detection of 5-FU and melatonin was developed and validated.

Results and Discussion

Both 5-FU and melatonin were found to inhibit TDO activity. The administration of melatonin led to an increase in brain serotonin levels, while 5-FU resulted in significant decreases in the levels of serotonin, norepinephrine and dopamine. This could be through 5-FU's inhibitory effects on transcription required for the expression of the enzymes necessary for the formation of serotonin. Low levels of these neurotransmitters are known to be associated with depression, suggesting that 5-FU treatment could precipitate this disorder. The co-administration of melatonin was shown to significantly counter 5-FU-induced reduction in brain neurotransmitter levels, thereby possibly alleviating depression.

Neither 5-FU nor melatonin was found to have an inhibitory or inductive effect on cytochrome P450. The co-administration of melatonin was found to not alter the concentration of 5-FU metabolised per µg protein per minute. This absence of a metabolic drug interaction supports the recommendation for combination therapy, as the administration of melatonin will not alter plasma concentrations of the antineoplastic and is thus unlikely to cause inefficacy or toxicity of the latter.

Muscarinic cholinergic inhibition unmasks antidepressant-like properties of sildenafil: behavioural and neuroreceptor evidence

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Background:

Sildenafil, a selective phosphodiesterase type 5 (PDE5) inhibitor, is registered for the treatment of male erectile dysfunction. PDE5 catalyses cGMP to inactive cGMP and is found in the endothelium of blood vessels in the penile corpus cavernosum, but also brain and other peripheral tissue. Sildenafil crosses the blood-brain barrier, leading to side-effects such as headache and dizziness, as well as behavioural manifestations including depression, anxiety and aggression. Recent *in vitro* studies in our laboratory suggest that sildenafil may potentiate cholinergic muscarinic receptor signalling, while in *vivo* studies in rats indicate that sildenafil has anxiogenic and stressogenic actions. The latter, plus its cholinergic actions, suggest that sildenafil may be depressogenic.

Objectives:

The current study investigated the behavioural and neuroreceptor properties of sildenafil in a rat model of depression. We also investigated a hypothesis that sildenafil displays antidepressant-like properties, but which are masked by its potentiation of the cholinergic system**.**

Methods:

In a first phase, Sprague-Dawley rats were treated for 3, 7 or 11 days with vehicle (control) or 20 mg/kg fluoxetine to establish the time-dependency of the onset of antidepressant-like effects in a rat model of depression. We measured decreased immobility in the rat forced swim test (FST), as well as changes in β-adrenergic receptor (β-AR) concentration in rat frontal cortex. In a second phase, rats were treated for 7 days with vehicle (control), 20 mg/kg fluoxetine, 10 mg/kg sildenafil, 1 mg/kg atropine or various combinations of these drugs.

Results:

Effects associated with antidepressant actions (↓ immobility in FST and decreased β-AR concentration) were observed in rats after 7 and 11 days of treatment with fluoxetine, but not after 3 days. After 7 days of treatment atropine and sildenafil alone did not exert any significant antidepressant-like behavioural effects or changes in β-AR concentration. However, a combination of atropine and sildenafil exerted a significant antidepressant-like behavioural effect, comparable with fluoxetine. Moreover, a triple combination of fluoxetine, sildenafil and atropine was superior to fluoxetine alone.

Conclusion:

Muscarinic cholinergic mechanisms mask an antidepressant-like effect of sildenafil in a rat model of depression. It remains to be established whether this effect is linked to PDE5 inhibition or another property of the drug. The data also suggest that muscarinic cholinergic-cGMP interactions may play an important role in antidepressant action. The superior efficacy of the triple combination of fluoxetine, sildenafil and atropine warrants further investigation.

Investigating the Potential Neuroprotective Effects of Statins on DNA Damage in Mice Striatum

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Introduction:

Parkinson's disease (PD) is caused by the idiopathic loss of dopaminergic neurons in the substantia nigra pars compacta and striatum. Oxidative damage to the DNA and decrease in mitochondrial complex I activity are currently under investigation as possible causes of this neuronal degeneration.

The widening role of the statin drugs, used in the treatment of dyslipidaemias, has been the subject of recent studies and they have as such been shown to reduce LDL oxidation, preserve endogenous superoxide dismutase, increase α-tocopherol (a proposed antioxidant), reduce lipoprotein oxidation in a number of oxidative systems and protect against DNA damage caused by antineoplastic agents.

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), a neurotoxin which causes selective neuronal death in the striatum through the inhibition of mitochondrial complex I, was used to replicate Parkinson's disease.

The DNA damaging effects of MPTP were measured and the potential effect of selected statin drugs (pravastatin, simvastatin and atorvastatin) on the DNA damage and repair processes were investigated.

Methods:

Groups of ten mice were treated with 70 mg/kg of pravastatin, simvastatin, atorvastatin or no drug (control group) for five consecutive days. Five mice from each group received an "immediate onset PD model for rapid degeneration with necrotic cell death" dose of MPTP (50 mg/kg) intraperitonially. After decapitation the striatum was isolated and analysed.

The immediate state of DNA damage in the tissue (baseline damage) was determined using the microgel electrophoresis (comet) assay. Further DNA damage was induced by treating the sample with H_2O_2 for thirty minutes after which the process was stopped and the DNA damage determined. Two more comet assays were performed at twenty minute intervals to determine the amount of repair that took place.

Results:

MPTP increased the baseline level of DNA damage and also increased the damage induced by H_2O_2 and subsequent repair that took place when compared to the control group.

The baseline level of DNA damage in mice receiving pravastatin treatment were comparable to that of the control, but when treated with MPTP the baseline levels increased significantly. There was also a marked increase in H_2O_2 sensitivity and subsequent repair, but only in mice not receiving MPTP.

Simvastatin treatment also greatly increased the baseline level of DNA damage but increased H_2O_2 induced damage and repair to a lesser extent in mice treated with MPTP and untreated mice.

The baseline DNA damage, H_2O_2 induced damage and repair of atorvastatin treated mice was comparable to that of the control groups in mice treated with MPTP and untreated mice.

Acknowledgements:

Our sincere gratitude to the North-West University for their support and the National Research Foundation (NRF) for their financial contribution in funding this study.

Polycyclic indole derivatives as novel structures for neuroprotection

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Background:

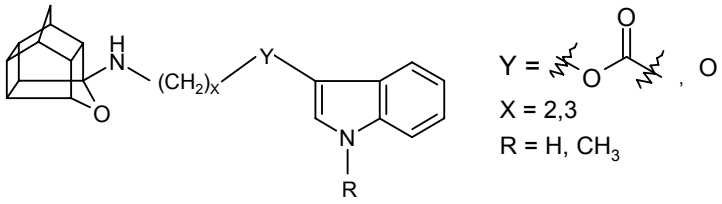
Several studies suggested that indazole and melatonin derivatives have activity on neurodegenerative cascades. Neuronal nitric oxide synthase (nNOS) is a key enzyme in these processes. The indazoles, specifically 7-nitroindazole, has been shown to be a selective nNOS inhibitor and has potent inhibition activity. Another small molecule, 3-bromo-7-nitroindazole, has profound neuroprotective activity in mouse models of stroke and Parkinson's disease. Melatonin, an antioxidant, has been demonstrated to be neuroprotective due to the fact that it is a direct free radical scavenger. Besides its ability to directly neutralize a number of free radicals, it stimulates several antioxidative enzymes which increase its efficiency as a neuroprotectant.

Objective:

In this study novel polycyclic indole derivatives were synthesised by combining the indole moiety with the known protective pentacyclo[5.4.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecane.

Method:

A method, where the known polycyclic cage compound was conjugated to the indole structure via linkers, was followed. The figure summarises the compounds that were synthesized:



The target compounds were evaluated by testing them for NOS inhibitory activity and by comparing it with the activity of aminoguanidines (known selective nNOS inhibitors).

The selected compounds were tested as MAO-B inhibitors and scavengers of reactive oxygen species (ROS).

Results:

NOS assay: The selected compounds had IC_{50} values that compared to that of aminoguanidines.

MAO-B assay: The compounds tested exhibited significant MOA-B inhibition.

Anti-oxidant assay: Substantial anti-oxidant values were obtained using the nitroblue tetrazolium assay. Most of the compounds tested were found to be scavengers of ROS. The potencies compare with that of the known anti-oxidant, Trolox.

According to these results, these compounds can have important applications in neuroprotective studies.

Acknowledgements:

NRF for financial support

Standardization and preparation of *Artemisia afra* herb in tea bag dosage form for use in clinical trials.

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Introduction:

Artemisia afra is a popular traditional herbal medicine in South Africa commonly administered as an infusion of the leaves. However, quality dosage forms with release profiles comparable to the traditional dosage forms are required in order to enable clinical trials to be conducted on the herb. For this purpose, a tea bag dosage form containing standardized dried leaves or freeze-dried aqueous extract of the leaves was prepared.

Methods:

Three plant batches were collected from the Western Cape province, the leaves removed, dried in an oven at 30˚C for 3 days and then blended to produce the standardized dried leaves. To produce the freeze-dried aqueous extract powder, the standardized dried leaves were infused in distilled water (at 80˚C for 10 minutes), filtered and then freeze-dried. Levels of the flavonoid luteolin as marker compound in the plant materials were determined using HPLC. 4 g or 0.55g of standardized dried leaves or freeze-dried aqueous extract powder, respectively, were dispensed into 36cm^2 tea bags made from Dynapore 117/7/0 tea bag paper and sealed using a hot iron. The stability of the plant materials in the tea bags was evaluated under conditions of room temperature and humidity and 40˚C/ 75% RH. The infusion profiles of luteolin from the loose leaves and the tea bag preparations were determined using the BP dissolution apparatus I (basket) at 100rpm and a modification of the BP dissolution apparatus II (paddle incorporating a holding cell for the tea bag) at 50rpm, respectively.

Results:

The standardized dried leaves and freeze-dried aqueous extract powder had an average total luteolin content of 2.065 ± 0.2347 and 13.870 ± 1.2460 µg/mg, respectively and a reduced intra-batch variation in luteolin (%R.S.D) of 11.36 and 6.70%, respectively, from an initial %R.S.D of 21.24, 30.00 and 16.77%, for each of the 3 collected plant batches. Tea bags containing the standardized dried leaves were stable under conditions of room temperature and humidity, while tea bags containing the freeze-dried aqueous extract were not. The rate of infusion of luteolin from the tea bag preparations was 3 times slower than that from the loose leaves and in addition, the f_1 and f_2 values for the infusion profiles were 73.52% and 13.85%, respectively, indicating that the infusion profiles for the tea bag and loose leaves preparations were not similar.

Discussion and Conclusion:

The results suggested that standardization could reduce the inherent variability in phytochemical constituents of the herbal materials with preparation of a freeze-dried aqueous extract reducing it further. Also, the tea bag dosage form is a suitable dosage form for the *A. afra* standardized dried leaves, but not for the freeze-dried aqueous extract. However, the tea bag preparation does not have similar luteolin infusion profiles to the loose leaves and therefore the tea bags may not be suitable for use in clinical trials.

Cortical/hippocampal M1 receptor changes and acoustic startle response in an animal model of posttraumatic stress disorder

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Purpose:

Posttraumatic stress disorder (PTSD) is an anxiety disorder that may follow exposure to severe emotional trauma, and presents with various symptoms of anxiety and hyperarousal. Interestingly, only 10-30% of an exposed population will go on to develop PTSD. Cholinergic neurotransmission is implicated in anxiety as well as other typical manifestations of PTSD, particularly cognitive changes. The frontal cortex and hippocampus regulate and in turn are affected by stress, and have also been implicated in the underlying neuropathology of PTSD. These areas are densely innervated by cholinergic neurons originating from the basal forebrain. The purpose of this study was to evaluate startle response behavior following repeated trauma and whether this demonstrated any correlation with cholinergic receptor changes in the above-mentioned brain areas.

Methods:

2 groups of Male Sprague-Dawley rats were exposed to a time dependent sensitization (TDS) stress paradigm with behavioral/neuroreceptor assessments performed on day 7 post re-stress (duration of experiment in whole is 14 days). Acoustic startle (AS) reflex was used to determine emotional state (hyperarousal) and the development of habituation. Muscarinic receptor binding studies were performed in frontal cortex and hippocampus. Moreover, as has been described in PTSD, we attempted to separate stress sensitive and stress-resilient animals based on predetermined AS response criteria.

Results:

Based on our inclusion criteria 37.5% of exposed animals showed a significant increase in startle response, indicating a maladaptive response to TDS stress, compared to the 16.67% of the control group. After startle habituation analysis, 42.5% of TDS subjects showed no habituation compared to 25% of the control group, although not statistically significant. Muscarinic receptor densities (Bmax) in stressed animals were significantly increased in both the hippocampus ($p=0.019$) and frontal cortex ($p=0.0011$) when compared to controls, with no changes in K_D values observed in either one of the areas.

Conclusion:

Although assays to determine acetylcholine levels in the relevant brain areas were not performed to support the muscarinic receptor studies, it is possible to speculate that an increased muscarinic receptor density reveals an underlying diminished acetylcholine concentration. Ultimately, these data support an association between altered cholinergic receptor and hyperarousal/anxiety in an animal model of PTSD. The data also support the phenomenon of individual susceptibility to stress in animals that parallels that observed in the humans exposed to trauma.

The Synthesis of Stavudine Derivatives for Transdermal Delivery

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Purpose:

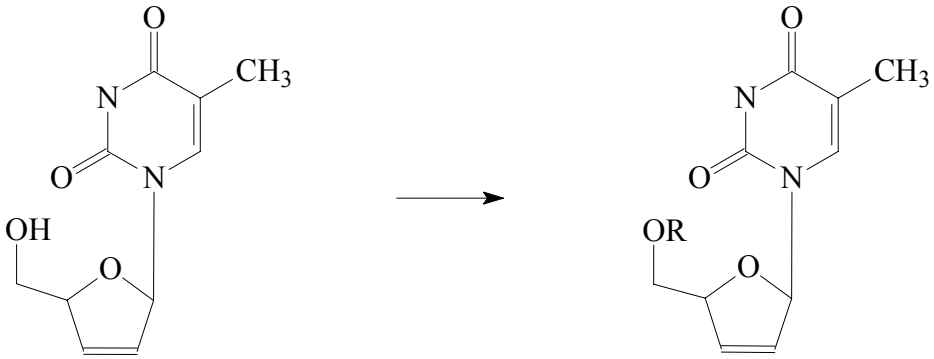
Stavudine **(1)** a synthetic antiretroviral agent is an inhibitor of the nucleoside reverse transcriptase and is used against the human immunodeficiency virus HIV-1 and HIV-2.

(1) is phosphorylated by cellular kinases to the active metabolite stavudine triphosphate which inhibits cellular DNA polymerase β and γ and markedly reduces the synthesis of mitochondrial DNA. Trandermal drug delivery has a lot of advantages over more traditional dosage forms such as fewer side effects, more consistent serum drug levels etc.

In this study a series of new derivatives of the anti-HIV drug **(1)** were synthesised with the aim to determine the transdermal penetration.

Methods:

The compounds were synthesised using standard organic chemical procedures to make ester derivatives. The products were then purified by re-crystallization. The structures were confirmed by using physical methods such as nuclear magnetic resonance spectroscopy (NMR) and Mass spectroscopy (MS). The transdermal flux through human skin will be measured using high performance liquid chromatography (HPLC).



(1) **Esters** R = $COCH_3$ **(2)**
= $COCH_2CH_3$ **(3)**
= $CO(CH_2)_2CH_3$ **(4)**
= $CO(CH_2)_4CH_3$ **(5)**
= $CO(CH_2)_6CH_3$ **(6)**
= $CO(CH_2)_8CH_3$ **(7)**

The confirmation of the structures of the synthesised products by NMR and the Log D values will be presented as well as the transdermal flux values.

Transport of Rooibos (*Aspalathin linearis*) across Percutaneous and Caco-2 cell monolayers

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Purpose:

Flavonoids are a group of polyphenolic compounds widely distributed in fruits, vegetables, and beverages. Epidemiological and *in vitro* studies reveal intake of flavonoids is strongly associated with prevention of coronary heart disease and stroke, and a variety of cancer cell development. Most of the beneficial effects of flavonoids attribute to their antioxidant abilities by scavenge of reactive oxygen species. Aspalathin is the major flavonoid component of the internationally popular health beverage Rooibos tea (*Aspalathus linearis*) tea. Commercially, the use of rooibos no longer merely focuses on the preparation of herbal tea but it is included in skin care products for its free radical scavenging properties in the prevention of "photooxidative stress". The purpose of this study is to investigate the percutaneous absorption. And bioavailability of aspalathin via *in vitro* intestinal epithelial (Caco-2) cell monolayer.

Methods:

Transport across Caco-2 cell monolayers: Green (unfermented) rooibos extracts (GRE) and aspalathin pure compound were used in the transport studies. A 5.0 mg/ml GRE and 1 mg/ml aspalathin were prepared in phosphate buffer saline (pH 7.4) and administered to the apical compartment of the cell monolayers. The apical to basolateral absorption of aspalathin was determined by sampling from the basolateral compartment and the apparent permeability coefficient (P_{app}) was calculated.

Percutaneous transport of aspalathin: Vertical Franz diffusion cells and human female abdominal skin were employed in this study. A 5.0 mg/ml GRE and 1.0 mg/ml aspalathin pure compound were prepared in phosphate buffer system (pH 5.5) and introduced to the donor compartment of the cell setup. Samples from the stratum corneum, the viable epidermis and dermis, and the receptor fluid phase were taken after 24 hours.

Results:

Aspalathin was found mostly distributed into the stratified layers of skin than in the receptor fluid phase. Greater amount of aspalathin was transported across the Caco-2 cell monolayer than across the skin. Better absorption was observed with the GRE than with aspalathin solution alone with P_{app} of $3.49 \pm 1.45 \times 10^{-6}$ cm/s and $2.48 \pm 0.03 \times 10^{-6}$ cm/s, respectively.

The antioxidant, N-acetyl cysteine (NAC), increases vacuous chewing movements in rats: Evidence for striatal oxidative stress

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Background:

N-acetylcysteine (NAC) bolsters glutathione (GSH) synthesis and is an effective antioxidant. The striatum, the brain region associated with regulation and control of movement, is highly susceptible to oxidative stress primarily because of the oxidative metabolism of dopamine in striatal neurons. Increased striatal oxidative stress has been implicated in neuroleptic induced tardive dyskinesia (TD), with antioxidants purported to have possible therapeutic value. The purpose of this study was to evaluate the behavioural and neurochemical effects of chronic NAC administration at various dosages under non-pathologic conditions. Oro-facial-buccal movements were determined as a behavioural measure of striatal toxicity. Brains were removed and striatal tissue used for assay of cellular levels of superoxide and lipid peroxidation.

Methods:

4 groups of male Sprague-Dawley rats (24/group) received either vehicle (water), or NAC 10mg/day, 100mg/day or 300mg/day administered orally for 21 days. Total cumulative vacuous chewing movements (VCM's) were determined, with behavior assessed on days 7, 14, 17, 19 and 21. On day 21 after the final behavioral session, animals were sacrificed by decapitation, and striatal tissue dissected out and fixed in liquid N_2 for assay of superoxide and lipid peroxidation.

Results:

Chronic administration of 300mg/day NAC, but not 10mg/day or 100mg/day NAC, significantly increased VCM's compared to control. Striatal levels of superoxide were significantly elevated in the former group, but not in those receiving 10mg/day or 100mg/day. Interestingly, levels of lipid peroxidation were unchanged in those groups receiving NAC 10mg/day or 100mg/day, but significantly reduced in animals receiving NAC 300mg/day.

Conclusion:

High dose NAC precipitates increased VCM's in rats with evidence suggesting a mechanism involving increased superoxide production. Paradoxical reduction in striatal lipid peroxidation suggests a complex protective mechanism evoked by the presence of reactive oxygen species. Under non-pathologic conditions, such as prevention of TD development, these pro-oxidant properties of NAC may in fact negatively affect striatal function. Studies in neuroleptic-induced TD are warranted.

The role of polyunsaturated fatty acids in refractory epilepsy

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Background and purpose:

Approximately 40% of all individuals suffering from epilepsy have medically refractory seizures. Medically refractory seizures are defined as those seizures that are not completely controlled by medical therapy. To date, no treatment except for a ketogenic diet (KD) has been effective against refractory epilepsy. The ketogenic diet has been used to treat epilepsy since the 1920s, but even today little is understood about the actual physiological and biochemical mechanisms behind its dramatic results. The aim of the study involved the investigation of the role of fatty acids in refractory epilepsy. We have reason to believe that this resistance in drug therapy may be due to a defect in the polyunsaturated fatty acid pathway. Identifying the defective enzyme will help us not only to understand why these patients do not respond to therapy, but also to optimize the clinical use of the ketogenic diet and to develop novel antiepileptic treatments. This study was a pilot study posing the following research questions:

1. Are there any differences in the blood concentrations and concentration ratios of the fatty acids in patients with drug resistant epilepsy versus patients with a normal drug response epilepsy and versus normal individuals?
2. Are there any differences in the urinary organic acid concentrations in patients with drug resistant epilepsy versus patients with a normal drug response epilepsy and versus normal individuals?

Methods:

For the determination of urinary organic acids and fatty acids, a standardised method employing gas chromatography-mass spectrometry (GC/MS) was used. Fatty acid concentrations were determined and expressed in consequential ratios because these fatty acid ratios could give an indication of enzyme defects in the fatty acid biosynthesis pathway.

Results:

We report that patients with drug resistant epilepsy and drug responsive epilepsy had significantly lower portions of plasma stearidonic, arachidonic and docosapentaenoic acid versus control subjects. Using an indirect method we could also speculate that the difference in total fatty acid concentrations was due to the epileptogenic status of the patients and not their medication.

Formulation of a Non-Alcoholic Mouthwash for the Treatment of Halitosis

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Introduction:

Halitosis, oral malodour or bad breath is a common problem. There are numerous causes of halitosis including, renal failure, liver cirrhosis, diabetes mellitus, cancer, and disorders of the upper respiratory tract. Research has shown that most cases of halitosis originate from the dorsal part of the tongue and involves Gram negative sulphur producing anaerobes. Contemporary dental practices emphasize routine control of these microorganisms and their natural accumulations, occurring as dental plague for maintenance of oral health.

Traditionally mouthwashes contain alcohol as a solvent at about 10 to 30% (v/v). At these concentrations alcohol contributes to xerostomia (dry mouth) which exacerbates halitosis. Thus, the aim of this project was to develop a non-alcoholic mouthwash with triclosan as the active ingredient.

Methods:

Triclosan was solubilised in water. The active solution was added to a prepared mouthwash base and the pH of the final product was adjusted to 6.5. The final product was packed in two different plastic bottles for stability studies. The mouthwash was compared to commercially available mouthwashes against anaerobic microbes known to contribute to halitosis.

Results:

Preliminary results indicated that a stable non-alcoholic mouthwash formulation was obtained after six months of stability testing in both types of plastic container. The formulated mouthwash exhibited larger zones of inhibition against the tested organisms when compared to commercially available mouthwashes.

The α_2-lytic properties of mirtazapine is not important for a putative earlier onset of antidepressant action

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Background:

Mirtazapine is an atypical antidepressant with α2-adrenergic receptor (α2-AR) antagonist properties, while it also antagonizes serotonin 5-HT2/3 receptors. Recent clinical data suggest that mirtazapine may have an earlier onset of action than conventional antidepressants. This has been suggested to be related to its α2-lytic properties. Recent results from our laboratory showed that mirtazapine is either a neutral antagonist or an inverse agonist at α2-ARs (Khoza, 2005).

Objectives:

The current study investigated the importance of the α2-lytic properties of mirtazapine for its putative earlier onset of antidepressant action. The study also explored whether the mode of antagonism (i.e. inverse agonism versus neutral antagonism) is of importance in this regard.

Methods:

In a first phase, Sprague-Dawley rats were treated for 3, 7 or 11 days with vehicle (control) or 20 mg/kg fluoxetine to establish the time-dependency of the onset of antidepressant-like effects in a rat model of depression. We measured decreased immobility in the rat forced swim test (FST), as well as changes in β-AR and 5-HT1A-receptor densities in rat frontal cortex and hippocampus, respectively. In a second phase, rats were treated for 3 or 7 days with vehicle (control), 20 mg/kg fluoxetine, 15 mg/kg mirtazapine, 3 mg/kg idazoxan (α2-AR neutral antagonist), 3 mg/kg yohimbine (α2-AR inverse agonist) or a combination of fluoxetine with the other drugs.

Results:

Rats showed antidepressant-like effects after 7 and 11 days of treatment with fluoxetine but not after 3 days. Only mirtazapine (alone and in combination with fluoxetine) exerted behavioral antidepressant-like effects after 3 days of treatment, while all other treatments produced such effects only after 7 days (except idazoxan, exerting no effect). In general the data from β-AR and 5-HT1A receptor binding studies supported the behavioral data, but with some discrepancies.

Conclusion:

It can be concluded that in this model mirtazapine indeed shows a faster onset of action compared to fluoxetine. However, α2-AR antagonism is not important/essential for early onset of antidepressant action, since yohimbine and idazoxan failed to hasten the onset of antidepressant action of fluoxetine. Other pharmacological properties of mirtazapine therefore seem to be of greater importance for its rapid onset of antidepressant action.

Reference

Khoza, K. 2004. The characterisation of the a2A-adrenoceptor antagonism by mirtazapine and its modifying effects on receptor signalling. Potchefstroom. (Dissertation (M.Sc.) – North-West University - PUK).

Pulmonary effects of traditionally prepared *Artemisia afra* steam inhalation, nebulized aqueous extract and the possible involvement of flavonoid luteolin

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Introduction*:*

Artemisia afra is traditionally advocated for the treatment of respiratory conditions such as asthma, bronchitis, e.t.c. In such use the plant leaves are generally boiled in water and the resultant steam inhaled while the head and the bowl are covered with a towel, continuing until the mixture had cooled down. The effectiveness of this steam inhalation has however not yet been evaluated particularly w.r.t how the lung responds to this inhaled steam. Therefore the aim of this study was to evaluate the effect of the inhaled *A. afra* steam on lung function and the isolated perfused rat lung model (IPL) that allowed both the administration of the traditionally prepared *A. afra* steam and simultaneous monitoring of lung function parameters was used.

Methods:

Lungs from anesthetised adult Wistar rats was mounted in an artificial glass thorax, perfused with albumin-containing Krebs-Henseleit buffer and ventilated under negative pressure solution while tidal volume (TV), lung compliance (CL) and resistance (RL) and arterial pressure (PAP) were monitored. After a 20 min equilibrium period (baseline readings), the perfused lungs inhaled steam from saline (control) or *A afra* decoctions (10 and 50mg/ml) or nebulised A afra or luteolin solutions for 3 mins where after lung function parameters monitored for further 30 mins. The pre- and post-inhalation parameter values were compared to determine the effect of the inhaled steam.

Results:

The isolated lungs remained stable during the equilibration period; afterwards inhaled saline (i.e. steamed and nebulized) had negligible effect (i.e. very low insignificant percentage changes) on lung function indicating that saline had essential no pulmonary effects. The *A. afra* steam inhalations however produced significant and dose dependent improvements in lung function (e.g. 7.59% increase in TV, ($p < 0.001$ & reversal of negative trend); 11.76% increase in CL, ($p<001$); 10.98% decrease in resistance, ($p<0.05$) for the 50mg/ml *A afra* steam inhalation. The effects lasted longer with the bigger doses of the plant and nebulized *A. afra* solution (100mg/ml) produced bigger effects on TV, CL and RL than nebulizeed luteolin solution (250ug/ml).

Conclusion:

A afra steam inhalation produced significant dose dependent improvements in lung function of the IPL, that supports its traditional use in respiratory disorders.

Mechanisms by which acyclovir reduces the oxidative neurotoxicity and biosynthesis of quinolinic acid

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Purpose:

The concentration of the endogenous neurotoxin quinolinic acid (QA) is increased in the central nervous system of mice with herpes simplex encephalitis (HSE). These levels were found to be co-incident with neurological signs and were high enough to induce neuronal damage. It has been shown that the antiherpetic agent acyclovir (AC) has the ability to reduce QA-induced neuronal damage in rat brain, by attenuating lipid peroxidation. The mechanism by which QA induces lipid peroxidation includes the enhancement of the iron (II) mediated Fenton reaction and the generation of free radicals, such as the superoxide anion. Thus, the present study aims to determine whether AC has the ability to reduce iron (II)-induced lipid peroxidation and QA-induced superoxide anion generation, and to bind free iron. Superoxide anion and iron (II) are also cofactors of the enzymes, indoleamine-2,3-dioxygenase (IDO) and 3-hydroxyanthranilic acid oxygenase (3-HAO) respectively. These enzymes catalyse steps in the biosynthesis of QA; thus, the effect of AC on their activity was also investigated.

Methods:

Male Wistar rats were used for all the biological assays. The thiobarbituric acid and nitroblue tetrazolium assays were used to measure the extent of lipid peroxidation and superoxide anion generation, respectively, on rat brain homogenate. The activities of the enzymes were determined by measuring the formation of the products after incubation of homogenate with the substrates. The ability of AC to bind iron (II) and iron (III) was investigated using the ferrozine assay and adsorptive stripping voltammetry respectively.

Results:

AC significantly attenuates iron (II)-induced lipid peroxidation and QA-induced superoxide anion generation, and strongly binds iron (II) and iron (III). It also reduces the activity of both IDO and 3-HAO, which could be attributed to the superoxide anion scavenging and iron binding properties, respectively, of this drug.

Conclusion:

The findings of this study strengthen the argument that AC is neuroprotective, which may contribute to the effectiveness of this antiviral drug in the treatment of HSE.

The Introduction of Process Analytical Technology, using Near Infrared Analysis, to a Pharmaceutical Blending Process

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Purpose:

Process analytical technologies are systems for the analysis and control of manufacturing processes to assure acceptable end-product quality. This is achieved by timely measurements of critical parameters and performance attributes of raw and in-process materials and processes. The blending of solids is a critical step in the production of many pharmaceutical products. Poor mixing or over mixing, after the addition of lubricants to granule, could result in poor compression and or dissolution profiles. There is therefore a need to move from the conventional time-determined blending end-points to process-determined blending end-points. The aim of the study was to assess if blend uniformity of a lubricant in a granule matrix could be predicted using near infrared (NIR) technology.

Methods:

The SP15 NIR Laboratory Blender fitted with a near infrared (NIR) probe was utilized for the study. A software method was developed to monitor the standard deviation (SD) of the absorbance at the wavelengths that were specific for Ridaq® granule and magnesium stearate. Each blend was carried out by loading 3 kg of Ridaq® granule into the blender, rotating for 8 rotations and then adding 22.18 g of magnesium stearate and blending for the required time period. To validate the prediction of end-point using NIR, results were correlated with standard sampling methods and tested using atomic absorption at times corresponding to three states of the blend namely before end-point (1 minute and 30 seconds), at end-point (6 minutes) and after end-point (17 minutes), determined by preliminary investigations. Blends were conducted in triplicate at each time interval. An additional 6 blends were run and sampled when the SD had reached a value below 0.000003 at the magnesium stearate wavelength at four consecutive data points (SD value extrapolated from blends carried out to predetermined time intervals).

Results:

Blends sampled at the predetermined time intervals demonstrated a homogeneous state when the SD of the absorbance was low and a non-homogeneous state when the SD of the absorbance was high, thus the NIR prediction on the state of the blend was validated by standard analytical methods. The series of blends sampled when the SD was below 0.000003 were homogeneous with the exception of one blend that was marginally out of specification. Blend durations were significantly lower than the standard blend durations currently used in the facility and ranged from 112 to 198 seconds. With a process driven end-point approach, it becomes possible to have blend durations that are specific for each blend, without the possibility of de-mixing or denaturing of the granule. This approach will benefit the pharmaceutical industry as there will be significant time saving and will also improve the quality of the final product.

The Antioxidative Properties of 4-Hydroxyquinolines

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Purpose:

Reactive oxygen species can attack a series of essential biological molecules within the human body. This attack is associated with various pathological and neurological disorders such as Parkinson's and Alzheimer's diseases. It is thus of great importance to identify compounds with antioxidative activity, which can counteract this attack, preventing such diseases. In this study a series of 4-hydroxyquinoline derivatives were selected, synthesized and assayed in order to determine the antioxidative properties thereof.

Methods:

4-Hydroxyquinolines with a nitro-, amino- or dibuthylamino-group in the 6- or 7-position, respectively, were synthesized according to the Gould-Jacobs reaction and characterized by MS, IR, NMR. The antioxidative properties of the compounds were assayed in terms of: the oxygen radical absorbance capacity (ORAC), the ability to reduce free chelatable iron, which was proven to play an active role in the Fenton reaction, producing the highly toxic hydroxyl anion (FRAP), the ability to scavenge superoxide anions (NBT assay) and the ability to reduce potassium cyanide induced lipid peroxidation (TBARS assay), *in vitro.*

Results:

In the ORAC assay only the 6-amino-4-hydroxyquinoline had significantly more antioxidative capacity as compared to all the other compounds (7119±2020.314 μM trolox/μM sample units, $p<0.001$). In the FRAP assay it is evident that both the amino-compounds had significantly more ferric reducing power than the rest of the compounds. Also, the 6-amino-4-hydroxyquinoline had significantly more activity than the 7-amino-4-hydroxyquinolines. All the tested compounds significantly reduced the 1mM KCN induced level of superoxide anions at all concentrations used (0.25; 0.5 and 1mM). In the TBARS assay, all the compounds significantly reduced the 1mM KCN induced lipid peroxidation. There wasn't a significant difference in the reduction of lipid peroxidation between the 6-or 7-amino-4-hydroxyquinolines.

Conclusion:

The results obtained in this study clearly indicate that the 4-hydroxyquinolines have antioxidative activity. The nitro-4-hydroxyquinolines have shown to be the best radical scavengers as they exerted the best reduction in the NBT values. Although it seems from the ORAC, FRAP and TBARS assays, that the amino-4-hydroxyquinolines have the most promising potential for antioxidative activity. From this study it is possible to conclude that these compounds may be used in the development of antioxidant strategies against oxidative stress induced diseases.

Pentacyclo[5.4.0^{2,6}.0^{3,10}.0^{5,9}]Undecane as Carrier Molecule for Neuroprotective NSAIDS

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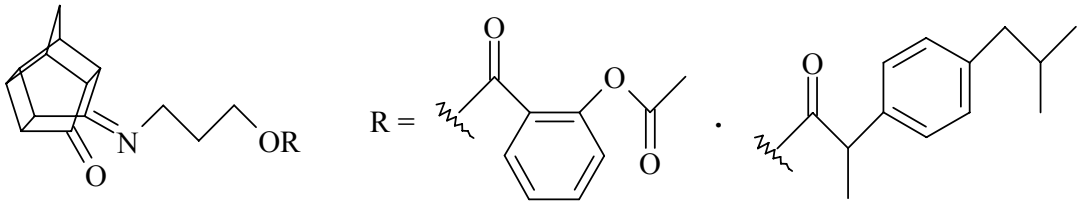
Background:

Neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases, have a negative influence on the quality of life of millions of people around the world. In the quest to successfully prevent and treat these disorders, the blood-brain barrier (BBB) poses a major obstacle by preventing the entrance of certain substances into the central nervous system (CNS). Recent studies on the polycyclic structure, pentacyclo[5.4.0.02,6.03,10.05,9]undecane, indicated favourable distribution thereof to the brain. In all of these studies, it was concluded that this polycyclic structure and its derivatives penetrate the BBB readily. It has also recently been described that non-steroidal anti-inflammatory drugs (NSAIDs), which includes acetylsalicylic acid and ibuprofen, may have neuroprotective properties and may therefore be used in the treatment and even prevention of neurodegenerative diseases. These drugs are highly hydrophilic and as a result, demonstrate extremely low BBB penetration.

Objective:

In this study we synthesised prodrugs by conjugating two NSAIDs, namely acetylsalicylic acid and ibuprofen, to the polycyclic amine pentacyclo[5.4.0.02,6.03,10.05,9]undecane using 3-aminopropanol as a linker. This was done in order to improve the CNS delivery of the particular NSAIDs.



Methods:

The test model developed in our laboratory was applied to determine BBB permeability of the synthesised compounds. This was done by comparing brain and blood concentrations of the relevant drug at specific time intervals using male C57Bl/6 mice. A highly sensitive LC-MS.MS procedure was used to quantify the drug concentration.

Results:

The prodrug structures exhibited favourable BBB penetration when compared to the free drugs. The more efficient delivery of the drug to the brain would afford higher concentrations of the neuroprotectant and thus better neuroprotection.

Acknowledgements:

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The evaluation of low-density lipoprotein cholesterol goals achieved in patients with established cardiovascular disease and/or hyperlipidaemia receiving lipid–lowering therapy *(Preliminary results)*

The South African Not At Goal Study *(NAG)*

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Background:

Dyslipidaemia is a common condition that leads to the clinical sequelae of cardiovascular disease. Cardiovascular disease (CVD) is the leading cause of morbidity and mortality worldwide. As a result, it has become essential for South Africa to update its clinical guidelines for cardiovascular disease management, and the scientific community has thus adopted the European Guidelines on cardiovascular disease prevention in clinical practice. Treatment, according to these guidelines, separates patient treatment into two groups (i) established CVD, diabetes, severe genetic lipid disorders or those at persistently high risk and, (ii) asymptomatic patients or patients at lower risk.

Objectives:

The South African Not at Goal Study (*NAG*) is a survey to determine the percentage of patients, on lipid-lowering therapy, who are not at target LDL-C goal, as defined by the updated South African Guidelines. The study is currently in progress and aims to enroll 1400 patients, recruited by physicians and GPs, who are currently on lipid-lowering therapy for >4 months. Blood samples from these patients are analysed to obtain a fasting lipogram and fasting blood glucose levels. Currently, 860 patients (age 58±11.6 yrs) from across South Africa have been enrolled.

Results:

Under the new guidelines, 66% of patients are defined as high risk (HR) and 34% of patients are low risk (LR). 75% of patients at HR and 62% of LR patients did not achieve LDL-C target goals of 2.5 and 3.0 mmol/L, respectively. Patients at HR and LR patients, who did not achieve the LDL-C goal, were, on average, 46% (1.14 mmol/L) and 27% (0.83 mmol/L) above the LDL-C target levels, respectively.

Conclusion:

Preliminary results, in light of the new guidelines, suggest that a considerable percentage of patients will fall into the category of "not at goal" LDL-C. The adoption of the new guidelines will necessitate enhanced disease management to reduce the disease burden.

Anti-oxidant effects of novel gold compounds

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Auranofin (AF), the orally administered gold-based anti-arthritic agent, emerged as a clinically useful therapeutic drug for the treatment of rheumatoid arthritis in the late 1970's.

The toxicity of anti-rheumatic gold compounds has limited their use, yet both the toxicological and therapeutic actions of these drugs remain unclear. To improve the bioavailability, pharmacokinetics and to obtain a safer profile, three derivatives of auranofin viz. Asa-fin, Mpta-fin and Pta-fin, with varying degrees of lipophillicity have been designed and synthesized. Reactive oxygen species (ROS) play an important role amplifying the inflammatory process and in tissue injury. It has been documented that AF inhibits many aspects of immune cell function, including superoxide and hydrogen peroxide production.

The in vitro anti-oxidant efficacy of the gold compound AF and the three novel AF derivatives, were compared by studying their effects on the generation of ROS using N-formymethionyl-leucyl-phenylalanine (FMLP) and phorbol myristate acetate (PMA) stimulated human neutrophils. The production of ROS was measured using a luminol enhanced chemiluminescence and a flow cytometry procedure to determine superoxide release with Hydroethidine, hydrogen peroxide release with Dihydrorhodamine 123 and nitric oxide release with2'7'-Dichloroflourescin diacetate.

AF, Asa-fin and Mpta-fin showed a biphasic effect on the hydrogen peroxide produced by the FMLP stimulated neutrophils on the chemiluminometer. Addition of low concentrations of AF, Asa-fin and Mpta-fin ($\leq$ 0.5 μM) enhanced, while higher concentrations (0.5-12.5 μM) inhibited hydrogen peroxide release. Pta-fin had no effect on the hydrogen peroxide produced.

Concentrations of AF and Asa-fin ($\geq$ 12.5μM) decreased the release of hydrogen peroxide and superoxide released by the PMA stimulated neutrophils on the flow cytometer. Mpta-fin and Pta-fin exerted no effect on the hydrogen peroxide and the superoxide produced by the PMA stimulated human neutrophils. All the drugs had no effect on nitric oxide released by the PMA stimulated human neutrophils.

These findings suggest that the gold compounds AF, Asa-fin and Mpta-fin inhibit respiratory bursts and the generation of inflammatory reaction products by neutrophils. Further testing could prove these compounds to be promising anti-inflammatory drugs.

L-Dopa Administration Enhances 6-Hydroxydopamine Generation

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Purpose:

Parkinson's Disease (PD) is a neurodegenerative disease that affects a number of aged individuals. Although a great deal is known about the neurochemical changes that occur during PD, the etiology of the disease is still not known. Current treatments of Parkinson's disease rely on the dopamine replacement approach. However, dopamine cannot cross the blood brain barrier so its immediate precursor, levodopa (L-Dopa) is used to treat Parkinson's disease. L-Dopa is effective in early treatment, however chronic L-Dopa therapy is limited by the development of a number of side effects, including dyskinesia. A possible reason for this may be the formation of 6-hydroxydopamine (6-OHDA) in the brain, a potent neurotoxin. This study sought to investigate whether chronic or acute L-Dopa treatment resulted in the endogenous formation of 6-OHDA in rat brain. The effect of L-Dopa on iron-induced lipid peroxidation was also investigated using *in vivo* studies. The ability of melatonin to offer neuroprotection against iron-induced lipid peroxidation and 6-OHDA formation was also investigated.

Methods:

Male Wistar rats were used in the study. Rats were treated with L-Dopa for a period of 7 and 28 days to determine whether L-Dopa treatment results in 6-OHDA formation in the rat striatum. The study also investigated the interaction of L-Dopa with iron by performing lipid peroxidation studies and the detection of 6-OHDA in iron-infused rats. Striatal samples were assayed for 6-OHDA content using high performance liquid chromatography with electrochemical detection and analysis for lipid peroxidation was done using the TBA assay. In each study melatonin was used to determine whether it could offer protection against 6-OHDA formation and lipid peroxidation.

Results:

The results of the research show that both acute and chronic L-Dopa administration results in the endogenous formation of 6-OHDA in rat brain. Chronic treatment with L-Dopa resulted in higher levels of endogenous 6-OHDA than acute treatment. L-Dopa treatment also enhances iron-induced lipid peroxidation *in vivo*. Melatonin was able to inhibit L-Dopa enhanced lipid peroxidation as well as L-Dopa induced 6-OHDA formation and therefore has a neuroprotective effect.

Conclusion:

This study provides evidence to support a plausible hypothesis that the long term side effects related to L-Dopa treatment is due to the conversion of the neurotransmitter dopamine into the neurotoxin 6-hydroxydopamine in the brain. Administration of melatonin with L-Dopa should be considered as a means to counteract the side effects associated with long term L-Dopa treatment in Parkinson's Disease.

A Novel Multi-Polymeric Crosslinked Glycosidic Platform for Site-Specific Delivery of Nicotine

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Purpose:

This study aimed to develop a novel reinforced crosslinked multi-polymeric drug delivery device to deliver newly researched neuroprotective agents to retard the progression of PD. To date, no such device exists for application in PD. Nicotine was considered as a model neuroprotectant for the intended drug delivery system. Several studies have demonstrated its effectiveness in controlling PD symptoms as well as delaying disease onset. The study also aimed to elucidate new mechanisms of interaction between the various components of the developed composite crosslinked multi-polymeric device.

Methods:

Preparation of Gelispheres: Ionotropic gelation was employed to formulate the gelispheres in accordance with a Plackett-Burman Statistical Design. A polymeric dispersion comprising alginate and hydroxyethylcellulose (HEC) into which $1\%^{w}/_{v}$ nicotine was incorporated. This polymeric solution was titrated dropwise into the gently vortexed crosslinking solution composed of Polyacrylic acid (PAA) and either $BaCl_2$, $CaCl_2$ or a binary combination of the two and cured for 30 minutes.

Drug Entrapment Efficiency (DEE) Tests: Were conducted in triplicate on 200mg of drug-loaded gelispheres. The gelispheres were homogenized in 300mL 0.1M PBS (pH 7.4, 21ºC). Filtered samples were analyzed employing UV spectroscopy for the presence of nicotine.

In Vitro Drug Release: In vitro dissolution studies were performed over 48 hours on 100mg of drug-loaded multi-polymeric gelispheres placed in 100mL 0.1M PBS of pH 7.4 in sealed glass jars which were placed in a shaker bath (37°C, 50rpm) and analyzed employing UV spectroscopy.

Textural Analysis: A Texture Analyzer was employed to conduct textural analysis of the unhydrated and hydrated gelispheres in 100mL PBS (pH 7.4, 37°C) over a period of 48 hours.

Optimization: Optimization was carried out through the application of Design of Experiments response optimizer function in Minitab (Release 14, Minitab Inc., USA) employing simultaneous optimization of responses to maximize DEE and minimize drug release.

Results:

Drug Entrapment Efficiency: DEE ranged between 18.75 - 53.67%. Higher entrapment efficiencies were observed with formulations with a high alginate and high HEC component. Both crosslinking ions and high PAA concentrations of were required to produce compact matrices.

In Vitro Drug Release: All formulations demonstrated first-order drug release kinetics. High alginate and high HEC formulations attenuated the burst effect.

Textural Analysis: Alginate and Ba^{2+} concentrations had the most significant impact on the formation of compact and robust gelispheres.

Optimization Studies: Formulations composed of high alginate and high HEC concentration and crosslinked with both crosslinking ions and high PAA concentrations demonstrated high DEE and slow drug release with a minimal burst effect.

Conclusions:

The interaction between alginate and HEC is significantly synergistic in producing a highly compact matrix when crosslinked with Ba^{2+} ions. Exploiting this system to achieve a drug delivery system that has the capacity to prolong and sustain drug release can revolutionize the future of biodegradable implantable devices, particularly in the field of neuroscience and PD therapy.

Cross-linked Chitosan Matrix Systems for Sustained Drug Release

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Purpose

Conventional single unit oral dosage forms release a drug immediately after administration (i.e. burst release effect) causing the drug blood concentration to rise quickly to a high value ("peak") followed by a sudden decrease to a very low level ("trough") as a result of drug elimination. This may give rise to sub-therapeutic and side-effects. Finding a suitable cross-linking agent by formulating novel dosage forms that elicit sustained drug release profiles over an extended period of time, usually 8 to 12 hours, to address these problems was the focus of this study.

Method

Casting of chitosan matrix systems: Chitosan (3% w/v) was gelled in acetic acid (2% v/v) and ibuprofen (3% w/v) was dispersed in the gel. Aliquots of gels were cross-linked either with tripolyphosphate (TPP) or a surfactant: sodium lauryl sulphate (SLS), oleic acid or tween 80 for 1 hour under continuous stirring. The disintegrant Explotab® was added to a second batch of each formulation. The cross-linked chitosan gels were dried in an oven for 60 minutes at 37 ºC and then forced through a 2 mm sieve while still damp to obtain wet granules. Accurately weighed aliquots of approximately 0.550 g (limited by the size of the moulding well) of the chitosan gels were loaded, using a spatula, into 24 wells on polystyrene plates (Conning Costar®, USA), diameter 1.55 cm and depth 2.00 cm. Three light hand-compressions were applied using the flat face of a metallic margin marker casing, diameter 1.50 cm. The mouldings were dried in a drying oven at 37 °C overnight, and were then evenly hand-compressed thrice before further drying in their plates at 37 °C for 48 hrs.

Physical evaluation of cross-linked chitosan matrix systems: Physical tests that were performed on the matrix systems according to various published procedures included determination of weight variation, hardness and dimensions, swelling ratios, friability, drug content, surface and cross-sectional scanning micrographs.

In vitro drug release studies: Drug release studies (USP 1995 paddle method) at 60 rpm on the matrix systems were performed in triplicate in 900 ml of buffers at pH 5.8 and pH 7.4, respectively at 37 °C. Autosampling was done at predetermined intervals up to 8 hours and samples were analysed by UV spectroscopy at 264 nm.

Statistical analysis of data: Data were statistically analysed using several indices, including t50% and mean dissolution time (MDT) values by one-way repeated analysis of variance (ANOVA, $p<0.05$) as well as the similarity factor, ($f2<15$) and the difference factor, ($f1>50$).

Results

Results showed that SLS (especially 5% w/v) significantly cross-linked chitosan (comparable to TPP 10% w/v) better than tween 80 and oleic acid within 0.1-15% v/v concentrations range. SLS at concentrations ≤5% w/v is a promising candidate for sustaining the release of drugs. Ibuprofen release was slower in matrix systems that did not have Explotab®, as was predicted by swelling studies, and also at higher dissolution media pH (7.4). An inverse concentration-bonding strength relationship was predicted for SLS as was reflected by scanning micrographs, which could have accounted for the low drug release rates and loss of surface-active properties observed for the 5% w/v SLS matrix systems compared to the 10% w/v SLS formulations.

Inhibition of Monoamine Oxidase B by Substituted Benzimidazole Analogues

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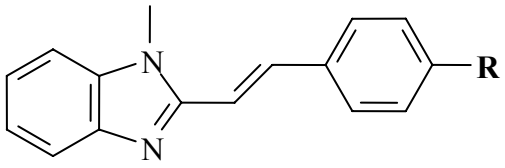
Background:

The enzyme monoamine oxidase B (MAO-B) is of significant pharmacological interest in view of the fact that it is the principle enzyme that catalyzes the oxidation of dopamine in the brain. Inhibitors of MAO-B are frequently used as therapy for the treatment of Parkinson's disease (PD). The beneficial effects of MAO-B inhibitors may be dependant upon the inhibition of the MAO-B catalyzed oxidation of dopamine in the central nervous system, consequently conserving the depleted supply of dopamine and delaying the need for levodopa in patients diagnosed with early PD. MAO-B inhibitors are also reported to exert a neuroprotective effect by blocking apoptic cell death, and accordingly may be used clinically to postpone the emergence of symptoms in PD patients. For these reasons MAO-B is considered to be an attractive target for the treatment of neurodegenerative diseases. Recent studies identified benzimidazolyl analogues as potential inhibitors of MAO-B. In this study we investigated additional benzimidazolyl analogues for their ability to inhibit MAO-B.

Objective:

A series of novel benzimidazolyl analogues was synthesized and characterized as reversible inhibitors of MAO-B. The analogues chosen are illustrated by the structure below.



Electron-withdrawing: **R** = Cl, Br, F, CF_3
Electron-donating: **R** = CH_3, OCH_3
Unsubstituted: **R** = H

Results:

All of the benzimidazolyl analogues synthesized were found to be moderately potent reversible inhibitors of MAO-B. The modes of inhibition were found to be competitive with enzyme-inhibitor dissociation constants (K_i values) in the low micro-molar range.

The Preparation and Evaluation of Xanthine Analogues as Inhibitors of Monoamine Oxidase B

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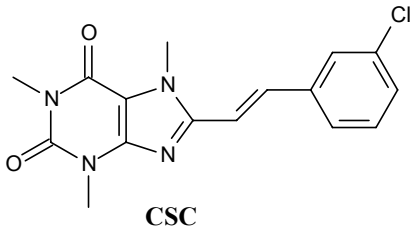
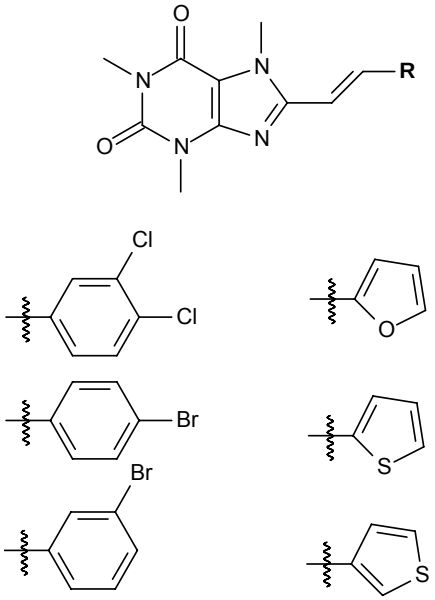
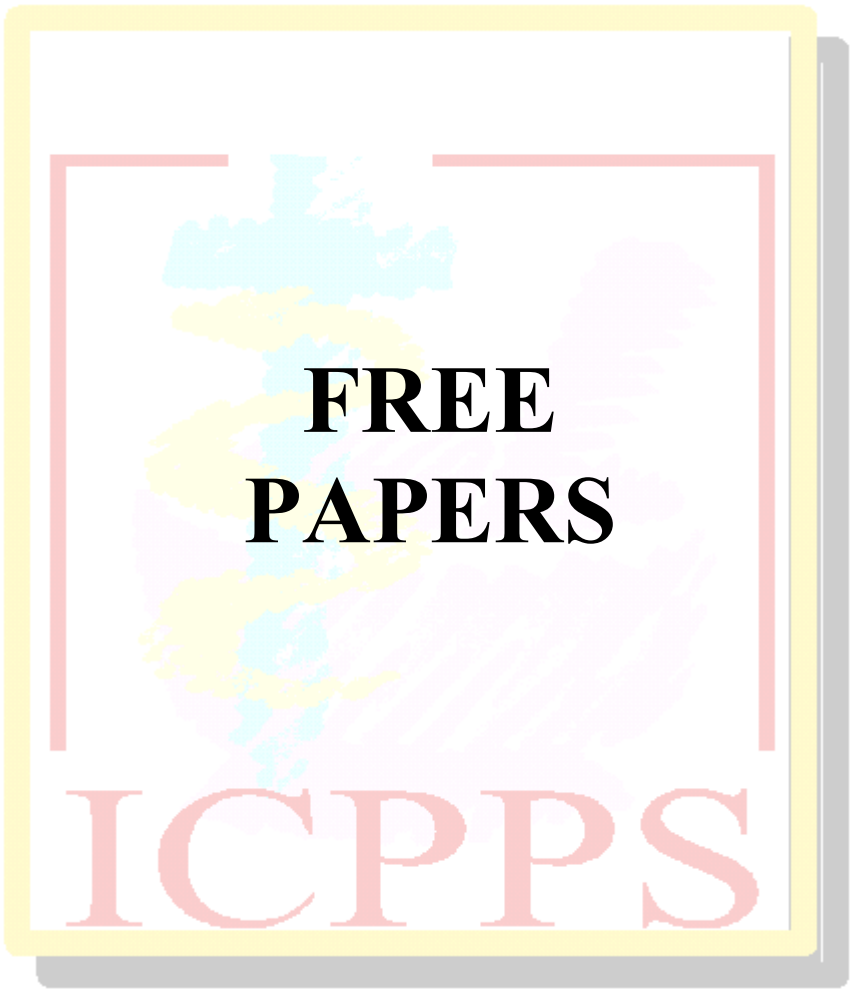
Monoamine oxidase B (MAO-B) is a drug target for the treatment of neurodegenerative diseases such as Parkinson's disease. For example, the mechanism-based inactivator of MAO-B, (*R*)-deprenyl, is frequently used in combination with levodopa as dopamine replacement therapy in Parkinson's disease. In contrast with reversible inhibitors, following treatment with inactivators such as (*R*)-deprenyl, enzyme activity can only be regained via *de novo* synthesis of the MAO-B protein. For this reason, several studies are currently underway to develop safer inhibitors of MAO-B as an alternative to (*R*)-deprenyl. These inhibitors are required to be reversible while retaining selectivity towards MAO-B. We have recently identified (*E*)-8-(3-chlorostyryl) caffeine (CSC) as a exceptionally potent reversible inhibitor of MAO-B with a enzyme-inhibitor dissociation constant (K_i value) of 70 nM.



CSC

In an attempt to identify the structural features that are responsible for the high inhibition potency of CSC, we have synthesized additional analogues of CSC and examined their MAO-B inhibition potencies *in vitro*. The analogues chosen for this study are illustrated below. All of the analogues were found to be reversible inhibitors of MAO-B with K_i values in the nano-molar to low micro-molar range.

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Bronchodilator and Anti-Inflammatory Activities of *Adhato* ***schimperiana*****, Acanthaceae**

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Background:

A.schimperiana is a plant believed to have several therapeutic effects including anti-asthmatic properties. We have studied the acute toxicity, bronchodialatory and anti-inflammatory effects of the hydromethanolic extract of leaves of this plant.

Methods:

The isolated guinea-pig trachea pre-contracted with histamine and acetylcholine was used to study the relaxation of hydromethanolic extract of leaves *A.shimperiana.* Salbutamol and Atropine were used as standards. The effect of the hydromethanol extract of leaves of *A.schimperiana* on carageenin-induced acute inflammation was evaluated by the rat hind paw edema method. Oral and interaperitoneal acute toxicity studies of the extract were performed on mice.

Results:

The extract inhibited contractions of guinea pig tracheal chains induced by histamine and acetylcholine. The inhibitory activity of atropine was lower than the hydromethanol extract and Salbutamol. More than one mechanism of actions are involved in the relaxation of pre-contracted tissue by the hydromethanol extract of leaves of *A. schimperiana*. The hydromethanolic extract of *A.schimperiana* exhibited a moderate degree of anti-inflammatory activity. The wide safety margin of the extract indicates that human exposure to hydromethanolic extracts of *A.schimperiana* leaves is very unlikely to occur.

Conclusion:

The results of this study show anti-inflammatory activity and a relatively potent relaxant (bronchodialatory) effect of *A.schimperiana* on the tracheal chain of the guinea pig. These activities justify the traditional use of this plant in the treatment of bronchoconstrictive diseases.

Key words*:* *A.schimperiana,* anti-inflammatory, bronchodialator

N-terminal Genetic Variations of Regulator of G Protein Signalling 2 (RGS2) in Hypertensive Patients: Decreased Expression and Function of the Mutant RGS2-Q2L

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Hypothesis

Hypertension is a leading risk factor in cardiovascular disease with a worldwide estimated prevalence of 1 billion people and 7.1 million deaths per year (Chobanian et al., 2003).

Regulator of G protein signalling 2 (RGS2) is a 25 kDa ubiquitously expressed protein and plays an important role in modulating signalling of physiological vasoconstrictors (Tang et al., 2003) such as the octapeptide angiotensin II. Mice models deficient in RGS2 exhibit enhanced vasoconstriction and a strong hypertensive phenotype (Sun et al., 2005), but the relevance to hypertension in humans is not known. Recently, several rare N-terminal genetic variations of RGS2 were identified in a Japanese cohort (Yang et al., 2005). Two N-terminal second position mutations (Gln to Leu or Arg) may impact the N-end rule pathway of protein degradation by the proteasome (see Varshavsky, 1996 for a review).

In this study we investigated the expression, proteasomal regulation as N-end rule pathway component, and function of wild-type RGS2 (RGS2-WT) and the genetic variants RGS2-Q2L and RGS2-Q2R.

Methods and Results

Protein Expression and Proteasomal Regulation—In transiently transfected human embryonic kidney 293T (HEK293T) cells, we demonstrate by Western blot that RGS2-Q2L is expressed at 12-fold lower levels than WT and is more robustly regulated by the proteasome as demonstrated by a 17-fold higher expression with proteasomal inhibition (20 μM MG-132; 4 h).

Protein Function—Inositol phosphates accumulation was measured with angiotensin II (varying concentrations; 2h) in HEK293T cells transiently cotransfected with the $G\alpha_{q/11}$-coupled angiotensin II type 1 (AT_1) receptor and we demonstrate that RGS2-Q2L has a 3-fold lower functional activity in modulating angiotensin AT_1 receptor-mediated signalling.

Conclusion

Our results demonstrate that RGS2-Q2L, but not RGS2-Q2R, is unstable and degraded by the proteasome and has significantly reduced functional activity. This effect likely reduces inhibition of vasoconstrictor signalling and may explain the mechanism contributing to hypertension in some patients. In individuals with this marked post-translational control of RGS2 expression, pharmacological means to potentiate RGS2 function, inhibit proteasomal degradation or arginine tRNA transfersase enzymes involved in the N-end rule could be useful therapeutically.

References

A. V. Chobanian, G. L. Bakris, H. R. Black, W. C. Cushman, L. A. Green, J. L. Izzo Jr., D. W. Jones, B. J. Materson, S. Oparil, J. T. Wright Jr. and E. J. Rocella, Seventh report of the Joint National Committee on prevention, detection, evaluation and treatment of high blood pressure. Hypertension 42(6), 1206-1252 (2003).

X. Sun, K. M. Kaltenbronn, T. H. Steinberg and K. J. Blumer, RGS2 is a mediator of nitric oxide action on blood pressure and vasoconstrictor signaling. Mol. Pharmacol. 67(3), 631-639 (2005).

K. M. Tang, G. R. Wang, P. Lu, R. H. Karas, M. Aronovitz, S. P. Heximer, K. M. Kaltenbronn, K. J. Blumer, D. P. Siderovski, Y. Zhu and M. E. Mendelsohn, Regulator of G-protein signaling-2 mediates vascular smooth muscle relaxation and blood pressure. Nat. Med. 9(12), 1506-1512 (2003).

A. Varshavsky, The N-end rule: functions, mysteries, uses. Proc. Natl. Acad. Sci. U S A 93(22), 12142-12149 (1996).

J. Yang, K. Kamide, Y. Kokubo, S. Takiuchi, C. Tanaka, M. Banno, Y. Miwa, M. Yoshii, T. Horio, A. Okayama, H. Tomoike, Y. Kawano and T. Miyata, Genetic variations of regulator of G-protein signaling 2 in hypertensive populations and in the general population. J. Hypertens. 23(8), 1497-1505 (2005).

Acknowledgements

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Manufacture and optimization of tubular nickel membrane supports

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Purpose:

Casted porous supports made by a centrifugal dispositioning system are presented. The most widely used material for this manufacturing method is usually ceramics, but for this study elementary nickel was used for manufacturing purposes. The shortcomings of the ceramics, for example brittleness and inadequate separation module sealage, are compared to the virtues of the nickel supports.

Method:

Nickel powders are prepared by hydrothermal reduction method to produce very fine powders of size 0.2 to 0.8μm in diameter. These particles are spherical in shape as confirmed by SEM. The starting material of nickelchloride hexahydrate is reduced in a two step manner to solid nickel by the reducing agent hydrazine. After a brief preparation procedure, the powder is triple washed and boiled in distilled water to ensure purity and to remove exipients used in the preparation procedure.

The centrifugal casting method is unique when compared to pressing of porous structures with respect that smaller particles of a powder will concentrate on the inner side of the support, and the larger particles on the outer side during support formation that occurs when dispersion is spun around its axis in a tubular cast. This is the case because the centrifugal forces deployed, forces larger particles to the outer side, and retain smaller particles on the inner side. The approximate rotational speed the centrifuge used is 17000rpm. This method produces asymmetrical supports that prosess higher flux, inherent of membrane structures that have a thin top layer and more porous outer layer.

Dispersing the powder to meet experimental requirements is achieved with polymeric dispersing agents. This include the polymer poly(acrylamide)-co-diallyldimethyl-ammoniumchloride(PAAco), which proved to be a good dispersing agent for the nickel powders, even at low polymer concentration. Other less successful polymeric dispersants were also studied, which include poly(acrylamide) and poly(ethelyneglycol) 300.

After the support is spun up, the remaining polymer is evacuated form the cast which leaves the membrane situated on the inner surface of the cast. The support is dried at room temperature for 48 hours to ensure easy removal of the greencasted (unsintered) support. Sintering of the membrane follows for it to gain strength and for the particles to fuse together. An array of sintering experiments follows at varying temperatures to determine optimal sintering conditions.

To characterize the new support, several characterization methods and techniques are deployed. This includes mercury intrusion porosimetry (Autopore IV, Micromeritics, USA), scanning electron microscopy (FEI Quanta 200 ESEM), water permeation and strength testing of the support itself.

Results:

An asymmetrical centrifugally casted nickel support was successfully manufactured. A suitable polymer has been found to disperse the powder and supports are currently undergoing sintering experiments. A successful in-house manufacturing procedure has been installed to produce the required nickel powder for membrane manufacture.

A novel controlled release PLGA alginate-pectinate polyspheric drug delivery system

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Purpose

This study focused on developing PLGA microparticles incorporated into a platform consisting of a combination of crosslinked alginate and pectinate matrices referred to as "polyspheres" to achieve prolonged rate-modulated drug release.

Methods

Design of Experiments: A 2^3 full factorial design was employed to evaluate and optimize the drug entrapment efficiency (DEE) and *in vitro* drug release from PLGA microparticles encapsulated in a complex crosslinked alginate–pectinate matrix (polysphere). Surface morphology and internal structure of optimized PLGA microparticles and polyspheres were examined by Scanning Electron Microscopy (SEM) and a Texture Analyzer (StableMicroSystems, UK) was employed to profile the physicomechanical properties of polyspheres namely, matrix resilience, matrix tolerance, and energy absorbed.

Drug Entrapment Efficiency: 100mg of drug-loaded polyspheres were dissolved in 100mL of phosphate buffered saline (PBS) (pH 7.4; 37°C) and agitated over a period of 24 hours. Samples were analyzed using UV Spectroscopy for the presence of model drug diclofenac.

In vitro drug release: Investigations were performed in PBS (pH 7.4; 37°C) on native PLGA microparticles and polyspheres. Kinetic modeling was performed on WinNonlin Version 5 (Pharsight, USA) using the Gaussian-Newton approach.

In vivo studies: 56 adult male rats were purchased from Harlan Sprague Dawley Inc., (Indianapolis, IN) in order to assess *in vivo* drug release. Three polysphere formulations (model drug diclofenac) each of which had a blank formulation (control groups) were studied.

Results

Preliminary polyspheric formulations exhibited ideal zero-order release (R^2=0.99) while native PLGA microparticles behaved as expected with an initial burst effect (39% at t_{24} hours) followed by a lag phase. Formulations were not highly dependent on polymeric erosion as a mechanism for drug release (R^2=0.35), however, matrix relaxation and swelling were principle mechanisms involved (R^2=0.98). PLGA concentrations and its interaction with the external phase had a significant impact on DEE ($p<0.05$).

Textural profile analysis of polyspheres indicated a close correlation between matrix tolerance and energy absorbed (R^2=0.93). Formulations with a decreased tolerance absorbed less energy, leading to rapid surface erosion, lower matrix integrity and hence a burst effect. *In vivo* studies demonstrated steady-state drug levels maintained over a period of 20 days.

The novel application of a combination of polyesters and celluloses employed for the development of an intelligent system that may possibly enable superior rate-modulated drug delivery.

How much stress is bad for you?

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Mental disorders such as depression and anxiety disorders are often associated with chronic exposure to stress. We have previously shown that maternal separation (MS), as a model of stress during childhood, lead to anxiety-like behaviours and neuroendocrine abnormalities in rats. Similar results were obtained when rats were subjected to time dependent sensitization (TDS), a model of trauma during adolescence. In the present study we combined both these models to see whether a combination of these stressors would yield greater behavioural and hormonal aberrations.

Sprague-Dawley rat pups were separated from their mothers 3 hours daily, between 08h30 – 13h00, from postnatal day 2-14. On postnatal day 28 these same animals were exposed to 3 different stressors i.e. 20 minutes restraint, 20 minutes swimming, and finally exposure to ether vapours until loss of consciousness. On postnatal day 35 and 60 the animals were again exposed to swim stress.

The results show that MS and TDS caused similar behavioural effects with increased rearing being most prominent. A combination of the two stressors however resulted in no significant behavioural changes. Both MS and TDS led to increased baseline corticosterone release, while repeated exposure to stressors resulted in decreased corticosterone secretion.

Contrary to expectation the data indicate that the combination of childhood stress and adolescent trauma did not lead to exaggerated responses but rather suggested that subsequent stressors may in fact lead to the development of resilience and better coping strategies against stressful events.

Evaluation of Novelly-Formed Enterospheres for Targeted Delivery of an Anti-Tuberculosis Drug

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Purpose:

Oral enterosoluble multiparticulate matrices, henceforth referred to as *enterospheres,* were designed for the targeted delivery of the anti-TB drug, isoniazid, to the small intestine. The novelly-formed enterospheres were obtained by inducing separation ('salting-out') of the pH-sensitive poly (methacrylic acid-co-ethylacrylate) copolymer as a polymer-rich enteric film and ionotropically cross-linking the internal enterosphere matrix following extrusion and curing of a partially neutralized aqueous dispersion of the copolymer into a concentrated electrolyte solution. Because there is an unequivocal relationship between the properties of a crosslinked enterosphere and its structure in such a way that both characteristics cannot be considered in an isolated way, and because the polymeric composition and synthesis method decisively influence the structure of the enterosphere as well as the final properties that the structure will have; in depth analyses on drug-free and drug-loaded enterospheres was undertaken.

Methodology:

Critical formulation and processing variables for enterosphere manufacture were previously identified employing a Box-Behnken experimental design approach. In order to gain clarity into the relationship between the structure and the properties of optimally synthesised drug-free and drug-loaded enterosphere formulations, expound on the mechanism of the crosslinking interaction between the cations and the three-dimensional methacrylic acid copolymer structure, and elucidate the crystalline structure of the copolymeric enterospheres Fourier Transform Infrared (FTIR) Spectroscopy and X-Ray Powder Diffraction were systematically undertaken.

In addition, because of the intrinsically important influence of incorporated plasticiser on enterosphere behaviour, relatable effects of incremental increases in plasticizer levels (0%, 2%, 6% and 10%$^{w}/_{w}$) in the enterosphere on the thermal, gravimetric and morphological transitions (visualised by scanning electron microscopy) at simulated gastrointestinal pHs were characterised.

Results:

The IR spectra of the amorphous enterospheres revealed that the intensity of the characteristic band of the carboxylic acid group was greatly diminished in the drug-free and drug-loaded enterospheres. When ionization occurred, with formation of the COO^{-} group, resonance was possible between the two C---O bands. As a consequence, the characteristic pendant anhydride absorption is replaced by the band in the 1550–1556 cm^{-1} region indicative of a degree of ionisation and crosslinking in the enterosphere. The enterosphere demonstrated an overall increase in the glass-transition temperature (T_g) compared to the pure copolymer, while increasing degrees of plasticisation caused incremental decreases in the T_g and more rapid erosion of the enterospheres at small intestinal pHs, as observed morphologically and gravimetrically.

The effects of trauma in previously maternally separated rats

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Clinical studies indicate that early life adverse events increase the susceptibility of developing psychiatric disorders later in life. These findings suggest that stress during childhood causes neurochemical alterations that may render individuals vulnerable to subsequent insults.

The aim of the present study was therefore to investigate the effects of severe trauma during adolescence, in animals that were previously subjected to maternal separation during childhood. Maternally separated (MS) rats were subjected to Time-Dependent Sensitisation (TDS stress entails the exposure of animals to a triple stressor on postnatal day (PND) 28 consisting of 2hrs restraint stress followed by a 20min swim stress, where after animals are exposed to ether vapours until loss of consciousness. Additionally, on PND 35 and 60 animals are subjected to 20min swim stress which serves the purpose as situational reminder of the trauma experienced on PND 28). On PND 67 behavioural responses of the animals were evaluated using the elevated plus maze and the open field tests. Trunk blood was collected for ACTH and corticosterone determinations, and brain tissue (dorsal and ventral hippocampus) for neurotrophin (brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF) and neurotrophin-3 (NT-3)) measurements the following day.

Our results showed that there were no significant differences in behaviour between rats that were subjected to MS compared to those receiving MS + TDS. Subjecting animals to MS led to significant decreases in ACTH and corticosterone levels. The combination of MS and TDS yielded similar results. Neurotrophic factors were significantly up-regulated in the MS + TDS group. These included NGF ($p=0.007$) and NT-3 ($p=0.05$) in the dorsal hippocampus; BDNF ($p=0.014$), NGF ($p=0.006$) and NT-3 ($p=0.001$) in the ventral hippocampus.

The behavioural data suggest that when both the stress paradigms are applied in combination that later stressors ameliorate the detrimental effects of earlier stressors. This occurred in the presence of sustained endocrine irregularities in both groups. The increased growth factor levels observed in stressed animals may represent compensatory mechanisms by the brain to neutralise the damaging effects of repetitive stress exposure. In contrast to our hypothesis, our data suggested that multiple exposures to stress could have beneficial outcomes as stressors may mitigate some of the effects of the early life stressors.

Plasmodium falciparum kinases as drug targets

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Introduction

Infections caused by the malaria parasite *Plasmodium falciparum* remain a major health burden in the underdeveloped countries of the world. This is compounded by the fact that chloroquine resistance is now commonplace and the parasite rapidly develops resistance to newly introduced drugs. The *Plasmodium* genome project has provided researchers with a valuable tool to identify novel drug targets. It is essential that new drugs selectively target the parasite and have a minimal effect on the host. In recent years kinases have become attractive drug targets and several selective inhibitors are currently in clinical use. Imatinib mesylate has brought about a dramatic change in the management of chronic myeloid leukaemia and several other promising agents are currently undergoing clinical evaluation. Contrary to expectations, the adverse effect profile of the kinase inhibitors has been mild. Results obtained from the screening of a large panel of kinase inhibitors in a *P. falciparum* growth assay showed that *Plasmodium* kinases are potential targets for new drugs. In this context two *P. falciparum* kinase genes (PFL2280W and PFL0080c) were identified *in silico* and investigated as potential tools for high throughput drug screening. PFL2280 shows some similarity to cyclin G associated kinase and PFL0080c shows similarity to a NIMA related kinase. The two kinases are referred to as PfcGAK and PfNek3 respectively.

Methods

The *P. falciparum* 3D7 strain was maintained in culture according to standard procedures. DNA was isolated by a modified phenol extraction procedure and used in PCR reactions. The catalytic domain and flanking region of the PfcGAK gene and the complete gene sequence of PfNek3 was amplified by PCR and the sequence confirmed by partial DNA sequencing. The PfcGAK PCR product was cloned into a vector that yielded a fusion protein with a N-terminal HA and a C-terminal His (6X) tag. The PfNek3 PCR product was cloned into an expression vector that yielded a fusion protein with a N-terminal His (6X) tag. Insert positive colonies were sequenced to determine if the sequence was inserted in the correct reading frame. Protein expression was induced by the addition of IPTG and the expressed protein isolated by affinity chromatography on Ni^{+} beads. The protein samples were analysed by SDS-PAGE and Western blotting. The fusion proteins were used in a standard kinase assay to determine the activity of the recombinant enzymes.

Results

Recombinant PfcGAK showed kinase activity using casein as substrate. No kinase activity was observed when myelin basic protein and histone H1 protein were used as substrates. PfcGAK was also shown to undergo autophosphorylation. PfNek3 was expressed at very low levels and only one clone displayed kinase activity using casein as substrate. Structural analysis of PfcGAK revealed a unique repetitive nanomeric amino acid sequence which occurs outside the catalytic domain with an unknown function. This nanomeric sequence repressed the ability of *E. coli* to produce the recombinant protein. PfNek3 contains a PEXEL secretory motif and a transmembrane region that indicates that the protein is exported to the parasitophorous vesicle and plays a role outside the parasite. This makes the protein more susceptible to inhibitors. Both PfcGAK and PfNek3 have the potential to be important drug targets in *P. falciparum* and warrant further investigation.

Aripiprazole: Role of partial agonism and its relevance in psychiatry

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All currently used antipsychotic agents bind to dopamine-2 (D2) receptors. Historically, D2 binding has been directly related to their clinical efficacy. However, in recent years a new generation of antipsychotic has been introduced that have in many ways turned our attention away from the so-called dopamine hypothesis of schizophrenia. These agents, which in many ways owe their development to the archtypal atypical antipsychotic, clozapine, have introduced the importance of especially serotonin in antipsychotic drug design, as well as the putative role of a number of other binding sites for which clozapine has high affinity. While retaining substantial D2 affinity, these new atypicals such as olanzapine, sertindole, quetiapine, ziprasidone and risperidone, in addition demonstrate a higher affinity for the serotonin 5HT2A receptor. These agents have since proved to be a major advance in the treatment of psychosis, especially their ability to more effectively address negative symptoms and their lower propensity to induce motor side effects, especially dystonia, parkinsonism and tardive dyskinesia.

Over the ensuing years, it has become apparent that serotonin plays a minor role in the antipsychotic actions of these drugs, playing more an ancillary role in improving tolerability. Moreover, new lead compounds for application in schizophrenia, but devoid of D2 receptor affinity, have repeatedly been found to be of no clinical value in schizophrenia. This has prompted researchers to re-look at the pharmacological profile of clozapine. The apparent low affinity or "loose" binding to D2 receptors now appears essential for the unique clinical profile of this drug. This new interpretation of antipsychotic action invites a closer scrutiny of how antipsychotics interact with the D2 receptor at the molecular level. Aripiprazole (Abilify®) is a new generation atypical antipsychotic that presents with a complex pharmacology with both partial agonist and full antagonist properties at D2, 5HT2A and 5HT1A receptors. The drug has demonstrated clear antipsychotic efficacy against both negative and positive symptoms, as well as having a low incidence of motor, endocrine and other side effects. Its receptor binding chemistry is unique in that it allows it to respond to different synaptic levels of endogenous transmitter in different regions of the brain, thus acting as a neuronal stabilizer. This review will take a closer look at new concepts in antipsychotic action, especially aripiprazole and the relevance and important of partial agonism in psychiatry.

The Antimalarial Activity and Cytotoxic Effects of Solvent Extracts of South African *Salvia* Species and Isolated Compounds from *S. radula*

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Purpose:

About 80% of the population in developing countries, including South Africa rely on traditional medicine for their primary care needs. The objectives of this paper are to investigate the *in vitro* antimalarial and cytotoxic effects of *Salvia* species which are currently used in traditional medicine in South Africa and to isolate the compounds responsible for the antimalarial activity from the most active species.

Method:

The hypoxanthine radioisotope and the sulforhodamine B methods used to investigate the antimalarial and anticancer activity respectively. The cytotoxicity of the extracts (methanol:chloroform) were tested on a human kidney epithelium cell line using the 3-(4,5-dimethylthiazol-2-yl)-2,5 dimethyl tetrazolium bromide (MTT) method and on three human cancer cell lines (SF-268, MCF-7 and HT-29). The active compounds were isolated using a combination of TLC and column chromatography and their characterization was done using NMR and mass spectrometry.

Results:

Solvent extracts displayed antimalarial activity with the IC_{50} values ranging from 3.91 ± 0.52 to 26.01 ± 2.95 µg/ml. Among the plants screened, *S. radula* displayed the most favorable activity, while *S. lanceolata* was the least active. Since the most active extract was that obtained from *S. radula*, this species was selected to isolate the compound(s) which may account for the observed biological activity. Two compounds were isolated, identified and characterized as; 5-hydroxy-6,7-dimethoxy-2-(4-methoxyphenyl)-4H-1-benzopyran-4-one and dammarane-3,12,25-triol, 20,24-epoxy-, (3α,12β,24R)- (9CI) and tested for antimalarial activity. The plant extracts also exhibited the ability to inhibit cell proliferation against the human kidney epithelial cells (12.12 ± 2.02 < IC_{50} < 53.34 ± 3.90 µg/ml) and three human cancer cell lines. The concentration required to inhibit 50% of cell growth (IC_{50} values) ranged between 9.69 ± 0.92 and 43.65 ± 8.38 and between 8.72 ± 1.52 and 54.40 ± 4.20 µg/ml against the MCF-7 and SF-268 cell lines, respectively. Against the HT-29 cell line, the IC_{50} values ranged from 17.05 ± 3.50 to 57.00 ± 11.67 µg/ml; with *S. lanceolata* being the most active plant. *S. africana-caerulea* was the most active against SF-268 and *S. radula* showed the best activity against the MCF-7 cell line. Results obtained in this *in vitro* study may support the traditional use of indigenous *Salvia* species as coveted ingredient in traditional headlining.

Manufacturing and development of a zeolite X coated membranes.

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Purpose:

The application of zeolite membranes in the separation of chemical compounds is becoming an increasingly important phenomenon, especially within the petrochemical industry. In this study, various methods have been considered in order to achieve the synthesis of a closed membrane with the desirable properties. The high thermal and chemical stability, cost-effectiveness in terms of energy usage and composition, as well as the better selectivity of the sodium X-membrane are only a few of the major advantages that motivate the deeper investigation into its synthesis. In summary, the main objectives of this study include the following:

- The synthesis of zeolite X-crystals that are consistent with X-ray diffraction spectra present in literature, i.e. zeolites that are without impurities.
- To obtain a pure intact zeolite membrane that attach properly to the ceramic support.
- To optimise the membrane, obtaining a high flux over, as well as a good selectivity of it.
- To investigate the zeolite's gas separation properties.

Method:

By making use of existing synthesis methods, crystals of the zeolite have been synthesised. X-ray diffraction analyses of these crystals confirmed their crystallinity and purity. After comparing these methods, the three synthesis routes that resulted in the purest products have been chosen as basis for further investigation. Minimal manipulations to these methods were applied in order to determine which of these would most easily result in a closed membrane.

An in-depth investigation has been performed into both a gel and aqueous system for the synthesis of the membrane. This was achieved by varying different parameters in the synthesis procedures, evaluating the effects using scanned electron microscopic photos. These parameters include composition of the synthesis solution, aging period, etc. The best results were fine-tuned until a closed zeolite membrane was eventually obtained.

Characterisation of these membranes was done with a simple gas flow bubble meter, measuring the flux of different gasses across the membranes. Ideal selectivities were also calculated for the gasses investigated, in order to determine the theoretical selectivities of the membranes in separating these gases.

Results:

A closed zeolite X-membrane was synthesised in a single step mechanism onto a ceramic support. Satisfactory reproducibility was obtained. XRD, SEM and gas separation studies confirmed the crystallinity and purity of the membrane.

Using a Popular Autobiography as a Teaching Tool; Medical Students' Perceptions

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Introduction:

Medical teachers participating in the problem-based learning (PBL) programme are encouraged to use innovate non-traditional teaching methods to stimulate students' interest and reflective learning.

Objectives:

In this study we assessed the feasibility and medical students' perceptions of using a popular autobiography as a teaching tool.

Methods:

Groups of third year medical students were requested, after receiving lectures on 'Cytotoxic Drugs' using Lance Armstrong's autobiography entitled; "Its Not About the Bike; My Journey Back to Life", to complete originally constructed questionnaires, with statement graded on 5-points Likert scale and open-ended questions (qualitative measures).

Results:

Majority (90%) of participants agreed/strongly agreed that the exercise stimulated their interest in cancer drugs and 85% felt that the exercise was a valuable use of their time.

Conclusion:

Using a popular autobiography to teach cancer and cytotoxic drugs is feasible, worthwhile and rewarding exercise, from the point of view of medical students. It is a stimulating, interactive and innovative method, which "facilitates and improves understanding of key pharmacological concepts".

Modification of cAMP levels and PDE4 activity through SSRI and NRI treatment in a putative animal model of OCD

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Obsessive compulsive disorder (OCD) is characterized by intrusive thoughts (obsessions) and various repetitive behaviours or mental acts (compulsions) that are performed to reduce distress caused by obsessions. Our group validated the spontaneous stereotypic behaviour of the deer mice (*Peromyscus maniculatus bairdii*) as a potential animal model of obsessive compulsive disorder. For this reason validity of empirical validity (pharmacological isomorphism) was evaluated through the differential response of the model to drugs of known efficacy in OCD, namely fluoxetine (positive control) and desipramine (negative control). Moreover, the down-stream neural messengers known to be up-regulated by antidepressants, namely cyclic adenosine monophosphate (cAMP) and activity of phosphodiesterase 4 (PDE4), were also examined. The role this second messenger and its cellular regulator in the both the frontal cortex and striatum were explored since these areas are thought to be involved in OCD pathophysiology.

The behavioural study was performed using a Digiscan Animal Activity Monitor (DAAM). This method provides automated and continual computerized monitoring of the animal and array of beams enables the computerized collection of all stereotypic and locomotor activity by a digital analyzer. Mice that generated less than 1000 counts per hour (Cph) were classified as non-stereotypic mice and those that generated more that 1000 Cph were classified as stereotypic mice. A further distinction was made between low (LSB; Cph of 1000 – 2000) and high stereotypic mice (HSB; Cph > 2000). HSB and LSB mice received 10 mg/kg dose of either fluoxetine, desipramine or vehicle for 21 days. Subsequent to treatment, mice were sacrificed, the frontal cortex and striatum dissected and cAMP levels and PDE4 enzyme activity determined.

A 10 mg/kg fluoxetine dose significantly reduced cAMP levels in the frontal cortex of HSB mice, while desipramine was unable to significantly modify cAMP levels in both LSB and HSB mice. In the striatum however, 10 mg/kg desipramine significantly reduced cAMP levels in both HSB and LSB mice. PDE4 activity was not altered by either drug treatment in the frontal cortex of stereotypic deer mice, although its doings was altered in the striatum of both stereotypic groups. Fluoxetine increased PDE4 activity in LSB mice compared to controls and interestingly, had the opposite effect in HSB mice. Desipramine, moreover, had a significant influence on PDE4 activity, with the drug increasing activity in LSB and decreasing activity in HSB.

Our data suggest that spontaneous stereotypic behaviour in deer mice respond in a similar fashion to pharmacological treatment in OCD patients, namely that stereotypy is selectively reversed by fluoxetine and not desipramine. Distinct changes in molecular parameters linked to antidepressant response, including cAMP may provide new avenues to further understand the pathophysiology of the stereotypic aspect of OCD. Regulation of PDE4 activity may be particularly appealing since drugs targeting this molecule have been used in treating disorders such as depression and Parkinson's disease. As with the differential effects on behaviour, fluoxetine and desipramine also differ in their ability to modulate cAMP and PDE4, which may reflect their differing actions on monoamines, different roles for monoamines in stereotypy, or both.

A Novel and Scalable Biocatalytic Process for the Production of Chiral Pharmaceutical Intermediates

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Purpose:

The chiral drug industry's phenomenal growth is rooted in the inherent chirality of the targeted biological systems and the consequent concern of the FDA and its international counterparts regarding the development of chiral drugs in their single-isomer *versus* racemic forms. Against this backdrop, drug companies are now also using chirality as a tool to extend the patent lives and manage the product life cycle of their blockbuster drugs, specifically by means of "racemic switching". Biocatalysis has become a major tool in the environmentally friendly production of chiral drug intermediates such as optically active epoxides and vicinal diols, which are highly versatile and are utilised in the production of chiral pharmaceuticals, agrochemicals, ferro-electric liquid crystals, flavours and fragrances. Epoxides and vicinal diols, however, often possess an inherent enzyme deactivating capacity at the substrate levels required for industrial processes, thereby limiting the full potential for the industrial application of these intermediates. Additionally, the high cost of production of the epoxide hydrolase (EH) biocatalyst employed in their production results in the continual use of *chemical* chiral methodologies. The development of a scalable biocatalytic process with acceptable EH activity, stability and volumetric productivity is thus an apt topic for research.

Methods:

A comprehensive screening of more than 500 indigenous wild-type yeast strains for enantioselective EH activity towards an extensive range of epoxide substrates from different structural classes was performed. The enantioselective EH activities identified in wild-type yeasts were amplified in a novel recombinant *Yarrowia lipolytica* yeast expression vehicle, the latter being employed either as an engineered whole-cell EH biocatalyst, or for EH enzyme over-production. The resulting whole-cell biocatalysts were formulated and dried for use as "off-the-shelf" dry powder catalysts. The reactions were scaled-up to 5L scale and volumetric productivities were optimised under biphasic conditions, often in the presence of a third phase inhibitor for substrate solubilisation and/or enzyme stabilisation.

Results:

This project demonstrated the applicability and scalability of a biocatalytic process employing genetically engineered epoxide hydrolase enzymes of yeast origin, for industrial production of optically active epoxides and vicinal diols. These products can be successfully used as intermediates in the production and/or racemic switching of a number of chiral blockbuster drugs.

The Design of Patient Information Leaflets for the Unique South African Situation: Suggestions from a Document Design Perspective

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Purpose:

Package inserts are the only information provided with the majority of medicines sold in South Africa. However, one document cannot satisfy the print information needs of both health professionals and consumers. Our Constitution specifically provides for the right to information, with the Promotion of Access to Information Act coming to force in 2001. In 2004, the Medicines Control Council published its "Guideline on the requirements for patient information leaflets", requiring the inclusion of a patient information leaflet (PIL) with all medicines sold. However, the few PILs currently available differ widely in text quality and the readability levels are in general too high, considering the literacy levels and linguistic diversity of the readership. The purpose of this multidisciplinary study was firstly to determine the communicative effectiveness of a PIL included with a well-known anti-malarial product, and secondly to determine the predictive validity of *expert-focused* research and the possibility of omitting other, more time-consuming methods. The feedback was intended to provide cues for revision, resulting in a redesigned document that provides sufficient information for safe and effective use, in a format that is maximally efficient for the unique South African target audience.

Methods:

The chosen PIL was evaluated by combining a *reader-focused* evaluation method with an *expert-focused* method, both well-known procedures in the field of document design. Experts in the field of pharmacy were invited to respond to the style and content of the PIL and its appropriateness for an audience ranging from low-literate to highly literate. Respondents for the *reader* research comprised two groups, representing the two extremes of the literacy spectrum. A questionnaire was distributed, comprising questions pertaining to comprehension, as well as questions that elicited the opinions and experiences of participants regarding the PIL content and style. A set of heuristics was subsequently distilled to serve as a style sheet for designing PILs across the literacy spectrum. The PIL was redesigned according to this set of heuristics and *effect research* was conducted among participants with similar socio-demographic profiles as the original group, using the same questionnaire.

Results:

This study indicated that it is sufficient to perform only an *expert* testing of PILs. Whereas *reader*-testing usually yields the most useful suggestions for revision in the evaluation of other documents, *experts*, specifically those in the fields of pharmacy practice and pharmacotherapy, were competent in identifying the problems that authentic readers – especially low-literates with concomitant underdeveloped metacognitive reasoning abilities – experience with comprehension. They were also able to translate them into concrete suggestions for revision. The reader-focused comprehension test proved the original PIL to be inadequate as an instructive and informative document, since the respondents were unable to find and understand some of the essential information. On the other hand, the newly designed PIL was proven to greatly enhance comprehension. In conclusion, this study demonstrated that the evaluation strategies employed in the document design process are useful both as diagnostic tools and as criteria for *design protocols*.

Isolation of selected Bio-active Lactones from South African *Helichrysum* species

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Purpose:

Several species from the plant genus *Helichrysum* (Asteraceae) are used in traditional medicine to treat a range of conditions[1]. The purpose of this study is twofold. Firstly, uninvestigated species from this large genus (*ca*. 245 species) with possible biological activity was identified and their chemical constituents were isolated. This was followed by the identification of potential bio-active molecules and the selection of one of these for organic synthesis and biological testing.

Methods:

Both column and centrifugal chromatography were used in the isolation of compounds from the chloroform:methanol extracts of *H. montanum* and *H. excisum*. Structures were elucidated using various 1D and 2D NMR techniques as well as mass spectrometry. Overlapping proton signals prevented the unambiguous determination of the stereochemistry by coupling constants, NOESY and other two-dimensional NMR experiments. Molecular modelling was thus done with Spartan® to assist with determination of the stereochemistry. Minimum Inhibitory Concentrations (MIC's) were determined with a 96 well microplate method and determination of the antimicrobial fraction of the extract from *H. excisum* was done with an autobiography assay.

Results:

An unusual group of compounds, namely guaianolides, have been isolated from *H. montanum.* The chloroform:methanol extract of *H. excisum* exhibited promising antibacterial activity. Fractionation of this extract resulted in the isolation of several flavonoids and an interesting lactone, lepidissipyrone, which is responsible for the antibacterial activity of the extract. Lepidissipyrone exhibited MIC values of 78 µg/ml against both *Bacillus cereus* and *Staphylococcus epidermidis*. The activity of similar lactones against HIV-1 protease[2] prompted the selection of this compound for synthesis. The synthesis of this lactone is currently in progress.

References:

1.J.M. Watt & M.G. Breyer-Brandwijk The Medicinal and Poisonous Plants of Southern and Eastern Africa. 2nd ed. Livingstone, London, (1962).

2. S. Thaisrivongs, L. Romero, R.A. Tommasi, M.N. Janakiraman, J.W. Strohbach, S.R. Turner, C. Biles, R.R. Morge, P.D. Johnason, P.A. Aristoff, P.K. Tomich, J.C. Lynn, M-M. Horng, K-T. Chong, R.R. Hinshaw, W.J. Howe, B.C. Finzel, K.D. Watenpaugh, Structure-based design of HIV protease inhibitors: 5,6-dihydro-4-hydroxy-2-pyrones as effective, nonpeptidic inhibitors, J.Med. Chem. 39, 4630-4642, (1996).

Quantity and Quality in Pharmacy Education can be maintained with a Problem-Based Learning Approach

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Background:

With the change to a democratic government in South Africa in 1994, there was a move to redress previous unequal access to education, whilst maintaining the quality of graduates to meet the stringent requirements of statutory bodies. The BPharm programme at the University of Limpopo (Medunsa Campus) in partnership with Tshwane University of Technology (TUT) follows an integrated, thematic, problem-based, modular approach. Student selection aims at redressing previous educational inequalities. Students are accepted on their academic results, appropriate training, potential tests and interviews. Final selection is based on Medunsa's policy of demographic representation of all racial groups of South Africa.

Method:

Quantity: The selection data from 1999 and 2001 were compared against the final marks of the first year and final (4^{th}) year groups. Selection results were compared with academic performance. Pass rates and attrition rates were calculated of the 1^{st} to 2^{nd} and 1^{st} to 4^{th} years. Experiential Learning Tool to prepare BPharm students for practice.

Quality: July 2003 and 2004 SAPC pre-registration examination results used to measure graduates in terms of their attaining the knowledge and skills required of entry-level pharmacists (based on the Unit Standards set by SAPC).

Results:

The Table shows pass and attrition rates from First to Second Year and First Year to Graduation for the 1999-2001 student intakes.

Table: BPharm Admission and Pass/Attrition Rates by Year of Study

	Number enrolled	First to Second Year			First Year to Graduation			
		Number passed	Pass rate	Attrition rate %	Year of graduation	Number graduated	Pass rate	Attrition rate %
1999 intake	30	29	97%	3	2002	28	93%	7
2000 intake	35*	32	91%	11	2003	30	83%	17
2001 intake	44*	44	100%	4	2004	31	93%	7
Overall	**109**	**105**	**94%**	**6**		**89**	**90%**	**10**

* Repeaters were excluded from the figures.

Conclusion:

The high pass rate did not result from a low standard of assessment, but from the combination of the following factors: selection mechanism, education, learning/teaching, assessment, pre-emptive support systems, dedicated, skilled, committed, motivated staff and students. The positive performance of the BPharm students before and after graduation when compared to pharmacy interns from other Pharmacy Schools regarding progress tests and pre-registration examinations, confirms the effectiveness of the Medunsa/TUT BPharm curriculum. Hence quantity and quality in Pharmacy Education can be maintained with a Problem Based Learning Approach.

From Traditional Medicines to Traditional Rituals: the good, the bad and the ugly - Traditional Male Circumcision in the Eastern Cape

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Introduction

Just as traditional medicines, particularly in the wrong hands, may be harmful, so can traditional rituals pose a serious threat to health in the absence of experience, proper care and hygienic conditions. A case in point is the traditional male circumcision which is still widely practiced amongst the Xhosa population in the Eastern Cape. Yearly, botched circumcisions lead to mutilations and deaths. In 2001, the Application of Health Standards in Traditional Circumcision Act was passed to ameliorate the problem.

Methods

The present study was undertaken to investigate if the Act was indeed successful. The statistical data was provided by the Eastern Cape Department of Health. Hospital admissions, mutilations and deaths per circumcision season were recorded as well as the total number of initiates and the number of legal and illegal initiation schools, respectively. At the same time the ritual and its cultural significance were explored through an extensive literature review.

Results

The findings showed that the incidence of circumcision-related complications and fatalities has remained virtually unchanged in the observation period from 2001 to 2006. So far, therefore, the above Act has not yet achieved any decrease in botched circumcisions.

Discussion

Traditional male circumcision has retained its cultural significance as a rite of transition from childhood to adulthood. Unfortunately, botched circumcisions leading to gangrene and even fatalities still abound. Unqualified traditional surgeons, negligent traditional nurses, unhygienic conditions and youths medically unfit for the hardships of initiation are the main obstacles indicating an extensive lack of compliance with the above Act. One of the main underlying reasons is the perception that government interference in this traditional ritual is undesirable and widely rejected.

Conclusion

A solution is only possible if all the relevant stakeholders – traditional surgeons, traditional nurses, the House of Traditional Leaders, representatives of the Department of Health, medical officers, initiates, parents and the communities concerned, can be made aware of the problem and rendered willing to work together in preserving a cultural tradition in the spirit of the Constitution, that is, without violating fundamental human rights.

Experiential Learning – A practice-based learning approach in the UL (Medunsa Campus)/TUT BPharm Programme

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Objectives:

The objectives of this paper are i) to describe the experiential learning component of the University of Limpopo (UL) (Medunsa Campus) / Tshwane University of Technology (TUT) BPharm programme; ii) to describe the assessment methods used by staff and by mentors at the experiential learning sites and iii) to present the outcomes of the assessments

Method:

Experiential learning, which forms an integral part of the practice-based approach of the UL (Medunsa Campus)/TUT BPharm programme, is used to bridge the gap between pharmacy education and pharmacy practice. One module in each year of study focuses on experiential learning and is divided into three phases. Phase I: Students are introduced to the topics within the module at the School of Pharmacy and prepared for the experiential attachment. Phase II: Students spend four weeks at the experiential learning sites (Primary Health Care, hospital-, community- and industrial pharmacy). Phase III: Students return to the campus to compile their reports and prepare presentations for final assessment.

Results:

Students are assessed internally by staff and externally by mentors at the experiential learning sites. The results for the experiential learning modules (2004 and 2005) showed that BPharm students perform very well in a practice setting. The marks of external assessments by mentors were higher than the internal assessments by staff. It must be acknowledged that high marks from mentors could be attributed to mentors being too lenient in the allocation of marks. However, student reflection on the experience and very positive reports from mentors on the performance and professional behaviour of students within the practice setting, confirm these positive results.

Conclusion:

Positive comments from practising pharmacists about the competence and attitude of our students validate the preparedness of our graduates for practice. Experiential learning has proven to be important for an outcomes-based programme, as it does not only prepare students for practice but also gives students an opportunity to demonstrate their knowledge and competence at an early stage of learning.

Exploring Student Attitudes to Learning Pharmacology: A Qualitative Analysis

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Introduction:

Pharmacology, the science of drug action on biological systems, is a para-clinical discipline, connecting basic science to pharmacotherapeutic skills. It is a key subject in the curriculum design of the Bachelor of Pharmacy course, since mastery of the field is intrinsic to professional practice. In the Faculty of Health Sciences, University of the Witwatersrand, this subject is currently taught using a Behaviourist epistemological approach. Throughput rates confirm the effectiveness of this paradigm when measured using a multiple choice-style examination. However, the technologically-driven exponential growth in biomedical knowledge, coupled with novel psychological approaches to effective lifelong learning, give rise to concerns regarding Behaviourist methodology. As a result, there is now widespread interest in shifting pharmacy education from factual-based programmes to those which prepare students to locate, critically appraise and apply new information to their practice of the profession.

Objective:

As a pilot study preparatory to investigation of pedagogical issues in pharmacology, this study was carried out to interrogate students' perceptions of different learning experiences.

Methodology:

Third year Pharmacy students participated in an initial workshop to set personal objectives for a 20 hour psychopharmacology module and then synthesised group and class-wide objectives. These objectives were documented anonymously and returned to the lecturer for analysis. Teaching activities in the module were then divided equally between 2 techniques; traditional lectures and student self-prepared tutorials. After this exposure, students' perceptions of their learning experiences were interrogated by means of an anonymous questionnaire.

Results:

The majority of the class (72%) preferred the lecture format, stating inertia, lack of time for the self-preparation method, and lack of confidence in determining the importance of peers' information, as the reasons for their choice. Fourteen percent of the class chose the self-preparation tutorials as their preferred learning experience, clarifying that preparing the material before the tutorial improved their understanding and facilitated their participation. The remaining 14% of the group listed advantages of both methods. Correlation of student learning experience choices with negotiated learning outcomes highlighted a lack of alignment between stated outcomes, teaching and learning activities and assessment.

Conclusion:

The content-driven assessment method utilised in the Division of Pharmacology encourages surface or strategic learning, which is detrimental to the achievement of "higher order" learning outcomes perceived by students to be desirable in this subject. Further study of outcomes, curriculum, teaching and learning opportunities and assessment methods in this subject is warranted.

Comparative *in vitro* Permeability of Cyclosporin A and Tacrolimus across Human Vaginal Mucosa

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Objectives:

Immunosuppressants may be applied, topically, to mucosal surfaces for treating inflammatory diseases, e.g. vulvar lichen planus and Behçet's disease. The purpose of this study was to investigate, and compare, the *in vitro* diffusion of Tacrolimus (Tac) and Cyclosporin A (CsA) across human vaginal mucosa.

Methods:

Fresh human vaginal mucosa was snap-frozen in liquid nitrogen and stored at -85 ºC. Prior to an experiment, the tissue was defrosted to 20 ºC in PBS buffer, pH 7.4, and placed in the seven flow cells of a flow-through perfusion apparatus. Either tritiated Tac, or CsA, was then pipetted into the donor chamber of the flow cell. Samples from each flow cell were collected every 2 hours (1.5 ml/h) over a 24-hour period. Statistical analyses were carried out using an F-test.

Results:

CsA and Tac flux values progressively increased throughout the 24-hour period and steady state was not reached for either drug. The mean estimated flux values (mean at 16, 20 and 24h) for Tac (599 ± 44 dpm.cm-2.min-1) were greater than those for CsA (96 ± 5.0 dpm.cm-2.min-1). Whole curve comparison indicated statistically significant differences (P = 1.77x10-163).

Conclusions:

Both drugs diffuse across vaginal mucosa. Mean steady state flux values for Tac were approximately 6 times higher than those for CsA, indicating that human vaginal mucosa is less permeable to CsA than to Tac.

The Acetone Extract of *Combretum woodii* Leaf inhibits the Respiratory Burst of Isolated Human Neutrophils.

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Introduction:

Neutrophils form part of the first line defence of the body against invading pathogens. The cells contain bactericidal lysosomal enzymes and a membrane bound NADPH oxidase which mediates the respiratory burst. In vivo activation of neutrophils occurs by the binding of invading microorganisms to the specific receptors on the neutrophil surface which activates the NADPH mediated respiratory burst. The respiratory burst is an increased oxygen consumption and the formation of reactive oxygen species (ROS) that includes superoxide anion (O_2-), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$) and the singlet oxygen (O_2). *C. woodii* leaf extracts have antibacterial, anti-inflammatory and antioxidant activity.

The aim of the study was to investigate the effects of the acetone extract of *C. woodii* on the superoxide production of human neutrophils activated by N-formyl-methionyl-leucyl-phenylalanine (fMLP) and phorbol myristatse acetate (PMA).

Materials and method.

Dry, milled leaf material of *C. woodii* was extracted with an acetone:water (10:1) solvent. Blood samples were collected from consenting healthy volunteers and the neutrophils were isolated by the Percoll sedimentation method. Biochemiluminescence assay of the neutrophils was performed on the 1251 Luminometer from Bio-Orbit. The neutrophils were incubated with diluted solutions of the acetone-water solvent and the acetone-water extract, each in duplicate. The neutrophils were activated with PMA, a protein kinase C activator and fMLP, a chemotactic receptor agonist, and their superoxide production (SOP) was measured on the luminometer over a period of 30 minutes. The luminescence assay was performed six times each for both fMLP and PMA, on different samples of neutrophils each time.

Analysis of extract: Solid phase extraction (SPE) was performed on the crude acetone water extract on Supelclean ™ LC-18 SPE columns, using the solvents methanol-water (65:35), hexane and chloroform respectively. Complexity of the extract was investigated by thin layer chromatography (TLC) on Merck Kieselgel 60 F_{254} plates with three different mobile phases. High performance liquid chromatography (HPLC) was done on a Shimadzu HPLC system with a diode-array detector.

Results and discussion

The acetone water solvent inhibits the SOP at higher concentrations and has no inhibitory effect at 10% and 20%. The acetone water extract completely inhibits the SOP of neutrophils activated by both PMA and fMLP equally. Some minimal SOP occurs only at very low concentrations of the acetone water extract (1% and 2%).

TLC analysis of the extracts shows the presence of majority of non-polar and intermediate polarity compounds and only a few polar compounds.

HPLC confirms the presence of more non-polar and intermediate polarity compounds in the crude acetone-water extract. These compounds had retention times that reduced when the mobile phase was made to be more non-polar.

Conclusion:

The inhibition of the SOP, hence the respiratory burst of the neutrophils, is attributed to the many different compounds present in the extract. This effect, which is significant even at concentrations as low as 4% extract, may be due to the compounds acting in synergy. The isolation, purification and identification of these compounds will benefit the search for anti-inflammatory compounds.

Development of a Symptom Focused HRQOL Tool for Patients on Highly Active Antiretroviral Therapy

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Introduction and Purpose Of Study:

Health Related Quality of Life (HRQOL) shifts the assessment of health outcomes from the absence of disease to the assessment of the patients' state of physical, mental and social well being[(1)]. Emphasis on identifying and managing patients' symptom status could improve HRQOL.[(2)] Current methods of assessment with open ended questions are insensitive, patients response may be restricted to be socially desirable [(3)] , and time and physician resources are limited. Existing tools that have been developed for research are not reliable for use in a clinical setting [(3)(4)]. Development of a symptom - focused HRQOL tool to satisfy SA monitoring and pharmacovigilance needs is therefore needed.

Methodology:

Development began with a review of symptoms and HRQOL descriptions, and their weighted frequencies, in published research. The data was consolidated into a patient questionnaire administered to 48 patients at 2 academic hospitals in Gauteng. The questionnaire comprised evaluation of sociodemographic, HRQOL and symptom data. Open text field questions were included to assess comprehensiveness and comprehensibility.

Results and Discussion:

The patient group comprised 96%black, 67% female and 77% maintained on Stavudine, Lamivudine and Efavirenz. The average age was 35.4years and the average duration of treatment was 4.5months. 21% had a primary school education. In open ended questions patients felt that the questionnaire was understandable and covered the most relevant aspects with only a mental well being scale and weight loss scale to be added on. Analysis of Variance showed HRQOL scales as fairly consistent across the group except sexuality. (P=0.9229). This will need to be addressed directly as a symptom. The most common symptoms were sadness(67%) tiredness(65%), headaches(58%), anxiety(57%), dizziness(50%), tingling(46%) and sleep abnormalities(45%). Symptom frequency correlated well to symptom bother in most symptoms except the psychiatric symptoms. Rephrasing may be needed to overcome the stigma of psychiatric illness to gage the extent of bother in this cultural group. The late manifesting adverse effects were poorly represented and must be accounted for.

Conclusion:

Routine clinical evaluation of symptom focused HRQOL is imperative in the care of HIV patients especially since HIV treatments invoke significant ADR's that diminish HRQOL. The tool should be self administered and user friendly and should measure fatigue, depression, anxiety, sleep disturbances, pain, nausea, vomiting, sexual dysfunction, actual and perceived body image. The routine use should assist drug related toxicity identification which would impact patients' perception of health status, adherence and long term survival.

References:

(1)Spitzer WO(1987) Quality of life and functional status as target variables in research. Journal of Chronic diseases, *40*, 465-471

(2)Justice AC(1999) Sensitivity, specificity, reliability and clinical validity of provider reported symptoms Journal of Acquired Immune Deficiency Syndrome , *21*, 123-133

(3)Justice AC(2001) Development and validation of a self completed HIV symptom index. Journal of Clinical Epidemiology, *54*, 77-s90

(4) Wu AC (1992) Measuring health status and quality of life in HIV and AIDS. Psychology and Health ,*6*, 251-264

Peroral and nasal delivery of Insulin with Pheroid Technology

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Purpose:

Since its initial discovery in 1922 by Banting and Best, the formulation of an oral insulin delivery system has ever been so troublesome. Factors contributing to difficulties in oral formulations of insulin are the gastro-intestinal (GI) tract that is home to various protein digestive enzymes such as pepsins in the stomach and trypsin, chymotrypsin and carboxypeptidases in the small intestine that digests insulin and also the physical barrier of the GI tract i.e. the columnar epithelial layer, which lines the GI tract and is a tightly bound collection of cells with minimal leakage and is thus a sound barrier for the absorption of peptides and hormones. Insulin is also hydrophilic and has a high molecular weight, hence its poor absorption. Unfortunately insulin is indispensable in the treatment of diabetes mellitus (DM), which affects approximately 350 million people worldwide. The aim of this study is to partly develop an oral formulation for insulin to overcome these barriers and to successfully deliver insulin at the site of action.

Methods:

The phases of the study consisted firstly of the manufacturing of Pheroid, entrapment of Flourescein-isothiocyanate labeled insulin (FITC-insulin) into the Pheroid and analysis of the Pheroid-insulin complex with confocal laser scanning microscopy (CLSM) to determine drug loading. Secondly an *in vivo* administration experiment in Sprague - Dawley rats was done where blood glucose levels as well as insulin plasma levels were monitored. A standard reference was set by subcutaneous injection of insulin (0.5 IU/kg body weight of insulin) followed by a comparative study where administration to the stomach, colon and intestine (50.0 IU/kg body weight of insulin) were compared by means of plasma insulin levels and therapeutic effect between the control and Pheroid complexes (Pheroid vesicles and micro sponges) to determine the most effective site of absorption. Each study was done by direct injection into the stomach, ileum or colon through which the insulin in saline (control) or insulin–Pheroid complex was administered. Nasal administration of 8 and 12 IU/kg body weight of insulin in saline (control) or insulin-Pheroid complex was also made in the right nostril of Sprague - Dawley rats. Blood samples were taken just before administration and then at 5, 10, 15, 30, 60, 120 and 180 minutes. Blood glucose levels were measured after each blood sample was collected and plasma insulin levels were determined with a human insulin specific radioimmunoassay.

Results:

The ileum showed by far to be the most effective area of absorption where Pheroid vesicles showed an 18.7 % lowering in blood glucose levels after 5 minutes and a peak of 42.0 % lowering in blood glucose levels after 60 minutes and a peak plasma insulin concentration of 244.0 µIU/ml after 5 minutes. After nasal administration of Pheroid micro sponges (8.0 IU/kg body weight of insulin) a remarkable lowered blood glucose level of 19.2 % after 10 minutes and 36.5 % after 30 minutes and a peak plasma insulin level of 220.2 µIU/ml after 3 hours were obtained. Pheroid micro sponges administered with 12.0 IU/kg body weight of insulin showed a maximum blood glucose lowering effect of 72.4 % after 3 hours with a peak plasma insulin level of 154.8 µIU/ml after 3 hours, thus showing a long acting effect. In conclusion, the delivery system based on Pheroid technology shows a sufficient therapeutic effect for insulin and therefore shows to be promising for further *in vivo* evaluation and ultimately for pharmaceutical use to patients suffering from DM.

Training the Next Generation of Pharmacists

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Introduction:

The bachelor of pharmacy (BPharm) training programme that is presented by the Schools of Pharmacy at the Tshwane University of Technology in partnership with the University of Limpopo (Medunsa Campus) is based on outcomes- and problem based learning techniques. This presentation deals with the different teaching methods used in the programme and how innovative methods of higher education are employed and improved.

Teaching methods:

The application of innovative teaching methods such as real-life scenarios, workshops and debates are described as well as some of the improvements and changes implemented during the application of problem based education. The assessment of learners after small group discussions and the use of electronic aids in pharmacy education and their implications are also explored.

Conclusions:

The teaching philosophy of training learners to be better pharmacists than the facilitator and the results obtained during this problem-based programme indicates that the desired outcomes are achieved such as a high throughput rate of students with a satisfactory level of knowledge and skills.

The Antibacterial and Anti-oxidant Activity of South African Indigenous *Commiphora* Species and the Isolated Compounds from *C. glandulosa*

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Purpose:

Commiphora species (from which Myrrh is obtained) has been a source of several novel and bioactive natural compounds. The botanical diversity of this genus in South Africa warrants a study of this plant group, to provide scientific evidence for the traditional use of *Commiphora* species in African healing rites. Traditionally members of this genus are used in southern Africa for the treatment of ulcers, fevers, and as a remedy for snake and scorpion bites. The resin of some *Commiphora* species is applied topically for wound healing. Documented uses of *Commiphora* include antibacterial and antifungal properties, as well as anti-oxidant activity.

Methods:

In vitro antimicrobial efficacy was determined against Gram-positive, Gram-negative bacteria and yeasts using the MIC microtitre plate assay. Using death kinetics studies (time-kill studies), the rate at which the antimicrobial agent kills pathogens over a 24-hour period was determined. The *in vitro* anti-oxidant activity of the leaf and stem extracts of ten *Commiphora* species was investigated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and the 2,2'-azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid) (ABTS) assays. Isolated compounds were subjected to the DPPH assay to determine the anti-oxidant potential of each of the compounds, separately and in combination to determine possible synergistic, antagonistic or additive interactions.

Results:

Commiphora marlothii (stem) was identified as a suitable candidate for the death kinetics assay (MIC = 1 mg/ml against *S. aureus*). The antibacterial activity was observed to begin at ca. 30 min of the exposure of *S. aureus* to the different concentrations of plant extract, as observed through the reduction in colony forming units (CFU) over time. All concentrations exhibited antibacterial activity, with complete bactericidal effect achieved by all test concentrations by the 24^{th} hour. Extracts generally exhibited poor anti-oxidant activity in the DPPH assay, with the exception of *C. schimperi* (stem), *C. neglecta* (stem), *C. tenuipetiolata* (stem and leaf), and *C. edulis* (stem), which possessed IC_{50} values ranging between 7.31 μg/ml and 10.81 μg/ml. The flavonol, kaempferol (IC_{50} = 3.32 μg/ml) showed exceptional radical scavenging activity, in contrast to the activity displayed by dihydrokaempferol (IC_{50} = 301.57 μg/ml), their combination being antagonistic. The results obtained in the ABTS assay differed significantly from the results obtained in the DPPH assay, with a greater anti-oxidant activity observed for most of the species. The best activity was observed for the stem extracts of *C. neglecta* (IC_{50} = 7.28 μg/ml) and *C. mollis* (IC_{50} = 8.82 μg/ml).

A gender specific dose-related response shown towards 6-OHDA in developing rate CNS

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The injection of 6-OHDA into the brains of rats, is commonly used as an animal model to study Parkinson's disease. However most of these studies used male animals alone while the disease is also evident in females.

The present study therefore focused on possible gender-effects at various doses of 6-OHDA. In doing so, the medial forebrain bundle of 35 day old male and female Sprague-Dawley (SD) rats were stereotaxically lesioned with a high and low dosage (0.4 and 10.0 mg/kg body weight) of 6-OHDA dissolved in 0.1% L-ascorbic acid. Control rats received equivalent saline injections. Either of the solutions were administered to subjects that were randomly assigned to one of possibly 3 treatment groups (n=3 per gender, n=6 per dosage-group).One week post-surgery, the rats were subjected to an assessment battery, comprised of neurological tests which are sensitive to dopamine loss. These were repeated again at 2 weeks post.

Significant differences in locomotor activity were observed between the two genders when the higher dosage (10mg/kg) was administered. The results indicated that the single-limb akinesia test, the vibrissae-elicited forelimb placing test, as well as the behaviour in the Open Field maze, conducted under standard-conditions, can be useful measures to identify subtle motor differences between male and female rats treated with 6-OHDA. These differences may be important when studying Parkinson's disease.

Prenatal stress decreases the beneficial effects of exercise on motor function in rats given intracerebral injection of 6-OHDA

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Stress experienced during the perinatal period is known to have long-term effects. In order to investigate whether prenatal stress increases the vulnerability of dopamine neurons to toxic insult in adulthood, two models of mild prenatal stress were investigated. Dams were either 50% food-deprived or subjected to a series of mild stressors (single overnight food deprivation, 7-hour shift in the light-dark cycle and handling) during the third week of gestation. At 60 days of age (P60), offspring did not display any difference in behaviour in the elevated plus maze or in the corticosterone response to restraint stress, suggesting absence of anxiety. Both of the prenatally stressed groups displayed slightly blunted ACTH responses to 10-min restraint stress. However, whereas the food deprived group showed no detectable behavioural changes, the 'mild stressor 'group displayed decreased locomotor activity in the open field, suggesting lasting effects on neuronal circuits that control motor function. The mild stressor model was used to determine whether exercise protected against the toxic effects of 6-hydroxydopamine (6-OHDA) in prenatally stressed rats. At P54, offspring of dams subjected to either the mild stress regimen or non-stressed rats, were placed in individual cages with attached running wheels for one week prior to injection of 6-OHDA (5 µg/4 µl) into the medial forebrain bundle. Non-runner rats were housed in individual cages without running wheels. Fourteen days after infusion of 6-OHDA, rats were removed from their respective cages and tested for forelimb asymmetry and akinesia. As expected, voluntary exercise significantly reduced the behavioural effects of 6-OHDA. However, this ameliorative influence was significantly reduced by prenatal stress. We are currently exploring the neuroanatomical correlates of stress-induced blockade of the protective effects of exercise. This work was supported in part by NIH Fogarty International Center grant DA018087.

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Antiretroviral treatment programme in Grahamstown's public health sector

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Purpose:

South Africa is highly affected by the Human Immunodeficiency Virus. The prevalence rate determined by testing pregnant woman is estimated to be 29.5%. By the end of 2005 there were 983 000 people in need of antiretroviral treatment (ART) in the country while only 178,000-235,000 received this treatment. In Grahamstown the roll out of the national treatment programme was initiated in May 2004, and at present there are about 500 patients receiving ART from hospitals and local clinics in the town. By carrying out structured interviews with health care professionals, this ongoing study analyzes the follow up and care of HIV patients benefiting from the roll out of ART.

Method:

Participating observation: Prior to the interviews, observational studies were carried out in two hospitals and one clinic to gather background information.

Structured interviews: The sampling group for data collection included 54 health care professionals (HCPs) from all categories of staff working with HIV positive patients in the governmental hospitals and clinics. Structured interviews were carried out to gather data on the following topics: the role of different HCPs in HIV related work, training of staff involved, workload, patient and HCP interaction, and HCP cooperation within and between the clinics. The interviews provide information to analyze the use of human resources in treatment, follow up and care of HIV patients.

Results:

As the number of patients receiving treatment is still very low compared to the need, the programme is growing continually. Though the number of patients receiving ART in local clinics is small, HIV patients require special care and attention, and therefore this requires more time and focus in the clinics. This leads to a great challenge for HCPs who are already coping with stretched limits with regard to workload and opportunity to provide each individual with sufficient health care. All the interviewees pointed out that work with HIV patients requires specific training, but sufficient training has not yet been provided for all HCPs. The majority of the interviewees acknowledged the need for prioritising within their work for HIV patients due to time constraints being one of the limiting factors.

HPLC/MS/MS Technology for Analysing Substances of Abuse

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Objectives:

Substance abuse, worldwide, is an ever increasing problem. Positive characterisation of individual compounds within a particular chemical group may be difficult using routine methodology based on immunoassays. Because precise identification is often required for clinical and legal purposes, it was the objective of the present study to apply HPLC/MS/MS technology to compose a library of mass spectra for detecting and quantifying common substances of abuse.

Methods:

Standard samples of common substances of abuse were obtained and used to set-up an Agilent HPLC instrument coupled to an Applied Biosystems MS/MS 2000 system. Following HPLC separation, substances were identified on their HPLC retention times as well as on their respective precursor and product ions. The presence of a substance was confirmed by calculating the ratio of the peak area of the respective precursor and product ions. Urine samples from 50 patients, in which substances of abuse could not be clearly identified when assayed by immunoassay techniques, were re-analysed using the HPLC/MS/MS system.

Results:

HPLC retention times and mass spectra were obtained for 36 substances of abuse. Detection limits of the analytical system were found to be as low as 2 ng/ml. Substances of abuse in the urine samples from the 45 patients were positively and quantitatively identified by means of the HPLC/MS/MS system. In some instances abused drugs were identified which were not detected by the normal immunoassay methodology.

Conclusions:

Compared to immunoassay techniques, HPLC/MS/MS methodology is a powerful and efficient tool for identifying and quantifying substances of abuse at very low concentrations.

The effects of polymeric cross-linking on the *in vitro* dissolution of amitryptiline from a PLGA-based monolithic system

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Purpose:

Amitriptyline is a tricyclic antidepressant used in depressive illness of psychotic or endogenous nature and in selected patients with neurotic depression. It undergoes extensive first-pass metabolism with a systemic bioavailability and an apparent volume of distribution of about 45% and 19 L/kg, respectively. The aim of this study was to develop a novel monolithic oral formulation capable of displaying a 24-hour zero-order kinetics *in vitro* dissolution for amitriptiline hydrochloride. Furthermore, this study intended to verify the effect of cross-linking on drug release.

Methods:

This system is based on the salting-out and subsequent cross-linking of the polymer in the presence and in the absence of the drug. Formulations of either drug-free PLGA or drug-loaded PLGA samples were salted-out at various concentrations based on a Box-Benhnken statistical design. Discs were then formed by direct compression of the mixture of 300mg salted-out PLGA and 50mg amitriptiline for the drug-free PLGA and 350mg of each drug-loaded variants were also compressed. The dissolution studies were conducted in 500mL phosphate buffered saline pH 7.4 at $37^0C \pm 0.5^0C$ using a USP rotatable paddle at 50 rpm. Amitryptiline assays were performed with UV-spectroscopy (240 nm) over 24 hours. Drug release profiles were plotted as a function of time and the influence of cross-linking on the amount of drug released was elucidated.

Results:

Dissolution studies demonstrated the differences in drug release with 50% to 78% in 24 hours from the discs prepared by cross-linking the polymer on its own. Discs prepared by cross-linking the drug with the polymer in various salts showed a percentage release of less than 20% in 24 hours. Drug release kinetics were illustrated by $M_t/M_\infty=k_0t$, with release rate constant k_0 of 0.007 to 0.03. This study demonstrated that cross-linking significantly reduced drug release due to the strong bonds between the drug and the polymer.

Transplantation of Pre-differentiated Mouse Embryonic Stem (ES) Cells in 6-OHDA-lesioned Rats.

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Purpose:

Parkinson's disease (PD) is a neurodegenerative disorder characterised by a gradual loss of dopaminergic neurons in the nigrostriatal pathway. Transplantation of foetal ventral mesencephalic cells has been studied in rat models of PD and parkinsonian patients. Difficulties in obtaining sufficient donor brain tissue have limited the clinical application of this therapy and shifted the focus towards the use of embryonic stem cells. Undifferentiated mouse ES cells differentiate into functional dopaminergic neurons after grafting in hemiparkinsonian rats but induce the formation of tumours, a problem which might be circumvented by grafting pre-differentiated ES cells. Our aim was to study the fate of pre-differentiated mouse ES cells transplanted in 6-OHDA-lesioned adult rats.

Methods:

Mouse ES cells were differentiated on a PA6-feeder for 14 days. 25 – 30% of the obtained neurons were positive for tyrosine hydroxylase. The cells were labelled with PKH26, a fluorescent membrane dye, and grafted as a suspension in the striata of hemiparkinsonian adult rats. Graft recipients were immunosuppressed by daily injections of cyclosporine A (10mg/kg) to prevent xenograft rejection. Rats were challenged with amphetamine pre- and post-grafting to assess the extent of the lesion and functional effects of the grafted cells. The survival and integration of the grafted cells and the host responses were analysed at five weeks post-grafting using immunohistochemistry and in vitro receptor autoradiography.

Results:

Mouse ES cell-derived dopaminergic neurons survive intrastriatal transplantation in hemiparkinsonian adult rats, express tyrosine hydroxylase and vesicular monoamine transporter (both of which are involved in dopaminergic neurotransmission) and alleviate amphetamine-induced rotation in graft recipients. Cyclosporine A immunosuppression improves the survival of grafted dopaminergic neurons but is associated with increased risk of tumour formation and increased morbidity. Grafted dopaminergic neurons lose neurites over time and demonstrate limited reinnervation of the host striatum. The number of striatal D1 receptor binding sites was not altered by denervation and/or the grafting of dopamine-producing cells. In contrast, denervation induced a loss of dopamine transporter binding in the lesioned striatum. This loss was not reversed by ES cell grafts at five weeks post grafting.

Permeability of Human and Porcine Intestinal Mucosa to Lipophilic and Hydrophilic Molecules

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Purpose:

The oral route, among the non-invasive routes of drug administration, is still the most preferred and convenient one for the majority of patients. Because of the problem of availability of human tissue, *in vitro* permeability studies have often been performed on specimens obtained from laboratory animals, which are then extrapolated to the human situation.

Method:

In this study porcine intestinal mucosa was used as an *in vitro* permeability model for human intestinal mucosa using tritium-labelled permeants (17β-estradiol, r-arecoline, vasopressin, oxytocin and water). Fresh porcine and human intestinal tissues were frozen in liquid nitrogen and stored at –85^{o}C. *In vitro* permeability studies were performed using a flow-through diffusion apparatus (24 h, 20^{o}C, 1.5 ml/h).

Results:

The mean and estimated mean steady state flux values for water, 17β-estradiol, r-arecoline, vasopressin and oxytocin were approximately 19%, 126% and 20%, 21% and 15% higher, through porcine intestinal mucosa when compared to human intestinal mucosa, respectively. Using an F-test (comparing whole curves), statistically significant differences in the diffusion rates of all permeants tested were found when comparing human and porcine intestinal mucosa.

Conclusions:

Generally, porcine intestinal mucosa seems to be a good *in vitro* permeability model for human intestinal mucosa. However, the permeability of porcine intestinal mucosa to the compounds tested in the present study, were found to be higher when compared to the corresponding human tissue. Large differences were observed between the human and animal tissues for some permeants. The differences between the permeability characteristics of the two types of mucosa must therefore always be considered when using porcine tissue as an *in vitro* permeability model for human intestinal mucosa.

In Vitro Diffusion of the Immunosuppressant Tacrolimus through Human and Rabbit Corneas

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Purpose:

Cyclosporin A (CsA) and tacrolimus (TAC) may be used in the treatment of eye inflammatory disorders and for prevention of corneal graft rejection. *In vitro* flux values of TAC through rabbit and human corneas were compared in this study. **Method:**

A flow-through diffusion apparatus was used for all permeability experiments. Flux values for tritium-labelled TAC were determined over 24 hours and fractions collected 2-hourly (20^{o}C, 1.5 ml/h). Statistical analysis was performed using a F-test.

Results:

Steady state flux values for TAC across both types of corneas were not reached. Up to 12 hours, no statistically significant differences between the mean flux values were indicated between the two species. After 16 hours, the mean flux values achieved for TAC through human corneas became higher than those found through rabbit corneas. Whole curve comparison (F test), indicated statistically significant differences ($P<0.05$). Estimated mean steady state flux values of 1174±118 and 1009±59 $dpm.cm^{-2}.min^{-1}$ were reached for TAC through human and rabbit corneas, respectively. Results found previously for CsA through both tissues, indicated estimated mean steady state flux values of 125±11 and 136±12 $dpm.cm^{-2}.min^{-1}$ for human and rabbit corneas, respectively ($P>0.05$).

Conclusions:

Estimated mean steady state flux values for TAC were approximately 9x and 7x higher than those for CsA across human and rabbit corneas, respectively. Rabbit cornea can thus be used as a general good *in vitro* permeability model for human cornea.

The Effect of Temperature and pH on Valproic Acid and Acyclovir *In Vitro*

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Introduction and aim:

Valproic acid (VPA) is an antiepileptic drug that is widely used for treatment of epilepsy. Unfortunately, it has a narrow therapeutic concentration range that predisposes it to adverse drug interactions and hence the need for therapeutic drug monitoring. Acyclovir (ACV), on the other hand, is an antiviral drug indicated for treatment of various viral infections, and is often prescribed to patients on treatment with VPA. Parmeggiani and co-workers (1994) reported a pharmacokinetic interaction between ACV and VPA which was heralded by reduced plasma levels of VPA and break through convulsions. This was followed by Moattari and co-workers (2002) who described the possibility of a direct interaction between ACV and VPA. Unfortunately, the mode of interaction between ACV and VPA is still not well understood. Therefore, the aim of this study was to investigate the possibility of a direct interaction between VPA and ACV at different pH and temperatures *in vitro*.

Methods:

The concentrations of VPA and ACV, prepared separately or as a mixture of the two, were monitored in buffers at different pH and temperatures. The test samples consisted of 1 ml buffer at pH 7.4 or pH 3 or pH 10, containing either ACV, 112.6 μg/ml (5 mM), or VPA sulphate, 72.13 μg/ml (5 mM), or both in a 1:1 molar ratio. The samples were incubated at 25^{o}C for 2 hours and a further 1 hour at 37^{o}C. Aliquotes of 200 μl were drawn at 0, 2 and 3 hours to measure the concentration of VPA and /or ACV, and the experiments were repeated three times (n=3) on different days. The concentrations of VPA were determined using an Enzyme-linked Immunoabsorbent Assay from Abbott Diagnostics and the concentrations of ACV were analyzed by a validated HPLC method. Results were compared using ANOVA Tukey-Kramer Multiple Comparisons Test with the level of significance at $p < 0.05$.

Results:

The concentrations of VPA and ACV alone were not different ($P > 0.05$) from those in the mixture of both drugs at the different temperatures and pH. However, when the temperature and pH were evaluated separately, there was a trend whereby at high temperature (37^{o}C) the concentrations of ACV (percentage detected) tended to be higher in the mixture (87%) than when it was alone (84%), while those of VPA tended to be lower in the mixture (89%) than when it was alone (92%). This same trend was observed at acid or alkaline pH.

Conclusion:

Although temperature and pH did not induce significant effects on the concentrations of both ACV and VPA, increased concentrations of ACV were associated with reduced concentration of VPA when the two drugs were mixed under constrained conditions and these observations require further studies.

Mending the Myelin in Multiple Sclerosis: The Role Of 5,10 Methylene-Tetrahydrofolate Reductase (*MTHFR*)

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Purpose:

Increased homocysteine levels have been found in multiple sclerosis (MS) patients (Van Rensburg et al. 2006), leading to the hypothesis that mutations in enzymes of the folate-vitamin B12 methylation cycle, such as 5,10 Methylenetetrahydrofolate reductase (*MTHFR*), may be involved in the aetiology of MS. The aim of the current investigation was to screen for *MTHFR* mutations in MS patients and healthy control individuals.

Patients and methods:

Patients were diagnosed according to the criteria of McDonald (2001). Blood was analysed for folic acid and homocysteine concentrations. Mutation analysis of the *MTHFR* gene was performed using polymerase chain reaction (PCR), heteroduplex single strand conformational polymorphism (HEX-SSCP) detection and semi-automated DNA sequencing techniques. These procedures were applied to identify any known and/or novel variations in the *MTHFR* gene.

Results:

Two novel polymorphisms, 1056C/T and 1782G/C, and two previously described mutations, 677C/T and 1286A/C, were identified. A statistically significant difference in genotypic frequency was noted between patients and control cohorts ($p = 0.03$), implicating a possible role for *MTHFR* in the aetiology of MS.

Exploring the Antimicrobial Activity of Medicinal Aromatic Plants Used in Traditional Healing Practices

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Purpose:

The antiseptic properties of essential oils have been researched since the 1800's. However, due to the complex nature of essential oils and their specific chemical-physical properties many variables are involved in reproducing reliable results. This paper aims to highlight some of the aspects involved in validating the antimicrobial properties of plants used in traditional healing practices.

Methods:

Various microbiological techniques such as disc diffusion, minimum inhibitory concentration, time-kill and synergy assays employed in essential oil research will be presented with reference to their application in studies on South African aromatic medicinal plants.

Results:

The interaction of the major oil constituents of *Osmitopsis asteriscoides* is demonstrated where camphor and 1,8-cineole in combination enhance antimicrobial efficacy. Conversely, the major volatile constituents of *Artemisia afra* in various combinations have no significant role on the antimicrobial activity of the plant. Demonstrating synergistic antimicrobial principals, the *in vitro* efficacy of the essential oils of *Artemisia afra* and *Lippia javanica* in combination indicated enhanced activity. The antimicrobial activities of the non-volatile and volatile fractions of *Tarchonanthus camphoratus* singularly and in combination will be discussed to demonstrate that the volatile and non-volatile constituents collectively play an integral role in the total efficacy of the plant. The enantiomeric configuration may also impact on the antimicrobial outcome and this is demonstrated by the variable synergistic and antagonistic profiles determined for the combination of 1,8-cineol and limonene. The comparative evaluation of commercial essential oils with indigenous oils validates the use of South African aromatic plants for their anti-infective properties.

Surfactant treatment for respiratory distress syndrome: A study in preterm lambs

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Introduction:

A variety of animal models are used to test the efficacy of pulmonary surfactant developed for the treatment of respiratory distress syndrome (RDS). In the preterm lamb, RDS closely resembles that of the human infant with the advantages being the ability to measure multiple variables for the lungs and other systems over extended periods. Although it is not yet fully understood, current evidence indicates that besides the surface-active lipid composition of pulmonary surfactant, proteins also contribute to the efficacy of preparations used in clinical practise. The most satisfactory results have been obtained with preparations from animal lungs (Survanta® - bovine and Curosurf® - porcine). In a previous study in preterm lambs, we showed that surfactant replacement therapy (SRT) 30 min after birth with either Survanta® or a polymer containing surfactant (Synsurf) did not prevent systemic oxygenation from deteriorating over time.

The objective of the present study was to study systemic oxygenation ((PaO_2/FiO_2 ratio) and lung mechanics in preterm lambs when surfactant is administered before first breath. The efficacy of Synsurf in comparison to Curosurf® in RDS was also evaluated.

Methods:

A caesarean section was performed on time-dated pregnant Dohne-Merino ewes at a gestational age of 130 days. The head of the animal was delivered and a tracheostomy performed. Tracheal effluent was withdrawn (10-20 ml). Premature animals were delivered, dried, and weighed. Lambs were assigned into one of three groups (n=6 lambs/group) after delivery. Surfactant or placebo was instilled via the tracheostomy before starting artificial ventilation. Lambs were sedated and paralysed. Ventilation (expiratory tidal volume) was held constant throughout the study at 8-10 ml/kg. Arterial blood gases, ventilator indices and haemodynamic variables were monitored over a 5-hour study period.

Results:

After surfactant instillation a significant improvement occurred in PaO_2/FiO_2 compared to placebo treatment over the 5-hour period. Two lambs developed a pneumothorax, one each in the Synsurf and Placebo group of animals.

Conclusion:

In comparison to the other groups studied, only Synsurf had a sustained effect on PaO_2/FiO_2 at 5 hours in comparison to the 30-min PaO_2/FiO_2. We postulate that this beneficial effect may in part be ascribed to polymer-phospholipid complexes preventing surfactant inactivation or better access of these complexes to the air-liquid interface in the lung. Furthermore, surfactant administration before first breath may also play a role.

POSTER PRESENTATIONS

Standard of Pharmaceutical Services provided by Community Pharmacists in South Africa

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PURPOSE

The objects of the South African Pharmacy Council in terms of the pharmacy Act, 1974 (53/1974) as amended are*, inter alias*, "*to uphold and safeguard the rights of the general public to universally acceptable standards of pharmacy practice in both the private and the public sector*" as well as "*to establish, develop, maintain and control universally acceptable standards of practice of the various categories of persons required to be registered…*" One way of improving the practice of pharmacy is by means of pharmacy inspections, by inspectors appointed by the South African Pharmacy Council. ***The aim of this study was to investigate the standard of pharmaceutical services provided by community pharmacies in South Africa by using the inspection results of the South African Pharmacy Council.***

METHOD

Inspection results of community pharmacies were obtained from the South African Pharmacy Council and extracted for the time period 1 January 2004 to 31 May 2005, a 17 months period. To determine the demographic and geographic profile of these pharmacies, data of the Pharmacy Register of the South African Pharmacy Council for August 2005 was merged with the Census data of 2001. Data were analysed by using the SAS statistical programme. Compliance with the Good Pharmacy Practice (GPP) guidelines was determined for the following aspects: Registration details, premises and layout, equipment, storing, control of medicines and record keeping, thermolabile medicine, dispensing of prescriptions, provision of pharmaceutical care, standard operating procedures, references, promotion of public health and general aspects.

RESULTS

During the study period a total of 1178 community pharmacy inspections were carried out in 1103 community pharmacies (one or more than one inspection per pharmacy) which represent 43% (n = 2 550) of the total number of community pharmacies registered with the SAPC during May 2005. Nationally community pharmacies achieved a score of 92.27 ± 6.65 per cent for compliance with GPP guidelines for all above mentioned aspects. The lowest compliance score (73.34%). was obtained for the availability of written standard operating procedures and the highest was for the promotion of public health (99.02 ± 6.30 per cent). No practical significant differences ($d < 0.8$) were found between the overall compliance score obtained by community pharmacies of the different provinces. The highest compliance score was obtained by community pharmacies in the Free State (93.09 ± 4.90 per cent), followed by Western Cape, Eastern Cape, Kwazulu Natal, Limpopo, Northern Cape, Gauteng, Mpumalanga and the North West.

CONCLUSION

In general, it was found that overall compliance of community pharmacies inspected, with regard to Good Pharmacy Practice requirements is fairly adequate. Areas for concern in community pharmacies in general, were the availability of written standard operating procedures.

ACKNOWLEDGEMENT

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Standard of Pharmaceutical Services provided by Institutional Pharmacies: Public vs. Private Sector

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PURPOSE

The primary aim of Good Pharmacy Practice is to ensure that pharmacists as well as other health professionals provide quality pharmaceutical services to the whole population. ***The aim of this study was to compare the standard of pharmaceutical services provided by public and private institutional pharmacies in South Africa by using the inspection results of the South African Pharmacy Counc****il.*

METHOD

Inspection results of institutional pharmacies were obtained from the South African Pharmacy Council and extracted for the time period 1 January 2004 to 31 May 2005, a 17 months period. To determine the demographic and geographic profile of these pharmacies, data of the Pharmacy Register of the South African Pharmacy Council for August 2005 were merge with the Census data of 2001. Data were analysed by using the SAS statistical programme. Compliance with the Good Pharmacy Practice (GPP) guidelines was determined for the following aspects: Registration details, premises and layout, equipment, storing, control of medicines and record keeping, thermolabile medicine, dispensing of prescriptions, provision of pharmaceutical care, standard operating procedures, references, promotion of public health and general aspects.

RESULTS

During the study period a total of 343 institutional pharmacy inspections (one or more than one inspection per pharmacy) were carried out in public and state subsidized institutions (n = 245), private institutions (n = 90) and mine hospitals (n = 5). These pharmacies represented 46% of the total number of institutional pharmacies registered with the SAPC during May 2005. Nationally all institutional pharmacies (both private and public) achieved a score of 92.49 ± 8.33 per cent for compliance with GPP guidelines for all above mentioned aspects. Nationally public and state subsidized institutional pharmacies obtained lower compliance score (91.02 ± 9.08 per cent) than private institutional pharmacies (96.39 ± 3.91 per cent). The provision of pharmaceutical care obtained the lowest compliance score in private institutional pharmacies. The most important problems identified in public institutional pharmacies were registration details, the availability of references, the provision of pharmaceutical care and the availability of written standard operating procedures (compliance score < 90%).

CONCLUSION

In general, it was found that overall there is fairly adequate compliance with standards of Good Pharmacy Practice, for private institutional pharmacies. Public sector pharmacies are of concern as it was found that some of these pharmacies do not comply with certain key standards, namely: registration details, availability of references, provision of pharmaceutical care and availability of written standard operating procedures.

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Isolation and Structure Elucidation of Halogenated Monoterpenes from *Plocamium cornutum*.

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Biologically active halogenated monoterpenes have been isolated from the *Plocamium* genera from different parts of the world. We have previously reported the isolation and characterization of halogenated monoterpenes from *Plocamium corallorhiza*. These compounds exhibited potent activity towards oesophageal cancer cells. In our continuing search for biologically active metabolites from South African marine algae we have selected the Southern African endemic alga *Plocamium cornutum* for further study.

P. cornutum was collected from Kalk Bay on the west coast of South Africa and extracted with dichloromethane (DCM) and methanol. The extract was fractionated initially by solvent partitioning into four solvents: hexane, DCM, ethyl acetate and water. Isolation of pure compounds was carried out using column chromatography and normal phase HPLC of the crude fractions. The structures of the pure compounds were determined mainly by interpretation of one- and two-dimensional NMR, and MS data. Two known compounds, 4-erythro-7-dichloromethyl-3-methyl-3,4,8-trichloro-1,5 (E),7(E)-octatriene (**1**) and (7*E,5E,3R,4S*)-3,4-dichloro-7-(chloromethylene)-3-methylocta-1,5-dienal (**2**) and two new compounds (5*E*,7*Z*)-8-bromo-3,4-dichloro-3,7-dimethylocta-1,5,7-triene (**3**) and (5*E*,7*E*)-8-bromo-3,4-dichloro-7-(dichloromethyl)-3-methylocta-1,5,7-triene (**4**) were identified. Compound **3** was isolated as an isomeric mixture and all attempts to separate these stereoisomers were unsuccessful.

Preliminary *in vivo* Investigation of Nine Anti-malarial plants Traditionally used in and around Dollo Mena, Ethiopia.

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Background:

Malaria is a major health problem in many countries including Ethiopia, according to WHO more than five-hundred million infections occur per year and 2.7 million die each year, most of them children. The continuous infection and the spread of resistance among parasites to commonly used antimalarial drugs (quinoline) are causing a progressive increase in severity of the disease and mortality. Therefore, traditional method of malaria treatment could be a promising source of new antimalarial compounds.

Methods:

Twenty one extracts including 10 MeOH and 11 aqueous from different parts of nine medicinal plants used in traditional medicine for the treatment of malaria were subjected to pharmacological test. And their effect on chemosuppression of parasetimia in mice infected with chloroquine sensitive *Plasmodium bergie* was evaluated. In the present study, the four-day suppression test was used at a dose level of 400 mg/kg.

Result:

Sixteen of the 20 extracts show inhibition of parasetimia above 10 % (10- 60 %) and the remaining five plants show inhibition of parasetimia below 10 %, Chloroquine phosphate was compared as a positive control. Phytochemical screening and lethality bioassay is discussed. On the promising plants detailed work is undergoing to determine ED_{50} and LD_{50} values.

Key words*:* Malaria, Traditional Medicine, *Plasmodium bergie*

Spasmolitic activity of the aqueous extract *Solanum incanum*, Solanaceae

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Background:

The root of the common plant *Solanum incanum* L. (Solanaceae) (Commonly known as Enboy in Ethiopia) is widely used for the treatment of stomach complaints in Ethiopian traditional medicine.

Methods:

The guinea pig ileum pre-contracted by acetylcoline (*In-vitro*) and the charcoal travel methods *in-vivo* was used to determine spasmolitic activity.

Result:

The aqueous extract of the plant antagonizes in a dose dependent manner the action of acetylcholine in isolated guinea pig intestinal smooth muscle *in vitro*. The extract significantly decreased (P = 0.017) the distance traversed by charcoal in the gastrointestinal tract of albino mice *in vivo*. Acute toxicity study of the aqueous extract showed that the extract is safe up to 15000mg/kg orally and the mice did not show any sign of conventional toxicity. Phytochemical screening on the root of the plant revealed the presence of alkaloids, saponins, tannins and flavonoids.

Conclusion:

The plant might contain solanaceous alkaloids, which are responsible for the anti-colinergic activities observed. The study revealed that the traditional claim on the plant as spasmolitic has scientific bases.

Key words***: Solanum incanum*, anti-spasmodic, spasmolitic**

Evaluation of Pilot Dose Duration-Response Methodology for a Topical Corticosteroid Cream Containing Clobetasol 17-Propionate

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Purpose:

The United States Food and Drug Administration (FDA) recommends a method for the assessment of bioequivalence of topical corticosteroid products that utilizes a duration of exposure (dose duration) approach to control the dose of topical corticosteroid products. The main objective of this study was to evaluate the pilot dose duration-response methodology as recommended by the FDA Guidance i.e. to determine the appropriate dose duration for use in future pivotal studies. Furthermore, the blanching responses at different dose durations and the employment of visual and chromameter measurements of blanching responses were compared.

Methods:

The corticosteroid-induced skin blanching from a clobetasol 17-propionate cream formulation (Dermovate®) was assessed in a human skin blanching study. The blanching responses were assessed visually by three trained, independent observers and also recorded by a chromameter (Minolta model CR-300).

Results:

The visual and chromameter blanching profiles both showed similar blanching responses. The profiles for 0.5-, 1.0-, 2.0-, 4.0- and 6.0-hour dose durations indicated that blanching reaches a maximum at 12 hours after product removal. The visual and chromameter response profiles showed that skin blanching increases as the dose-duration increases. The greatest blanching response was found to occur at the 4.0- and 6.0-hour dose durations, which produced comparable profiles to one another. An E_{max} model was used to obtain the ED_{50} value which will be used in future pivotal studies. The ED_{50} values observed from the visual and chromameter data were at 0.5467- and 0.6582-hours respectively.

Conclusions:

The ED_{50} values from the chromameter and visual data in this study were at approximately 0.6 hours for this formulation. The visual and chromameter response profiles showed that skin blanching increased in line with the dose duration in the expected rank order. On the basis of these results both types of assessment methods are comparable to each other and should be equally applicable for the assessment of bioequivalence of clobetasol 17-proprionate creams.

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Formulation of a veterinary pour-on containing closantel sodium

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Purpose:

In veterinary medicine the use of topical pharmaceutical formulations are becoming a useful and popular alternative to the more traditional routes of drug administration. The purpose of this study was to formulate the anthelmintic drug closantel sodium into an aesthetically acceptable pour-on. Closantel sodium is a very useful anthelmintic especially in cattle and sheep; however its poor solubility makes it a challenging study.

Methods:

Formulation of closantel sodium pour-ons: Two sets of pour-ons were formulated. One set comprised of 2-Pyrrolidone, Polyethylene glycol 400 (PEG400), Benzylalcohol and Propylene glycol. The second set of formulations consisted of 2-Pyrrolidone, Glycerol, Benzylalcohol and Propylene glycol. For each formulation set the concentrations of 2-Pyrrolidone (transdermal vehicle), PEG400 and Glycerol (viscosity enhancers) were varied. Each formulation contained 5% w/v closantel sodium.

Physical tests: After completion of the formulation process the following tests were conducted: viscosity, relative density, pH and visual tests.

HPLC Assay: A HPLC assay was also performed on all the formulations to determine the amount of active drug present in the pour-ons.

Accelerated stability studies: The formulations were subjected to the following conditions: 25 ºC + 60 % relative humidity (RH), 30 ºC + 65 % RH and 40 ºC + 75 % RH, for a period of 0 -3 months. Each month the samples were removed from the stability chambers and both the physical tests and HPLC assays were performed on the samples.

Results and conclusion:

Results demonstrated that closantel sodium can be successfully formulated into a pour-on application. Although closantel sodium is a poorly soluble drug the application of 2-Pyrrolidone as transdermal vehicle/bridging solvent improved the ability of the drug to stay in solution for a longer period of time. Thus, sedimentation of the drug was eliminated. pH and viscosity values differentiated with variation in concentrations of the excipients and the HPLC method indicated to be an effective analysis for the closantel sodium pour-on formulations.

The Impact of Pharmaceutical Care Interventions on Cardiovascular Patients at Dr George Mukhari Hospital

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Objectives

To categorize and quantify drug related problem types encountered during provision of pharmaceutical care by documenting interventions, their outcomes and other observations.

To identify constraints during provision of pharmaceutical care.

Methods

Pharmaceutical care was provided to cardiovascular inpatients admitted to the Department of Internal Medicine at Dr George Mukhari Hospital, from the admission date until discharge. The study was done from May to November 2005, on week days. Documentation of pharmaceutical care interventions and other observations were done on pharmaceutical care data forms. Study data were entered into an electronic database, which together with spreadsheet software were used to analyze it.

Results

The study enrolled 128 patients. Forty-five (45) pharmaceutical care interventions were made on 30 patients. All interventions were accepted by other health care workers. The largest proportion (38%) of the total number of interventions was from the drug-related problem category, 'failure to receive therapy'.

Limitations encountered during the provision of pharmaceutical care were categorized into medical, administrative, pharmaceutical, diagnostics, nursing and 'other'. Medical constituted the largest percentage (25%).

Conclusion

The study revealed that more drug-related information needs to be provided and systems must be put in place to ensure adherence to medication dosage schedules by both patients and those responsible for medication administration.

Patient Pharmaceutical Distribution Systems: RSA experience 2003 – 2005.

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PURPOSE

Since 2004 the pharmaceutical distribution system in RSA have been constantly reported in the media via the introducing of the maximum single exit price (SEP), the introducing of licensing for dispensers in the private sector and the legal requirements in the public sector for registration as providers with the South African Pharmacy Council (SAPC). The objectives of this study were to investigate possible changes in the availability of pharmaceutical distribution structures in the private sector and to indicate if the public sector have met the legal registration requirements by the end of July 2005.

METHOD

Registration documentation of the SAPC for the periods Aug 2003 (pre-introduction of SEP or licensing requirements), Aug 2004 (after the initial introduction of SEP and licensing) and Aug 2005 (public sector registration requirements) were used for basic statistics. This was merged with geographic data from Statistic South Africa (Census 2001) using municipality, district council, provincial and national levels for data analysis purposes.

RESULTS

The results reveal that the non-availability of community pharmacies increased at municipal level from 81 to 83 municipalities (11.5% - 11.8% of the population) for the period 2003-2005. The availability of registered public pharmaceutical services increased from 85 to 87 municipalities (12% – 11.6% of the total population) from 2003 to 2005. In total 62 municipalities did not have any registered pharmaceutical services (public or private) representing 7.6% of the national population.

The average number of registered services points per municipality remained relatively static for the study periods. The availability of the community pharmacies declined from 191 to 189 municipalities, however the total number increased slightly (2524 to 2534) from 2003 to 2005. On the other hand the availability of private hospital pharmacies increased from 49 to 57 municipalities with a total number increase of 25 units.

CONCLUSION

This research project has shown that in the longer term it seems necessary to monitor the sustainability of community pharmacy services, for lower income groups, as the growth experience in the private pharmaceutical sector seems to be directed towards the higher income groups. In the public sector only 12 more hospitals / clinics have been registered between August 2003 and August 2005. A relative small number of the total public / state clinics are available at national level, and seems that most of the public clinics may be “illegal operations” .

The Availability of Private vs. Public Pharmaceutical Services in Different Geographical Areas in South Africa

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Purpose:

Some of the objectives of the National Drug Policy is to ensure the availability and accessibility of safe, good quality, essential drugs for all citizens of South Africa. Research has, however, shown that there has been a geographical maldistribution of pharmacies for many years [1,2,3] *The objective of this study was to investigate the availability of private vs. public pharmaceutical services in different geographical areas in South Africa from August 2003 to August 2005.*

Method:

The registers of pharmacies of the South African Pharmacy Council (SAPC) of August 2003, August 2004 and August 2005 were analysed on provincial, district and municipality level. Basic demographic information regarding the population served by the different pharmacies were analysed by using the Census data of 2001 and SAS®.

Results:

The results reveal that the number of pharmacies in both the private and public sector who provide pharmaceutical services directly to patients increased from 3153 in August 2003 to 3159 in August 2004 and to 3200 during August 2005. Public and private pharmacies who provided pharmaceutical services directly to patients increase respectively with 6.3% (n = 33) and 1.3% (n = 35) from August 2003 to August 2005. This was mostly the result of an increase in the number of community health centres (public health sector) and private institutional pharmacies (private hospital pharmacies) registered with the SAPC. Although less than 20% of the population of South Africa is situated in Gauteng, 34% (n = 872) of all community pharmacies are located in this province. Sixty-two municipalities (22.8%)(n = 272) where 3 405 147 persons live did not have any registered pharmacies during August 2005. These municipalities are mostly situated in the Kwazulu-Natal (29.03%) and Eastern Cape (17.74%).

Conclusion:

This research project has shown that there is still a geographical maldistribution of registered pharmacies in South Africa. Such maldistribution can affect the availability and accessibility of comprehensive pharmaceutical services, including pharmaceutical care.

References:

1. COMPETITION BOARD. Memorandum insake die reseptering van voorskrifmedisyne in die Republiek van Suid-Afrika. Pharmacy management, 8(2):25 -36, 38 -39, 41-44 (1986).
2. SERFONTEIN, J.H.P. Ondersoek na medisynedistribusiepunte in the RSA (registreer by die Suid-Afrikaanse Aptekersraad). Potchefstroom : PU vir CHO. (Unpublished). (1993)
3. SOUTH AFRICA. Commission of Inquiry into Health Services. Pretoria : Government Printer. 46 p. (RP 63/1986.) (Browne Commission) (1986).

Triamterene crystals obtained from DMF and DMF/water

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Purpose:

To investigate the different crystal forms and habits of traimterene in different DMF and DMF/water mixtures, and the thermal behaviour thereof.

Methods:

Triamterene DMF crystals are prepared by dissolving a fixed amount of triamterene in a DMF solution (A) and DMF water mixtures (B). The DMF/water mixtures are prepared in ratios of 9:1; 8:2; & 7:3 respectively. The solutions are then placed in a cool dry place away from heat or light. The following physico-chemical tests are carried out on the crystals: XRPD, DSC, TGA, IR and thermo-microscopy.

Results:

The physico-chemical properties of the crystals obtained from the 9:1; 8:2; and 7:3 DMF: water ratios are identical. For the purpose of this study the DMF: water (9:1) data will be presented. The crystals from the DMF solution are small bright, orange and shiny, the DMF/water crystals are long shiny and yellow. The XRPD, DSC, TGA and IR results show us that the triameterene crystals obtained from DMF and DMF water are different from the raw material. The melting point of the crystals obtained from solutions A and B were identical: 330°C. (raw material melting point = 331.9°C), but the desolvation peaks are different: 199°C and 189°C respectively. According to the XRPD there are major differences observed at 9 – 12 °2θ between the 3 discussed forms. The theoretical weight loss for a DMF solvate is 23.5% and DMF water hydrated solvate is 27.5%, respectively. The TGA results however indicate that both crystal forms obtained from solutions A & B were DMF solvates. The thermo-microscope shows us that the desolvation process starts at 135°C. Needle growth was observed at a temperature ranged between 261°C - 294°C, for the crystals obtained from solutions A and B.

Conclusions:

It can be concluded that the DMF (A) and various mixtures of DMF/ water (B) produce a solvate. This is contrary to previous reports. According to Dahl et al it was reported that DMF and DMF/ water mixtures produce hydrated solvates.

Reflexology in the management of tension headaches

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Background

Reflexology is a form of "alternative" or "complementary" medicine in which the reflex areas found in the feet and the hands are massaged. These reflex areas act as small "mirrors", reflecting the whole body structure. Reflexology is primarily a relaxation technique and can negate the effects of stress. Many health problems can be linked to stress. The primary aim of the study was to determine if reflexology is an effective method of treatment for the management of tension headaches.

Methods

The study was conducted during June and July 2005. Structured interviews were conducted with two reflexologists in the Nelson Mandela Metropole. The reflexologists completed consent forms prior to the interviews. Short structured questionnaires were also distributed by the reflexologists to patients who suffered from headaches and who were treated by them.

Results

According to the reflexologists, half or more of their patients suffered from headaches. Patients found reflexology to be an effective treatment method for headaches, especially tension headaches. The duration of the sessions varied. The reflexologists also gave patients advice on lifestyle management, such as diet, exercise and relaxation. Twenty respondents completed the short questionnaire. More females (80% of the respondents) visited the reflexologists for the treatment of their tension headaches. The age group in which the headaches were more prevalent was between 30 and 39 years. Most of the respondents (80%) experienced a significant decrease in the frequency of headaches after the initiation of reflexology treatment. Fourty percent of the respondents indicated that they used reflexology as a treatment method in order to reduce the use of over-the-counter medication. Reflexology also resulted in some respondents not having to use medication for their headaches at all.

Conclusion

It was concluded from the study that reflexology seems to be an effective method to manage tension headaches since treatments decreased the frequency and occurrence of headaches. The use of over-the-counter medicine for headaches was also reduced through reflexology treatment.

Studies on Ionotropic Gelation Based Controlled Release Spherical Matrices

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Purpose:

Multiparticulates are gaining increasing importance because of its attractive and convenient form to the patient. One such attempt has made to develop novel multiparticulate spherical matrices of biodegradable polymers such as sodium alginate and pectin by using Aceclofenac as a model drug. The purpose of using NSAID's is to control its highly ulcerogenic activity by prolonging its release in enteric environment.

Method:

1**.** Preparation of spherical matrices:

Spherical matrices were prepared by (forcing suspension of Aceclofenac with 2% medium viscosity sodium alginate and low methoxylated pectin in 0.10 M calcium chloride solution.) using Ionotropic gelatin mechanism in egg box junction model. 4 gm of Aceclofenac was dispersed an anionic biopolymers such as sodium alginate and pectin in a concentration of 2% and this mixture was forced out with the help of cylindrical tubing's having a diameter 2mm in a crosslinking solution. Each drop turned into sphere strong enough to withstand the handling. The residence time of the spheres in crosslinking solution was 1 hr and was then separated from cross linking solution and air dried.

2. Size analysis :

The size distribution of matrices was determined by using optical microscopy and mean diameter from each batch was measured.

3. Scanning Electron Microscopy:

Selected batches were sputter coated with gold for 3 min and then morphological examinations were done with the help of JSM Jeol 840 SEM.

4. Swelling behavior:

Because of its pH dependant solubility the swelling behavior of there sphere were done at different physiological pH medium and measured the size.

5.Release measurement:

The release of Aceclofenac from the spheres was measured using USP XXIII dissolution apparatus. The paddle was rotated at 50 rpm at 37 0C. The dissolution medium used were 0.1 HCl, and phosphate buffer (pH 4 and 6.8) and release of the drug was calculated from there spherical matrices.

Results and conclusion:

The prepared matrices were spherical and uniform in nature. The average sizes of different spheres were 1.5 mm. Morphological studies reveals that the spheres had uneven rough surfaces with some internal poles. Because of its pH dependant solubility of these biopolymers, swellability varies in different pH and is highest in phosphate buffer. Release behavior of spherical matrices 90-98% were in phosphate buffer pH 6.8 while 15-40% in pH 1-4 respectively. The equal proportion of Sodium alginate and Pectin were show controlled drug release over a period of 8 hrs.

The Characterisation of Pluronic® F127 as a Potential Vehicle for the Parenteral Delivery of Oxytocin

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Purpose

The characterisation of Pluronic® F127 (PF-127), a potential vehicle for oxytocin (OT) parenteral delivery was undertaken in order to understand and predict the release of OT from an extemporaneously prepared parenteral dosage form. Information obtained through characterisation studies were used to predict the behaviour of the vehicle during manufacturing, storage and clinical administration of OT in the extemperaneous dosage form. Aqueous solutions of PF-127 (>20 %w/w) form viscous fluid gels at low temperatures (e.g. 4°-8°C) and solidify upon warming to form stiff resistant gels.

Method

Solutions of different concentration of PF-127 were prepared by the "cold method". The surface tension of the solutions of concentration range 10^{-5} -10 %w/v was determined using a Version 2.0 Du Noüy Tensiometer (Cambridge Instrument Co. Ltd, London and Cambridge). The sol-to-gel transition temperatures for the PF-127 aqueous solutions (18 – 32.5 %w/w) were measured and the viscosities of the PF-127 solutions (18 – 32.5 %w/w) solutions were determined using a Brookfield Digital Viscometer Model DV-I+ (Brookfield Engineering Laboratories, Inc, Massachusetts, USA) at different temperatures in the range 5°C – 37°C.

Results

The surface tension of the Pluronic® solutions decreases in a linear manner over several concentration decades and two breaks are observed in the declining surface tension curve. The curve decreases until a plateau is reached at a concentration of 0.5 %w/v of PF-127. The Critical Micelle Concentration (CMC) was also found to be 0.5 %w/v. The sol-to-gel transition temperatures were found to be dependent on the concentration of Pluronic® in solution. The higher the concentration of the gel former in solution, the lower the temperature at which the transition occurred. As expected, the viscosity of the solutions was found to be dependent on the concentration of the gel former in solution and the higher the concentration, the higher the viscosity at any given temperature. The viscosity of the solutions showed a slight decrease in the viscosity on initial warming, but increased on further heating and underwent a dramatic increase in viscosity after the sol-to-gel transition.

Conclusion

PF-127 was found to be a potential vehicle for the sustained delivery of OT. Solutions of PF-127 stored in the refrigerator could be administered as viscous solutions, which would then solidify at body temperatures forming a depot for the sustained delivery of oxytocin. Solutions of PF-127 may be of value when solubilization of poorly water-soluble drugs or additives is required to promote solubility, as it has the ability to form micelles. The drug release would be expected to be higher from solutions made up of lower concentrations of PF-127, due to their inherent lower viscosity *in situ*.

Acknowledgements

The authors gratefully acknowledge financial support from the Rhodes University Joint Research Committee (RBW) and the Andrew Mellon Foundation (FAC). BASF (Ludwigshafen, Germany) are acknowledged for their generous donation of Pluronic® F127.

The Development of a Dissolution Test for Oyxtocin Release from *In Situ* Gel Forming Parenteral Formulations Manufactured with Pluronic® F127

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Purpose

In vitro release studies are an integral part of early stage formulation development studies. There is increasing interest in the use of polymeric materials for the controlled release of proteins and peptides. Currently there are no regulatory standards for the assessment of parenteral controlled release systems such as those manufactured using Pluronic® gels. The use of traditional USP dissolution test apparatus for these purposes is questionable and produces varying results. The objective of these studies was to develop an appropriate dissolution method for the *in vitro* assessment of a parenteral formulation of oxytocin (OT) manufactured using Pluronic® F127 as the gel matrix.

Method

Pluronic® F127 gels of concentration 20 %, 25 % and 30 %w/w were prepared using the "cold method" by addition of an appropriate amount of gel former to aqueous solution of OT (200 IU/ml). The gels were formed in molds of dimensions 23.6 X 11.0 X 8.0 mm (L X W X D) and were set at 37 °C, prior to dissolution testing. The USP Apparatus I (basket), USP Apparatus II (paddle) (Hanson Research SR8-Plus, Hanson Research Cooperation, Chatsworth, CA) and USP Apparatus III (reciprocating cylinder) (VanKel® VK Bio-Dis III, VanKel® Technology Group, Cary, North Carolina) were used in these studies and the resultant release rate profiles compared. The dissolution test medium was a 0.1 M phosphate buffer (pH 7.2). OT release was quantitated using a validated isocratic HPLC method and separation was achieved on a Phenomenex® Hypersil C_{18}, 5µm, 4.6 mm i.d. X 15.0 cm column with a mobile phase consisting of 20% v/v acetonitrile in 80mM phosphate buffer (pH = 5) at a wavelength of 220 nm.

Results

All three test apparatus were found to be suitable to monitor the release of OT from the gel matrices. Dissolution test results obtained using USP Apparatus I revealed that the methodology was able to discriminate between the three formulations tested, whereas only partial discrimination was achieved using USP Apparatus II (*viz.,* between the 20 %w/w formulation and the 25% and 30% w/w formulations but not between the 25% and 30 %w/w formulations) and using USP Apparatus III no difference between the 20% and 25 %w/w Pluronic® F127 formulations was observed. A comparison of the dissolution profiles indicated that rate of release observed when using USP Apparatus III was the most rapid and that observed using USP Apparatus I was the slowest.

Conclusion

The method of choice for the assessment of OT release from extemperaneous gels in the early stages of formulation development was found to be USP Apparatus I as the use of this apparatus permitted discriminatory analysis of drug release profiles from gels of three different concentrations of Pluronic® F127.

Acknowledgements

The authors gratefully acknowledge financial support from the Rhodes University Joint Research Committee (RBW) and the Andrew Mellon Foundation (FAC). BASF (Ludwigshafen, Germany) and PolyPeptide Laboratories s.r.o. (Hostiva-Czech Republic) are acknowledged for their generous donation of Pluronic® F127 and oxytocin, respectively.

In vitro antiplasmodial activity of corrinoid derivatives

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Purpose:

Haemoglobin is utilized as a source of amino acids for the growth of *Plasmodium.* However the parasite is unable to degrade the resultant haematin or ferriprotoporphyrin, which is toxic to the malaria parasite. In order to render this by-product inert, haematin subunits are sequestered into the crystalline haemozoin structure within the parasitic food vacuole. It is proposed that corrinoids which structurally resemble the porphyrins could interfere with the formation of haemozoin crystals and cause parasite death. With the high degree of drug resistance, it has become crucial to investigate the antiprotozoal activity of novel compounds.

Methods:

The effect of five corrinoids were tested on the *in vitro* growth of *P. falciparum* using the [^{3}H]-hypoxanthine incorporation assay, and the ferriprotoporphyrin biomineralisation inhibition test was carried out under acidic conditions to mimic the process of haemozoin formation in the parasitic food vacuole. The toxicity profile of the compounds were determined using the tetrazolium cell viability (MTT) assay.

Results:

Of the tested corrinoids, adenosylcobalamin (1.711 ± 0.098 μM) and aquocobalamin (8.377 ± 0.598 μM) were the most active in inhibiting parasite growth, however were not as active as the standard antimalarials. In combination, adenosylcobalamin displayed an additive/slightly antagonistic interaction with the 8-aminoquinolines. All the corrinoids, except dicyanocobinamide were approximately 40 times more potent than the 8-aminoquinolines in inhibiting β-haematin formation. The low toxicity and antimalarial activity of these corrin-ring containing compounds highlights their potential as templates for further investigation.

An investigation into the physicomechanical and erosion dynamics of a novel implantable polymeric matrix

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Purpose

This study focused on exploring the erosion dynamics, compressional relationships, and surface morphology of a novel polylactide co-glycolide (PLGA) implantable matrix.

Methods

Erosion studies: Matrix erosion was evaluated gravimetrically. Samples were weighed and erosion progressed in 4mL of phosphate buffered saline (PBS) (pH 7.4, 37°C). At predetermined intervals samples were removed, washed with deionised water and vacuum-dried. The initial and final masses were used to calculate the Mass Loss (ML%).

Scanning Electron Microscopy (SEM): Samples were prepared for photomicrographs and sputter-coated with gold-platinum under an electrical potential of 15kV. Scanning fields, selected at various magnifications (JEOL JSM-840 SEM, Tokyo, Japan) produced photomicrographs.

Textural analysis: A Texture Analyzer (StableMicroSystems, UK) was employed to determine the matrix indentation hardness using Force-Distance profiles generated to compute the Brinell Hardness Number (BHN).

Fourier Transform Infrared (FTIR) Analysis: FTIR was performed on native PLGA and compressed samples using a Nicolet Impact 400D apparatus to elucidate structural changes during compression.

Results

PLGA grades used in fabricating the matrices affected the erosion dynamics. PLGA grade, RG504 (high M_w) demonstrated a slower onset of erosion relative to RG502 (low M_w) after 8 weeks, 61.70±4.10 and 49.75±5.05 (%ML) respectively.

Textural profile analysis and the BHN values elucidated the compressional relationship of matrices. PLGA produced moderately soft compacts when compression forces increased. This led to increased crystallinity and higher BHN values (BHN_{2tons}=150.40N/mm^2; BHN_{7tons}=151.21N/mm^2). Furthermore, FTIR results showed minor vibrational frequency changes across characteristic finger print bands (C=O; 1700cm^{-1}-1800cm^{-1}).

SEM images revealed that at t_0 matrices appeared smooth with minimal pores visible. At 8 weeks superficial cracks emerged however, channel-like structures were absent, indicative of minimal diffusion of PBS into the system. At 12 weeks surface pores prominently engulfed the matrices, resulting in channel-like structures and signs of matrix size reduction. At 24 weeks an outstanding porous surface with numerous cracks and channel-like structures appeared although the veracity of the compact structure was retained.

This study proves that the inherent physicomechanical nature and erosion of the implantable matrices are desirable for application in biodegradable, prolonged drug delivery.

Non-steroidal anti-inflammatory agents, tolmetin and sulindac attenuate quinolinic acid (QA)-induced oxidative stress in primary hippocampal neurons and reduce QA-induced spatial memory deficits in male Wistar rats

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Accumulating evidence suggests that anti-inflammatory agents and antioxidants have neuroprotective properties and may be beneficial in the treatment of neurodegenerative disorders. In the present study, the possible neuroprotective properties of tolmetin and sulindac were investigated against quinolinic acid (QA)-induced neurotoxicity. The thiobarbituric acid assay was used to measure the extent of lipid peroxidation and the nitroblue tetrazolium assay was used to measure the levels of superoxide anions generated in primary hippocampal neurons that were established from one day old rat pups. QA, a metabolite of the tryptophan-kynurenine pathway, significantly induces lipid peroxidation, superoxide anion generation and decreases cell viability. However, co-incubation of the neurons with tolmetin or sulindac markedly reduces the oxidative stress and enhances cell viability. Animals were trained in a Morris water maze for four consecutive days and thereafter received 0.6μmoles of QA intrahippocampally. The animals were divided into groups and were treated with either tolmetin or sulindac (5mg/kg twice a day for five days). During test trials, the time taken for each rat to find the submerged platform was recorded over a period of two weeks. Animals were thereafter sacrificed and the hippocamppi were analyzed for the protein carbonyl and the glutathione content. The results show that both sulindac and tolmetin reduce the QA-induced spatial memory deficit and sulindac treated animals respond better in the water maze compared to the tolmetin treated animals. Both agents also reduce protein oxidation in rat hippocampus and attenuate the decrease in hippocampal glutathione content that was induced by QA. The findings of this study indicate that the antioxidant properties of tolmetin and sulindac may be beneficial in preventing or possibly delaying the onset of neurodegenerative disorders such as Alzheimer's disease.

Antileukemic Activity of the Resins of the Commiphora Sp.

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Background:

Extracts from the Commiphora sp. are known to possess antipyretic, anti-inflammatory and hepatoprotective properties.

Objectives:

The antileukemic effect of the resins was tested on the chronic myelogenous leukemic cell line, K-562, using the MTT (1-(4'5-dimethylthiazol-2-yl)-3,5-diphenylformazan assay, while the nitroblue tetrazolium (NBT) stain was used to determine potential for differentiation induction. The automated Coag-A-Mate machine was used to determine the direct effects of the resins on the blood coagulation pathways in human plasma.

Results:

IC50 values ranging from 37 µg/ml to 823.63 µg/ml and IC90 values ranging from 55 to 1800 µg/ml were obtained. The NBT stain was used to determine whether differentiation was induced. Resins from all species showed differentiation induction, with significant induction induced by Resin 4.

No significant effects on either the extrinsic or intrinsic pathway was evident. Furthermore, no effect on fibrinogen levels or Factor Xa activity was displayed.

Conclusion:

Resins of the Commiphora sp. display promising antileukemic activity, without adversely affecting either the intrinsic or extrinsic blood coagulation pathways.

Acknowledgements:

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Nasal delivery of insulin with Pheroid technology

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Purpose:

Approximately 350 million people worldwide suffer from diabetes mellitus (DM) and this number increases annually. Since insulin's discovery and clinical application in 1921, subcutaneous injections have been the standard treatment for DM. Because insulin is hydrophilic, has a high molecular weight, and low bioavailability, this molecule is poorly absorbed after oral administration. The aim of this study is to formulate a nasal delivery system for insulin, using Sprague Dawley rats as the nasal absorption model.

Methods:

Pheroid formulations and *N*-trimethyl chitosan chloride (TMC) with different dosages of insulin (4, 8 and 12 IU/kg bodyweight insulin) was administered in the left nostril of the rat by using a micropipette. Pheroid technology is a patented (North-West University) carrier system consisting of a unique oil/water emulsion, which actively transports drug actives through various physiological barriers. These formulations were administered nasally to rats in a volume of 100 µl/kg bodyweight in different types of Pheroids (vesicles, with a size of 1.71 – 1.94 µm and microsponges, with a size of 5.71 – 8.25 µm). Systemic absorption of insulin was monitored by measuring arterial blood glucose levels over a period of 3 hours.

Results:

The TMC formulation with 4 IU/kg body-weight insulin produced clinically relevant levels of insulin in the blood and as a result also the maximal blood glucose reduction. TMC, a quaternary derivative of chitosan, is able to enhance the absorption of various peptide drugs by the opening of tight junctions between epithelial cells. When insulin (12 IU/kg bodyweight) was entrapped in Pheroid microsponges, it showed a maximum blood glucose lowering effect of 55.69 % which was obtained 60 minutes after nasal administration, whereas Pheroid vesicles achieved a blood glucose lowering point of 65.82 % at 30 minutes after administration. Although the results in this study are encouraging, the nasal insulin bioavailability is still low with the Pheroid formulations and the long term safety of nasal insulin therapy has to be investigated.

Design and evaluation of placebo material for *Artemisia afra* leaves.

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Introduction:

Artemisia afra is a popular traditional herbal medicine used in South Africa and infusions of the leaves are commonly administered for conditions such as coughs, colds and asthma. Despite the popularity of the herb, clinical trials demonstrating its safety and efficacy in humans are lacking and one reason for this state of affairs is the lack of credible placebos for use in clinical trials. The objectives of this study therefore, were to design material with similar appearance, odour and taste to the *A. afra* leaves and devoid of pharmacological activity.

Methods:

15 solvent extractions on the *A. afra* leaves, using water, methanol/water (50/50 v/v), methanol or ethyl acetate with acid hydrolysis, were used to produce the placebo. The extraction process was monitored using UV spectroscopy and HPLC analysis. The odour and taste of the placebo and *A. afra* material was matched by inclusion of 100mg linalool and 0.15g sodium saccharin, respectively, to both materials. The placebo was evaluated for muscle relaxant activity against methacholine-induced contractions of an isolated guinea pig tracheal muscle preparation.

Results:

The solvent extractions produced approximately 98% reduction in absorbance of the leaf solutions. The HPLC chromatographic fingerprint of the placebo showed a reduced number of peaks indicating that most of the phytochemical constituents had been removed. Comparison of the placebo and *A. afra* materials showed close similarity in appearance, odour, taste and colour of teas produced. Cumulative concentrations of solutions of the placebo injected onto the guinea pig tracheal muscle produced a maximal relaxation of 3.955 ± 0.9622%, relative to an isoprenaline maximal relaxation, while similar cumulative doses of the *A. afra* material produced a maximum relaxation of 49.776 ± 2.51%.

Discussion and Conclusion:

The above results suggested that it is possible to develop pharmacologically inert and credible placebo materials for *A. afra* herb, although more pharmacological tests are required to prove inertness. In addition, the results of the muscle relaxation tests validated the traditional use of the herb in conditions requiring smooth muscle relaxation such as asthma.

The Brine Shrimp Test, Really!

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Purpose:

The brine shrimp (*Artemia salina)* test is used widely to determine the toxicity of drugs, industrial wastes, chemical formulations, additives and extracts of medicinal plants. The results are then extrapolated to humans. The aim of this study was to test the validity of the brine shrimp test using paracetamol and acetyl-salicylic acid as test compounds.

Methods:

The first assay is based on the inhibitory effect of the toxicant on the natural light emission (reflecting normal metabolism) of luminescent bacteria, *Vibrio fischeri*. The second assay reflects the ability of test compounds to stimulate the release of oxygen free radicals (OFRs) from isolated human neutrophils. The degree of luminescence is directly proportional to the quantity of OFRs produced. The third assay reflects the intracellular ATP concentration of isolated human neutrophils after incubation with the test compound. A decrease in intracellular ATP should reflect cell injury. The ATP concentration is linear to the emitted light and measured in mV. A dose response of KCN was used as control. The different stimulants were PMA (2 ng/ml), opsonized zymosan (2.5 mg/ml) and fMLP (1mM). Previously determined LC_{50}s of paracetamol (260 mg/l) and aspirin (284 mg/l) for the brine shrimp and then diluted to yield a dose response curve were used. Thirty ml of blood from each of seven healthy volunteers was collected in heparinized Vacutainer tubes from which the neutrophils were isolated and suspended in RPMI 1640 cell culture medium. In all cases 2×10^5 cells/ml human neutrophils were used.

Results:

The IC50 of aspirin in *Vibrio fischerii* was 80mg/l ($p<0.019$, $r=1$) compared to an IC50 of 284 mg/l for the brine shrimp. An IC50 of 260 mg/l of paracetamol in the brine shrimp increased the luminesence in *Vibrio fischerii* ($p<0.001$, $r=0.952$), indicating an increased metabolism. Neither aspirin nor paracetamol at an LD50 of 284 mg/l and 260 mg/l respectively for brine shrimps stimulated human neutrophils to release oxygen free-radicals. The highest concentration of KCN completely inhibited ATP production while a 5-fold dilution could still inhibit ATP significantly ($p=0.04$). Neither aspirin nor paracetamol could inhibit ATP production. Rather, from the dose-response curves it would seem that both aspirin and paracetamol stimulated the production of ATP.

Neither the brine shrimp test nor the *Vibrio fischerii* test should even be used as a screening test when testing for toxicity in humans.

Approaches to Fabricating Anti-TB Nanosystems Embodying a Salted-Out and Cross-linked Architecture

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Purpose:

The scientific arena is currently experiencing a nanotechnology revolution. A nanoparticulate drug delivery system that can be administered as a once-daily dose and maintain active levels for a longer duration than available forms will improve bioavailability and reduce dose frequency, which is a pertinent concern with the existing anti-tuberculosis (anti-TB) regimens. In this study, this technology was implemented in two different methodological approaches to achieve controlled release of the anti-TB drug, isoniazid.

Methodology:

Two comparative methods were employed; the first combining a salting-out and cross-linking methodology developed in our laboratories with spray atomisation to nanosize the polymeric matrix morphology. The synthetic approach followed the redispersion and partial neutralisation of the methacrylic acid copolymer effected by addition of 1 M NaOH, followed by atomisation of the drug-loaded dispersions into flow reactors of differing configurations containing the zinc sulfate heptahydrate ($ZnSO_4.7H_2O$) salting-out and cross-linking solution for induction of separation and formation of cross-linked nanoparticles. This method was compared with the developed emulsion-based salting-out approach. A 14-experimental set Plackett-Burman Design was generated for each formulatory approach for description of the effect of critical processing factors on nanoparticle morphology, drug incorporation efficiency, and drug release behaviour in phosphate buffered saline (pH 7.0).

Results:

Observation via scanning electron microscopy revealed irregular nano- to micro-sized polymeric matrices with spherical drug nanodeposits. Drug incorporation efficiency ranged from 8.12 to 17.74% when the salting-out and cross-linking methodology was employed for nanoparticle fabrication, and ranged from 14.16 to 18.69% for nanoparticles generated via emulsion-based salting-out. Assessment of drug release behavior in phosphate buffered media (pH 7.0) revealed an initial release of 24.79 to 63.60% of the incorporated isoniazid by t_{1h}, followed by a plateau in the release profile, with the remainder being released after t_{24h} for the salted-out and crosslinked nanoparticles. Emulsion salted-out nanoparticles released 80.19 to 90.43% of the incorporated isoniazid by t_{1h} with the remainder being released by t_{6h}. The cross-linked nanoparticles underwent a tubular morphological transition after incubation for $_{24\text{-}48h}$ in the phosphate buffered media, which coincided with the peak of the second release phase at t_{24h}. This is consequential of possible crystal growth and zinc oxide formation upon significant reaction of zinc cations with hydroxyl groups within the nanoparticle architecture and buffer medium.

Synthesis Of Selected Lamivudine Derivatives For Transdermal Penetration

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Purpose:

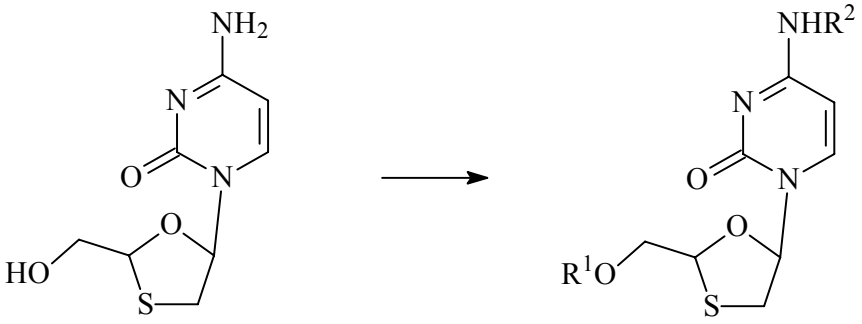
Lamivudine **(1)**, a nucleoside reverse transcriptase inhibitor, is used in the treatment of HIV infection. This virus invades cells of the immune system, particularly the CD4 T-helper lymphocytes and multiplies. Part of the process of viral multiplication involves the conversion of the virus genetic material, RNA, into DNA, which is achieved by a compound essential to the virus, called reverse transcriptase. **(1)** interferes with the conversion of viral RNA into DNA, through blocking the action of this enzyme and stopping the virus from multiplying.

The greatest advantages associated with the percutaneous delivery of drugs include a non-invasive treatment regimen, bypassing of first pass metabolism and quick interruption of treatment.

Six derivatives of **(1)** were synthesised with the aim to determine their transdermal penetration properties.

Methods:

Derivates of **(1)** were synthesised using standard chemical methods. The structures of the products were confirmed by physical methods like mass spectroscopy (MS), nuclear magnetic resonance spectroscopy (NMR) and differential scanning calorimetry (DSC). Aqueous solubility and log D (log partition coefficient in buffer solution) were determined experimentally for **(1)** and its derivatives (**(2) - (7)**) at two pH values, namely 5,0 and 7,0. *In vitro* penetration will be measured through excised female human abdominal skin in diffusion cells.



(1) **Esters:** R^1, R^2 = $COCH_3$ **(2)**
$COCH_2CH_3$ **(3)**
$CO(CH_2)_2CH_3$ **(4)**
$CO(CH_2)_4CH_3$ **(5)**
$CO(CH_2)_6CH_3$ **(6)**
$CO(CH_2)_8CH_3$ **(7)**

The aqueous solubility, log D, transdermal flux, as well as the NMR conformation of the structures of the synthesised derivatives will be presented.

What is it like to live with Epilepsy?

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Purpose:

People living with epilepsy have many challenges as epilepsy significantly impairs their quality of life. Furthermore the attitudes of society towards epilepsy have a wide-ranging influence on people living with epilepsy.The objective of the study was to explore and evaluate the experiences, feelings, knowledge, attitudes and perceived problems with epilepsy from the perspective of the person living with epilepsy.

Methods:

The qualitative study was performed at an institution caring for people living with epilepsy in the rural Mpumalanga. In-depth interviews were conducted with twelve randomly selected patients, six males and six females. During the interviews, participants' non-verbal communication and behaviour were also recorded. A three-step cyclical process of analysis was used to analyse the verbatim data.

Results:

Data were categorised into four categories. (1) **Knowledge** gaps were identified on the causes of epilepsy. Many respondents knew about trigger factors and the effects of their medications. (2) Seizure severity, stigma, fear, and the presence of cognitive or psychiatric problems impacted negatively on their **quality of life**. (3) Many experienced definite limitations to their **life fulfillment**. They experienced psychosocial problems resulting mainly from the consequences of living with a seizure disorder. This placed a threat on their life fulfillment. (4) **Social life**: discrimination was experienced, which leads to their exclusion from mainstream society, restricts their access to basic human and civil rights like for example to find and/or stay employed. These aspects generate a hidden burden which discourages people living with epilepsy from seeking the holistic care they need and deserve.

Conclusion:

People living with epilepsy are burdened with different problems. Non-medical aspects are important, yet are often ignored, misdiagnosed of untreated. Patients need care from a multi-disciplinary team. The knowledge gap must be bridged through effective patient education. The rights of people living with epilepsy must be promoted by empowerment of the individuals and communities.

Preliminary investigation into the synthesis of polymeric nanoparticles and scaffolds intended for implantation into the brain

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Purpose:

Nanotechnology enables the production of material in the nanometer scale, which has the potential for use in the design of novel drug delivery devices. This study aimed at developing polymeric nanoparticles and scaffolds by employing a salting-out approach and intended for implantation into the brain to treat neurodegenerative disorders.

Methods:

A polymeric dispersion was formed by agitating 0.8% pectin solution with a 24% zinc sulphate solution, containing 0.6g polyvinyl alcohol (PVA), which was used as the salting-out reagent. The rapid addition of deionised water to the polymeric dispersion induced the formation of nanoparticles. The nanoparticles were then lyophilised for 24 hours. 2%, 4%, 5%, and 10% w/v polymer solutions were formulated using acetone, dimethyl sulphoxide (DMSO) and dimethyl formaldehyde (DMF). 2%, 4%, 5%, and 10% w/v salt solutions were formulated using various sodium and calcium salts dissolved in deionised water. The polymer and salt solutions were agitated and left to stand for 24 hours. Salting-out of cross-linked polymer and salt particles occurred. The solution was filtered to remove the salted-out particles, which were then lyophilized for 24 hours, resulting in the formation of a polymer scaffold.

Fourier Transform Infrared (FTIR) spectroscopy was performed on the nanoparticles and polymeric scaffolds. The nanoparticles and polymeric scaffold were then analyzed on a JEOL-scanning electron micrograph (SEM). Several photomicrographs were obtained and analyzed to study surface morphology.

Results:

FTIR studies indicated that the basic polymeric structure was maintained, however surface interactions occurred in the formation of the nanoparticles, producing minor changes in the surface morphology of the nanoparticles. SEM measurements indicated the presence of particles in the nanometer range, ranging between 100-300nm.

Conclusion:

The salting out approach is a simple yet effective method for the synthesis of polymeric nanoparticles and scaffolds.

An *in vitro* investigation of the anticancer potential of *Sutherlandia frutescens*

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Objectives:

A well-known medicinal plant in Southern Africa, *Sutherlandia frutescens* (Family: Fabaceae/Leguminosa), has been used traditionally for a wide range of indications including cancer. Recent *in vitro* studies indicated that aqueous extracts of *S. frutescens* could induce apoptosis in cervical carcinoma cells whereas ethanol extracts were shown to have a concentration dependent anti-proliferative effect on several human tumour cell lines. No comparison of the *in vitro* effects of different solvent extracts of *S. frutescens*, on neoplastic cell growth has been reported. The differential solubility of extracts could aid in the possible identification of the active compound(s).

In this study we compared the growth of human cancer cell lines in the presence of *S. frutescens* extracts made from hot water, 70% ethanol, ethyl acetate and chloroform for samples collected from the same area six years apart.

Methods:

Two similar samples of *Sutherlandia frutescens* subsp. m*icrophylla* C. collected in 1998 and 2004 were used in this study. Equivalent hot water, 70% ethanol, ethyl acetate and chloroform extracts of these two samples were prepared. Cytotoxicity was determined in 96 well plates using CoLo 320DM (a human colon carcinoma cell line), DU-145 (a human prostate carcinoma cell line) and MCF-7 (a human breast cancer cell line) by a modified MTT cell enumeration assay after incubation with the extract.

Results:

Only the ethyl acetate extracts showed effective cytotoxicity. Significant differences in cytotoxicity was obtained between the equivalent extracts from the different collection dates with the older sample (1998) being at least three times more effective against all three cell lines tested.

Conclusion:

This is the first study to compare the effects of various solvent extracts of *S. frutescens* on neoplastic cell growth. An important observation is that ethyl acetate extracts from samples collected six years apart exhibit different anti-tumour activity. This needs to be investigated further as it might be due to chemical changes during storage, variation between harvests re concentration differences of active compounds depending on season/climate during the period preceding harvesting.

The effectiveness of Insulin Sensitizers in achieving improved glycaemic control in Type 2 Diabetics

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In this retrospective study, the addition of the thiazolidinedione, rosiglitazone (RGZ), in poorly controlled, type 2 diabetics was evaluated in order to prove the effectiveness by delaying the onset to insulin therapy.

Eligible participants (n=42), aged 27-69 years, (mean duration diabetes 8.8 years) who had RGZ 2 mg (n=2), 4 mg (n=26) or 8 mg (n=14) added to their existing oral antihyperglycemic agents (sulphonylureas/metformin) were assessed retrospectively for three months prior and three months after the addition of RGZ. Patients served as their own control. The parameters under investigation were: blood pressure, weight, random blood glucose, urine glucose and urine protein, serum cholesterol, triglycerides and HbA_1C data. Both males (n=25) and females (n=17) were included in the study.

In the three months after the addition of RGZ, mean and median weight change showed a significant increase. No significant change in blood pressure during the study was observed. The HbA_1C% decreased significantly (p=0.0001) from the pre-test (baseline average 8.5%) to post-test (average 6.5%). The proportion of patients that achieved glycaemic control increased by 23.8%. This increase was significant (McNemar's test, p=0.003). Random blood glucose values followed the same trend as the HbA_1C. Cholesterol and triglyceride values also showed a trend towards improvement.

The data suggest that significant glycaemic and lipid benefits were derived from the addition of rosiglitazone in patients with poorly controlled type 2 diabetes. Rosiglitazone can be considered as an effective treatment addition in poorly controlled type 2 diabetics.

Fluorescent structures for assay of neuroprotective properties

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Background:

In recent years polycyclic compounds have been shown to exhibit pharmacological profiles of importance in the symptomatic and proposed curative treatment of neurodegenerative diseases (e.g. Parkinson's and Alzheimer's disease). These structures also show modification and improvement of the pharmacokinetic and pharmacodynamic properties of drugs in current use.

The use of fluorescent techniques have found widespread applicability in receptor pharmacology and offer an attractive alternative to the use of radioligand studies such as multicolor detection, stability, sensitivity, low hazard and lower cost.

Aim:

The aim of this study was to synthesize a series of novel coumarin, indazole and other fluorescent derivatives. These compounds will find applicability in MAO-B and nNOS assays.

Methods:

Using pharmacophore models for inhibition of the above enzymes and docking techniques, a series of compounds incorporating the polycyclic structure and suitable fluorescent moieties were selected for synthesis. The polycyclic cage, obtained from photo cyclisation of the Diels-Alder adduct, was conjugated to the fluorescent moieties, using activation chemistry with EDC and CDI, esterfication, amination and ether formation. Structures were confirmed using NMR, MS and IR.

Results:

The final products were obtained as oils from chromatography or were crystallized from organic solvents. NMR and IR spectra showed the characteristic signals and MS confirmed the molecular masses of the compounds. The fluorencent properties of the synthesized structures correlated to that of the fluorophores.

Acknowledgements:

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Prevalence of Drug-Drug Interactions (DDIs) of Antiretroviral Agents (ARVs) in the Private Health Care Sector in South Africa

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Purpose:

Control over the usage of ARV drugs is probably one of the most difficult tasks faced by health officials. The use of multidrug therapy for HIV makes it imperative to understand how antiretroviral drugs interact with one another and with other drugs that were prescribed together. The aim of this study was to determine the prevalence of possible DDIs between antiretroviral agents themselves and other drugs on prescriptions claimed in the private health care sector in South Africa for 2004.

Methods:

This was a non-experimental, quantitative, retrospective drug utilization study. Data were obtained from a private medicine claims database for the year 2004. The study population consisted of all ARV prescriptions claimed during 2004. The focus was on the prevalence of possible DDIs between ARVs themselves and other medications on the same prescription. The possible DDIs found in this study were classified according to a clinical significant rating as described by Tatro (2005) in his book, "Drug Interactions- Fact and comparisons".

Results:

A total of 5 305 882 medicine items were prescribed, of these, 1.92%, (N = 101 938) accounted for ARVs. Of the total number of 2 595 254 prescriptions, 1.68%, (N = 43 482), were ARVs. A total number of 18 035 DDIs (81 different types) were identified, of these, 83.89%, (n = 15 130), were DDIs between ARVs and other medications, while 16.11% (n = 2 905) were DDIs between ARVs themselves. Possible DDIs with a clinical significance level of 1 (major) (n = 18) and 2 (moderate) (n = 1 436) represented 8.06% (n = 1 454) of the total number of identified interactions. Level 1 interactions were between: i) Efavirenz and simvastatin (n = 1), ii) indanivir and lanzoprozole (n = 3), omeprazole (n = 2), simvastatin (n = 1); iii) ritonavir and simvastatin (n = 4), digoxin (n = 5), fentanyl (n = 1); iv) Saquinavir and fentanyl (n = 1). The most prevalent (more than 100) clinical significance level 2 DDIs found between ARVs themselves were i) indinavir and ritonavir (n = 490); efavirenz and ritonavir (n = 274); efavirenz and indinavir (n = 198); didanosine and indinavir (n = 121) and efavirenz and lopinavir/ritonavir (n = 118).

Conclusions:

Since concomitant use of antiretroviral drugs and drugs used to treat complications of HIV is increasing, there is great need of understanding and anticipating these drug-drug interactions, andapplication of clinical findings in order to provide optimal therapy for patients infected with HIV.

Influence of Different Grades of Methocel on Verapamil Release from Mini Matrix Tablets

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Purpose:

The purpose of these studies was to investigate the effects of different concentrations and viscosity grades of Methocel®, *viz.,* K100M, K4M, alone and/or in combination on the *in vitro* release characteristics of verapamil hydrochloride (VRP) from mini matrix tablets.

Methods:

Tablet manufacture: VRP, Methocel® K100M, K4M, alone and/or in combination, Emcocel® 90M and anhydrous Emcompress® were blended using a planetary mixer on setting 1 for 15 minutes to ensure adequate mixing after which talc and magnesium stearate were added and blending continued for a further 3 minutes. Mini tablets, containing an equivalent of 80 mg VRP were manufactured and three tablets were enclosed in a size 0 capsule prior to dissolution testing using USP Apparatus I and a phosphate buffer pH = 7.4.

Results:

Formulations manufactured using the K100M grade released approximately 50 % VRP, whereas batches containing K4M or the K100M/K4M combination released only approximately 20 % over 12 hours. The tablets prepared using the K4M grade swelled immediately on contact with the dissolution media, illustrating that a glass/rubber polymer transition occurs with a subsequent increase in the polymer chain mobility [1] that facilitates VRP diffusion. The tablets occupied a larger volume, became buoyant and floated in the basket. Based on the *in vitro* release profiles, it is likely that this phenomenon contributes to another mechanism used to prolong gastric residence time, *in vivo*. As the concentration of K4M increased, the release rate of VRP decreased and at higher percentage compositions, the polymer matrix was thought to form a continuous network to the releasing surface thereby restricting drug diffusion. The dissolution curves obtained for tablets in which K100M was used exhibited typical diffusion controlled release profiles, characterised by an initial fast release phase followed by a decreased release rate. The tablets are cylindrical in nature and it is likely that the releasing surface area decreases significantly with time as compared to regular shaped tablets. The negative effect on VRP release observed with the K4M and K100M/K4M combination may be due to the compactness of the tablet that occurs as a result of increased cross-linking and the lack of penetration of the dissolution medium into the dosage forms. The tablets manufactured using K4M became gelatinous and adhered to the basket preventing tablet agitation by the dissolution medium. A test procedure that ensures tablets rotate in the basket without obstruction is required to ensure realistic *in vitro* release profiles are achieved.

Conclusion:

A simple matrix polymer tablet comprised of Methocel® with an optimum viscosity has been used to delay the release of VRP without achieving 100 % release. Studies to evaluate the impact of excipients that increase permeability of VRP in the tablets will be required.

References:

M. Grassi and G. Grassi. Mathematical modelling and controlled drug delivery: Matrix systems. Curr. Drug Del. 2 (1), 97-116 (2005).

Acknowledgements:

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The Biological Activity of Selected Histidine-Containing Cyclic Dipeptides

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Cyclic dipeptides are a reasonably unexplored class of peptides that possess interesting and possibly economically beneficial biological activities (Prasad 1995). The biological action of cyclic dipeptides is currently still speculative, with their therapeutic potential ranging from antibiotic, antitumour, tensoactive to muscle-relaxant activity. Structural differences between cyclic dipeptides have been shown to influence their biological activity and pharmacological quality, therefore necessitating their individual study (Monaghan & Tkacz 1990).

Two cyclic dipeptides, cyclo(His-Ala) and cyclo(His-Gly,) were synthesized from their linear counterparts and their structures elucidated using standard elucidation techniques. The biological potential of these compounds was then investigated with respect to their effects on tumour cells, bacteria and fungi, coagulation pathways and the isolated rat heart.

Molecular modeling and predictive NMR results indicated that the majority of energetically favourable conformers adopted a boat conformation with respect to the diketopiperazine ring. Cyclo(His-Ala), at concentrations of 100 μM inhibited the growth, *in vitro*, of various cancer cell lines, including HT-29, MCF-7 and HeLa carcinoma cells, while cyclo(His-Gly) inhibited the growth of MCF-7 cells. While the antibacterial potential of these two compounds was limited, both cyclic dipeptides significantly inhibited the gowth of C. albicans. Both compounds at a concentration of 100 μM resulted in a decrease in heart rate, coronary flow rate and left ventricular systolic pressure in the isolated rat heart. Inhibition of thrombin, amounting to a 63.3% and 36.7% reduction in the rate of fibrin formation, was noted for cyclo(His-Ala) and cyclo(His-Gly) respectively. While cyclo(His-Ala) showed no notable effects on platelet aggregation, cyclo(His-Gly) significantly inhibited both pathways tested with greatest effects on thrombin-induced platelet aggregation, yielding an IC_{50} of 0.0662 mM (R^2 = 0.989). The results of the anticancer and hematological studies indicate that histidine-containing diketopiperazines have potential as a novel group of cytotoxic agents with antithrombotic effects.

Monaghan, R.L., Tkacz, J.S. Biopharmaceutins. *An. Rev. Microbiol.* 44: 271-301. (1990)

C. Prasad, Bioactive cyclic dipeptides. *Peptides.* 16: 151-164. (1995)

Antioxidant properties of *Gymnosporia buxifolia* szyszyl

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Purpose:

Questions about the long term safety of synthetic antioxidants have increased the demand for natural antioxidants. Natural antioxidants have better long-term safety and stability and improve food quality capacity and can act as nutraceuticals to terminate free radical chain reactions in biological systems. The primary factor in various degenerative diseases, like Parkinson's disease and Alzheimer's disease, is oxidative stress induced by oxygen radicals. These reactive oxygen species is generated by normal metabolic processes and is capable of damaging a wide range of essential biomolecules. The oxidation of cellular oxidizable substrates can be prevented and delayed by antioxidants. Antioxidants scavenge reactive oxygen species by preventing the generation of reactive oxygen species by activating a battery of detoxifying proteins.

Methods:

A literature survey was done and 21 plants were selected for screening of antioxidant activity. Plant material was collected and extracted by Soxhlet extraction, using solvents in order of increasing polarity. The crude plant extracts were used for screening. The screening was done by assessing the total antioxidant capacity of the plants by measurement of the oxygen radical absorbance capacity (ORAC). The most promising plants were selected for further study. *Gymnosporia buxifolia* was selected due to high antioxidant capacity and availability of large quantities of the plant. Four solvents (petroleum ether, dichloromethane ,ethyl acetate and ethanol) were used with the soxhlet extraction and all four crude extracts were tested using the nitro blue terazolium assay. nitro blue tetrazollium is reduced to nitro blue diformazan in the presence of the superoxide anion radical. The capacity of the crude extract of the plant to scavenge the superoxide radical anion determine the antioxidant capacity of the extract.

Results:

G. buxifolia crude extracts showed very high total antioxidant capacity with oxygen radical absorbance capacity. It also showed that it reduced the transformation of nitro blue tetrazolium to nitro blue diformazan that indicated that there is reduction in superoxide radical anions. The diformazan (µmoles/mg protein) for the control group is 54.056±5.075 and for petroleum ether 52.374±6.960, dichloromethane 66.532±4.154, ethyl acetate 28.453±6.399 and ethanol 44.477±4.271. Of all four solvents used for extraction the ethyl acetate crude extract showed the best results.

Conclusion:

Ethyl acetate should be studied further and the active compound should be isolated and identified.

Development of a Novel Gastroretentive Drug Delivery System for Rate-Controlled Drug Delivery

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Purpose:

The bioactivity of some drugs is dependent on their site-specific absorption within the upper segment of the gastrointestinal tract. The development of drug delivery systems specific for this purpose by achieving high and consistent localized drug release, reducing side effects and potentially improving therapeutic outcomes is desirable. The objective of this study was thus to obtain a novel controlled-release polymeric matrix using the hydrophilic polymer called polyhexamethylene sebacamide (PS) employing low to high water soluble drugs namely theophylline and amitryptiline HCl to be liberated over 24 hours.

Methods:

Polyhexamethylene sebacamide (PS) was synthesized using stoichiometrically efficient combinations of the monomers as well as solvent systems using the Schotten-Bauman method. The synthesized PS was then dry-blended and used for the production of tablets. Each matrix system was produced in triplicate by the process of direct compression and was composed of a physical mixture of 300mg PS and 50mg each of the respective model drugs. In vitro dissolution and matrix erosion studies of the matrices were carried out using the USP 25 rotating paddle method at 50rpm in an acidic buffer medium of pH 1.2 maintained at 37^{o}C. The drug release and polymeric matrix dissolution rates from matrices was established using ultraviolet spectroscopy. Also, the buoyancy of the matrices was observed using buffer solutions of pH 1.2 over 24 hours. Fourier transform infrared spectroscopy (FTIR) was carried out to ascertain the presence of any chemical interaction between the polymer and the drug.

Results:

The matrix system showed the potential of effectively delivering both the soluble and insoluble model drugs in a rate-controlled manner. The two drugs generated closely related release patterns showing that the drug release process is modulated by the rate of polymeric disentanglement and not drug solubility. The matrices elicited some level of burst of about 10% (at the first hour) for each drug followed by a consistent release rate of approximately 4% per hour. Overall, a 100% of drug was liberated in 24 hours. The initial burst is considered an advantage as this may effectively provide an immediate therapeutic concentration which is maintained by the subsequent consistent, prolonged drug release pattern. Also, the device maintained its full buoyancy in the acidic medium as the controlled release matrix underwent gradual dissolution throughout the test duration. Furthermore, the matrix system showed potential to bioerode under the employed physiologically simulated media.

Conclusions:

The PS matrix system showed potential to be utilized as a rate-controlled Gastroretentive Delivery Device for the delivery of both soluble and insoluble drugs to the upper gastrointestinal tract.

Estimating the need for the involvement of the Community Pharmacy Sector in the ARV roll-out programme in South Africa

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PURPOSE

Efforts to increase access to life-saving treatment, including antiretroviral therapy, for people living with HIV /Aids is resource-limited settings has been the growing focus of national and international efforts. *The objective of this study was to estimate the usage of the community pharmacy sector as provider of ARV medication, as the pharmacist has been recognised as the "custodian of medicine*". Different statistics were used to estimate HIV prevalence as well as the total number of HIV patients who should be treated with antiretroviral medication per community pharmacy on municipality level.

METHODOLOGY

The register of pharmacies of the South African Pharmacy Council (SAPC) of August 2005 was analysed on provincial, district and municipality level. Basic demographic information (age group and gender) regarding the population served by the different community pharmacies were analysed by using the Census data of 2001 and SAS®. Statistics from the South African Medical Research Council[1] regarding HIV prevalence by sex and age group for 2004 were used for the estimations.

RESULTS

The results reveal an estimated HIV prevalence level of 4 876 333 and a total number of 590 036 patients who should received ARV medication in South Africa. The estimated average HIV prevalence per community pharmacy on municipality level was 3 633 ± 3903 patients. On municipality level (n = 189) an average number of 440 ± 472 HIV patients could be treated with ARV medication per community pharmacy. More female HIV patients (254 ± 299) than male HIV patients (186 ± 176) could be treated with ARV medication per community pharmacy on municipality level. The highest number of HIV patients who could be treated with ARV medication per community pharmacy on municipality level was in the agegroup 20 to 44 years (341 ± 354), followed by patients in the agegroups 45 to 59 years (46 ± 46), 0 to 9 years (26 ± 37), 10 to 19 years (24 ± 34) and older than 60 years (3 ± 3).

CONCLUSION

More research is needed to document the available pharmacist manpower in the private sector to assist the public sector in the ARV roll-out programme in South Africa.

REFERENCES

1. DORINGTON, R., BRADSHAW, D., JOHNSON, L. AND BUDLENDER, D. The demographic impact of HIV/Aids in South Africa. National indicators for 2004. Cape Town : Centre for Actuarial Research, South African Medical Research Council and Actuarial Society of South Africa. (2004)

Development of a quantitative HPLC method using a minibore column for the analysis of azithromycin

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Purpose:

Azithromycin (AZI) is the first member of the class of macrolide antibiotics called the azalides. It has been shown to be efficacious in the treatment of skin and skin structure infections when given as an oral dosage form. The objective of the study was to develop an accurate analytical method for the anlaysis of AZI using a minibore column and coulometric electrochemical detection. The method was then used to generate the pH solubility profile of AZI. The solubility profile shall be used for the determination of the appropriate conditions required to monitor the diffusion of AZI through artificial membranes and skin using microdialysis.

Methods:

A Coulochem detector was used with the analytical cell set at +0.4V and +0.9V for the screening and working electrodes respectively. The gain setting on the working electrode was set at 10 x 15. The minibore column was a Luna C_{18} silica based column with dimensions 150 x 2.0 mm I. D. and 5µ particle size. A 20mM phosphate buffer of pH 7.7 was used with acetonitrile in a 40:60 ratio respectively as the mobile phase.

The solubility study was conducted at ambient temperature using McIlvaine's buffer on a Juniour orbit shaker set at 150 rpm. The samples subsequently obtained were analysed using the previously validated quantitative method.

Results:

Of the five studies conducted to determine linearity, the correlation coefficients for all were found to be greater than 0.99. At the set level of signal amplification on the working electrode, the limits of detection and quantification were found to be 0.05 and 0.5µg/ml respectively. The relative standard deviation (%RSD) was used as an indicator of inter-day and intra-day precision for four standard solutions of AZI and all values fell below 5%.The pH solubility profile showed a decrease in AZI solubility with an increase in pH.

Detection of halogenated monoterpene aldehydes by chemical derivatisation and mass spectrometry

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Plocoraldehydes are halogenated monoterpene aldehydes that may be responsible for the significant antibacterial and cytotoxic activity of crude extracts of *Plocamium corallorhiza.* These compounds are produced in relatively small quantities and are subject to rapid decomposition. Obtaining mass spectrometry data using conventional methods for the Plocoraldehydes proved difficult. The compounds immediately degraded upon injection into a gas chromatograph hence no GC trace could be obtained. Similarly electron impact - mass spectrometry (EIMS) at 70 eV afforded no decipherable spectra.

The objective of this part of the project was to develop a method of derivatising the aldehydes in the crude algal extract with the aim of stabilising the compounds in order to obtain efficient and reliable mass spectra. Aldehyde groups react with nucleophilic reagents such as *O*-(2,3,4,5,6-pentafluorobenzyl-)hydroxylamine hydrochloride (PFBHA) quantitatively at room temperature to afford oximes. The fragmentation pattern for PFB oximes has been comprehensively documented and derivatives generate ion abundances in a typical fashion.

Initially the pure compound 4,6-dibromo-3,7-dimethyl-octa-2,7-dienal along with citral was selected for derivatisation. The molecular ion (M+1) for the oxime derivatives were detected by direct injection atmospheric pressure chemical ionisation (APCI) in the positive ion mode. Characteristic ion abundances for the PFBO were observed upon fragmentation of the molecular ion peak. The reaction was then performed on the crude algal extract, successfully yielding the mass spectra of three oximes. ^{1}H NMR and high resolution electronspray ionisation – mass spectrometry (ESI-MS) provided evidentiary support for the results obtained by APCI-MS

Solubilisation And Kinetics Of Degradation Of Nevirapine

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Purpose:

Nevirapine is part of a new class of antiviral agents – NNRTIs (non-nucleoside reverse transcriptase inhibitors). NNRTIs bind directly to the HIV reverse transcriptase enzyme and blocks the RNA-dependent and DNA-dependent DNA polymerase activities by causing a disruption of the enzyme catalytic site.

Nevirapine is practically insoluble in water and its solubility decreases even further with an increase in pH, thus, making it difficult to formulate a liquid dosage form at physiological pH. Further more, there is very little literature available about the stability and degradation kinetics of nevirapine.

The goal of the study is to gather information about the solubility and degradation kinetics of nevirapine that includes pH-stability, the influence of the type of buffer and buffer concentration, measuring the rate of degradation and isolate and identify degradation products. Solubilizing agents will be used to form complexes with nevirapine to try and increase nevirapine's solubility in an aqueous solution so that a liquid dosage form can be formulated.

Methods:

Phase-Solubility Study: An excess amount of nevirapine was added to solutions buffered at pH7 (5mg/ml). Polyvinylpirrolidone (PVP), Metylsulphonylmethane (MSM) and N-methyl-D-glucamine (Meglumine) was added to the nevirapine solutions separately as solubilizing agents. Different mixtures were made with each solubilizing agent varying in quantity to test the effect on solubility. The mixtures were placed in a water bath and shaken for 24h at 25°C. HPLC analysis was used to determine the concentrations in all the solutions.

pH-Stability Study: Different aqueous nevirapine solutions were made with concentrations of +/- 50µg/ml and buffered at pH2 through to pH8. The solutions were sealed in glass ampoules and stored at temperatures 25°C, 40°C, and 55°C respectively with samples taken monthly. HPLC analysis was used to determine the stability in all the solutions.

pH-Solubility Study: Aqueous solutions of nevirapine was made with concentrations of +/- 5mg/ml to exceed the expected solubility of nevirapine and buffered at different pH values from pH2 to pH8. The various solutions were placed in a water bath and shaken for 24h at 25°C. Then the solutions were filtered for HPLC analyses to determine the solubility.

Results:

pH-Stability Study: This study is currently in its ninth week. The greatest degradation can bee seen at pH2 and pH3 where +/- 17% decrease in original concentration had taken place. With a further increase in pH values the degradation decreases. At pH7 and pH8 there seems to be no degradation up until now

pH-Solubility Study: It was found that the solubility of nevirapine is +/-50µg/ml at pH4 to pH8. At pH2 and pH3 nevirapine's solubility was much higher with the solubility being >400µg/ml at pH2 and +/-90µg/ml at pH3.

Phase-Solubility Study: Not one of the solubilizing agents had an effect on the nevirapine solubility.

Synthesis and Evaluation of Selected Glycine-Containing Diketopiperazines

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A significant number of naturally occurring diketopiperazines, or cyclic dipeptides (CDPs), possess useful biological activities which is as a result of their ability to bind to multiple, unrelated classes of receptors with high affinity in both prokaryotes and eukaryotes (Horton *et al.*, 2002; Lambert *et al.*, 2001; Milne *et al.*, 1998).

The objective of the study was to synthesise and investigate the potential biological activities of selected glycine-containing cyclic dipeptides, cyclo(Gly-Thr) and cyclo(Gly-Ser). The CDPs were synthesised by cyclization from their linear dipeptide counterparts by heating in phenol (Kopple & Ghazarian, 1968). Qualitative analysis was performed by means of chromatography, scanning electron microscopy, x-ray powder diffraction and thermal analysis while structures were confirmed by means of standard elucidation techniques.

NMR and computational calculations on the CDPs were performed using Spartan '04 (build 121) (Wavefunction Inc.) and theoretically synthesised from glycine and L-threonine [(2S,3R)-2-amino-3-hydroxybutyric acid] or L-serine [(S)-2-amino-3-hydroxypropionic acid], respectively. High level density functional theory calculations showed, unexpectedly, that the most stable conformations for both CDPs involve boat-like ring shapes with the side-chains pseudo-equatorial and the hydroxyl groups within hydrogen bonding distances of the proximal carbonyl groups.

Both cyclic dipeptides demonstrated significant inhibition of Gram-negative bacteria as well as inhibiting the growth of HT-29 carcinoma cells in particular with limited inhibition of HeLa and MCF-7 cells. Both CDPs showed enhanced coagulation and platelet aggregation while their effects on the isolated rat heart revealed significant negative chronotropic activity, which may be beneficial in the treatment of atrial tachycardia and fibrillation.

D.A. Horton, G.T Bourne, M.L. Smythe. Exploring privileged structures: the combinatorial synthesis of cyclic peptides. *J. Comp. Mol. Designs.* 16: 415-430 (2002).

K.D. Kopple, H.G. Ghazarian. A convenient synthesis of 2,5-dioxopiperazines. *J. Organ. Chem.* 33: 862 (1968).

J.N. Lambert, J.P. Mitchell, K.D. Roberts. The synthesis of cyclic peptides. *J. Chem. Soc. Perkin Transact. 1*. 471-484 (2001).

P.J. Milne, A.L. Hunt, K. Rostoll, J.J. Van der Walt, C.J.M. Graz. The biological activity of selected cyclic dipeptides. *J. Pharm. Pharmacol.* 50: 1331-1335 (1998).

Effects of *Leonotis leonorus* aqueous extract on the isolated perfused rat heart.

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Leonotis leonurus (L.L.) is used in traditional medicine for treatment of various diseases including epilepsy, cardiac asthma, heart failure and hypertension. Traditional medicines, as effective and potent medicines, require evaluation by scientific methods in order to be used to their full effect and safely.

The aim of this study was to determine the effect of L.L. aqueous extract on the left ventricular systolic pressure (LVSP), left ventricular diastolic pressure (LVDP), heart rate (HR), and coronary perfusion pressure (CPP) in isolated rat hearts.

Hearts were perfused at constant flow and exposed for 3 min to the active compounds using the modified "Langendorff Perfusion Model". The effects of adrenaline (ADR) and digoxin (DIG) on the isolated heart were compared to that of the L.L. aqueous extract.

Results were expressed as a difference between the base line preceding the administration of each tested substance and at steady state (± 3 minutes after the beginning of the perfusion). Student's t-test for paired data was carried out on all data. Differences between two related means were considered statistically significant for P values equal or less than 0.05. ADR (1 µM) significantly ($p<0.05$) increased the LVSP by 40.6±2.67mmHg, the LVDP by 43.90±3.49mmHg and the HR by 22.49±5.58beats. The CPP was decreased by 8.02±5.30mmHg. DIG (2.5 ng/ml), significantly ($p<0.05$) increased the LVSD by 9.46±5.04 mmHg, the LVDP by 9.65±5.11mmHg, the HR by22.49±5.58bpm and the CPP by 5.80±2.31mmHg.

LL (1.0mg/ml and 2.0mg/ml respectively) significantly ($p<0.05$) increased the LVSP by 25.36±8.10mmHg, and 14.91±7.18mmHg, the LVDP by 29.40±2.11mmHg and 14.88±2.11mmHg. L.L. also decreased the HR by 34.73±3.70bpm and 42.71±8.02mmHg respectively. The CPP significantly ($p<0.05$) decreased by 12.09±3.49mmHg and 16.87±3.50mmHg respectively. There was no significant change when switching between the experimental and control side of the instrument when both sides contained a pure Krebs-Henseleit solution. LVSP changed by 0.03±0.63mmHg, LVDP by 0.52±1.09mmHg, HR by 0.05±1.23bpm and the CPP by 0.63±1.20mmHg. ADR (1µM) drastically increased the LVSP and the LVDP with corresponding acceleration of the HR, thus reflecting its positive inotropic and chronotropic effects. Digoxin shows a weaker positive inotropic effect and has little effect on the HR. At low concentrations LL produced a positive inotropic effect, a negative chronotropic effect, and decreased CPP. The latter could have been caused by coronary vasodilatation. At higher concentrations (2.0mg/ml) LL aqueous extract dropped the values of all parameters to zero. It appears that the extract at this concentration contains some constituents with toxic effects on the isolated heart.

There are some similarities in the effect of digoxin at the concentration of 2.5ng/ml and the aqueous extract of LL on the isolated perfused heart. Indeed both produced a positive inotropic effect and negative chronotropic effect. They however have opposing effects on the CPP.

Key words:

Leonotis leonurus, isolated perfused heart

Acknowledgement:

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Preliminary acute toxicity study of the aqueous extract of *Artemisia afra* in mice

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The objective of this study was to determine the preliminary acute toxicity profile of the aqueous extract of *Artemisia afra* (***AaAE***) in BALB/C mice via 2 routes of administration and when determined by the traditional "Litchfield and Wilcoxon" method compared with the new computer program-based method (AOT425statPgm, version: 1,0).

Mice were administered single oral (0.175 to 24 g/kg) or intra-peritoneal doses (0.175 to 5.5 g/kg) doses of ***AaAE***, monitored for mortality and toxic symptoms over two weeks, and the LD_{50} values for each route calculated. The post-administration symptoms were the same in both methods, and included minor to severe hypo-activity, pilo-erection, hyperventilation, salivation, dizziness, syncope and convulsions. When using the "Litchfield and Wilcoxon" method the LD_{50s} after acute i.p and p.o administrations were 2450 mg/kg and 8960mg/kg, respectively, and 3129 mg/kg (i.p) and greater than 5000 mg/kg (p.o) according to the "AOT425statPgm" method.

In addition, the latter method used fewer mice, viz. 20% and 50% of those used in the former method for the testing of the acute p.o. and i.p toxicity, respectively. Collectively, the results suggested that acute doses of ***AaAE*** are ***non-toxic*** in mice irrespective of the route of administration used and the "AOT425statPgm" is a potentially useful and cost-effective tool for the rapid screening of the acute toxicity of plant medicines in small animals.

Assessment of various approaches for nanoparticle formulation

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Purpose

This study focussed on the formulation of nanoparticles adopting various emulsification approaches.

Methodology

Emulsification/ solvent evaporation (ESE) approach: An aqueous solution comprising polyvinyl alcohol (PVA) (1.5% $^{w}/_{v}$) was added to an emulsified organic solution of PLGA (1000mg) dissolved in 33mL dichloromethane (DCM) and agitated overnight on a magnetic stirrer. Nanoparticles were recovered by centrifugation at 5000rpm for 10 minutes and subsequently lyophilized.

Emulsification/surfactant/solvent evaporation (ESSE) approach: PVA (1.5% $^{w}/_{v}$) was dissolved in deionised water. The organic phase comprised PLGA (1000mg) dissolved in 33mL DCM on a magnetic stirrer at 60°C (1.5% $^{w}/_{v}$) with span 20 (1% $^{w}/_{v}$) added as a surfactant. Nanoparticles were recovered by centrifugation at 5000rpm for 10 minutes.

Ionic gelification (IG) approach: A polymeric solution (0.06% $^{w}/_{v}$ Alginate) was crosslinked in a 0.5% $^{w}/_{v}$ solution employing calcium chloride as the crosslinking reagent. A 2mL solution of PLGA/DCM (0.05% $^{w}/_{v}$) was added, followed by agitation for 30 minutes at room temperature. An overnight curing time was required, subsequent to which nanoparticles were recovered by centrifugation at 5000rpm for 10 minutes.

Microscopic and Size Imaging: Samples were prepared for microscopic images with scanning fields selected at various magnifications.

Fourier Transform Infrared (FTIR) analysis: FTIR analysis was performed on native polymer and nanoparticulate samples using a Nicolet Impact 400D.

Results

Results demonstrated that addition of surfactant induced surface charge repulsion between nanoparticles, thus preventing aggregation and subsequent coalescence. This was evident from the high resolution microscopic images generated. The ESSE approach produced a superior quantity of particles in the sub-micron to nano-range (20μm and below) as opposed to the ESE approach. These preliminary investigations therefore show that the ESSE and IG approaches are the most suitable to produce a stable nanoparticle emulsion.

FTIR analysis elucidated that the polymeric backbone structure was maintained, however minor changes were noted over characteristic frequencies indicative of superficial morphological changes as a result of nanoparticle formation.

This study confirmed the presence of nanoparticles and provided evidence that the ESSE approach was the most suitable.

Development of a Symptom Focused HRQOL Tool for Patients on Highly Active Antiretroviral Therapy

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Introduction and Purpose Of Study:

Health Related Quality of Life (HRQOL) shifts the assessment of health outcomes from the absence of disease to the assessment of the patients' state of physical, mental and social well being. [(1)] Emphasis on identifying and managing patients' symptom status could improve HRQOL.[(2)] Current methods of assessment with open ended questions are insensitive, patients response may be restricted to be socially desirable [(3)] , and time and physician resources are limited. Existing tools that have been developed for research are not reliable for use in a clinical setting [(3)(4)]. Development of a symptom - focused HRQOL tool to satisfy SA monitoring and pharmacovigilance needs is therefore needed.

Methodology:

Development began with a review of symptoms and HRQOL descriptions, and their weighted frequencies, in published research. The data was consolidated into a patient questionnaire administered to 48 patients at 2 academic hospitals in Gauteng. The questionnaire comprised evaluation of sociodemographic, HRQOL and symptom data. Open text field questions were included to assess comprehensiveness and comprehensibility.

Results and Discussion:

The patient group comprised 96%black, 67% female and 77% maintained on Stavudine, Lamivudine and Efavirenz. The average age was 35.4years and the average duration of treatment was 4.5months. 21% had a primary school education. In open ended questions patients felt that the questionnaire was understandable and covered the most relevant aspects with only a mental well being scale and weight loss scale to be added on. Analysis of Variance showed HRQOL scales as fairly consistent across the group except sexuality. (P=0.9229). This will need to be addressed directly as a symptom. The most common symptoms were sadness(67%) tiredness(65%), headaches(58%), anxiety(57%), dizziness(50%), tingling(46%) and sleep abnormalities(45%). Symptom frequency correlated well to symptom bother in most symptoms except the psychiatric symptoms. Rephrasing may be needed to overcome the stigma of psychiatric illness to gage the extent of bother in this cultural group. The late manifesting adverse effects were poorly represented and must be accounted for.

Conclusion:

Routine clinical evaluation of symptom focused HRQOL is imperative in the care of HIV patients especially since HIV treatments invoke significant ADR's that diminish HRQOL. The tool should be self administered and user friendly and should measure fatigue, depression, anxiety, sleep disturbances, pain, nausea, vomiting, sexual dysfunction, actual and perceived body image. The routine use should assist drug related toxicity identification which would impact patients' perception of health status, adherence and long term survival.

Reference:

(1)Spitzer WO(1987) Quality of life and functional status as target variables in research. Journal of Chronic diseases, *40*, 465-471

(2)Justice AC(1999) Sensitivity, specificity, reliability and clinical validity of provider reported symptoms Journal of Acquired Immune Deficiency Syndrome , *21*, 123-133

(3)Justice AC(2001) Development and validation of a self completed HIV symptom index. Journal of Clinical Epidemiology, *54*, 77-s90

(4) Wu AC (1992) Measuring health status and quality of life in HIV and AIDS. Psychology and Health ,*6*, 251-264

Anti – Tumour Properties of Novel Gold Compounds

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Since the introduction of Auranofin in 1985 there has been no new clinically approved gold containing drugs introduced. Although promising results were achieved with a gold(I) phosphine complex [Au $(dppe)_2$]Cl,[1,2] this compound was never entered into clinical trials due to its toxicity to normal tissue such as the liver and heart.[3,4]

Six novel derivatives of [Au $(dppe)_2$]Cl were designed and synthesized to identify possible new candidates with improved tumour specificity compared to [Au $(dppe)_2$]Cl and cisplatin. Human cervical carcinoma cells (HeLa) were used for an initial toxicity screening. IC_{50}'s obtained for [Au $(dppe)_2$]Cl and cisplatin were 0.684 and 0.669 μM respectively. Mixed metal complexes incorporating similar coordination motifs, displayed IC_{50}'s ranging between 0.027 and 0.094 μM. These compounds were then tested further for selectivity and cytotoxicity on various malignant and normal cell lines. One showed selectivity for cervical, breast and ovarian cancer cells, while another was the most effective against cervical, prostate, breast and ovarian cancer cells. A third compound was most active against cervical, colon, prostate and ovarian cancer cells. The experimental compounds had much higher IC_{50}'s when tested on the normal cells, which indicates selectivity for cancer cells.

The octanol / water partition coefficient (lipophilicity) of all the experimental compounds were determined to establish if there is a correlation between the lipophilicity, IC_{50} and tumour specificity. No correlation was found between these parameters.

[Au$(dppe)_2$]Cl is known to have an effect on the mitochondrial membrane potential.[1,2] Three mixed metal complexes and cisplatin were compared to [Au$(dppe)_2$]Cl for effects on mitochondrial membrane potential. PHA stimulated human lymphocytes and a human undifferentiated leukemia T-cell line (Jurkat cells) were used in these experiments. [Au$(dppe)_2$]Cl and the three mixed metal complexes depolarized the mitochondrial membranes of PHA stimulated lymphocytes, while only [Au$(dppe)_2$]Cl depolarized the mitochondrial membranes of the Jurkat cells, indicating that a different mechanism of action might be operational.

The experimental compounds show low IC_{50}'s combined with increased tumour specificity. This indicates that the compounds have great potential and should be investigated further as anti-cancer agents.

References

[1] G.D. Hoke, F.L. McCabe, L.F. Faucette, J. O'Leary Bartus, C-M. Sung, B.D. Jensen, R. Heys, G.F. Rush, D.W. Alberts, R.K. Johnson, C.K. Mirabelli, Molecular Pharmacol. 39 (1990) 90.

[2] M.J. McKeage, L. Maharaj, S.J. Berners-Price, Coord. Chem. Rev. 232 **(**2002) 127.

[3] P.F. Smith, G. D. Hoke, D.W. Alberts, P.J. Bugelski, S. Lupo, C.K. Mirabelli, G.F. Rush, Pharmacology and Experimental Therapeutics 249(3) (1989) 944.

[4] P.J. Sadler, Z. Guo. Metal complexes in medicine: Design and mechanism of action. Pure and Appl. Chem. 70 (4) (1998) 863.

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Structure - Activity Relationship and Neurotoxicity Studies with the MAO-B Substrate, 1-methyl-3-phenyl-3-pyrroline.

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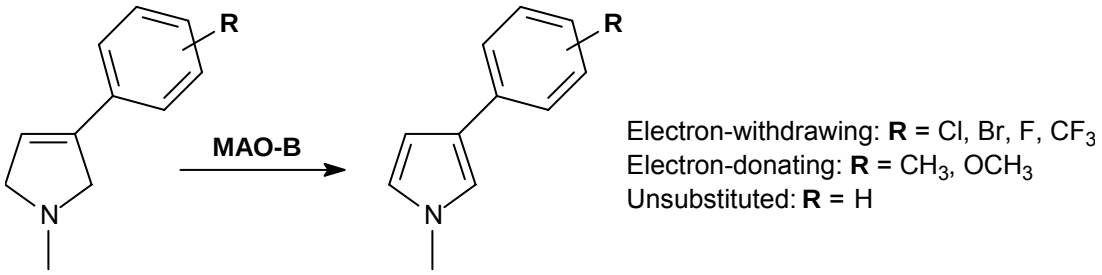
Purpose:

The enzyme monoamine oxidase B (MAO-B) is a drug target for the treatment of neurodegenerative diseases such as Parkinson's disease. In addition to being an important drug target, MAO-B also is of interest because of its role as the catalyst that mediates the bioactivation of the pro-neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Recently, it was shown that 1-methyl-3-phenyl-3-pyrrolines are also excellent substrates of MAO-B. These substrates are of considerable interest since they are not expected to be metabolized to neurotoxic end-products and therefore may be safe alternatives to tetrahydropyridinyl analogues for mechanistic studies of enzyme function. In this study we have synthesized and characterized additional 1-methyl-3-phenyl-3-pyrroline analogues as MAO-B substrates. Selected analogues were also evaluated as potential neurotoxic agents in C57BL/6 mice.

Method:

The target 1-methyl-3-phenyl-3-pyrroline analogues were synthesized according to procedures reported in the literature. An HPLC method was developed to determine the steady-state kinetic constants (K_m and V_{max} values). The rate data was fitted to the Michaelis-Menten equation using a non-linear least-squares fitting routine. Selected substrates were evaluated as potential neurotoxins by treating C57BL/6 mice with the test compounds and measuring the dopamine and DOPAC levels seven days later *ex vivo* in the dissected striatal tissue.



Results:

The synthesized analogues of 1-methyl-3-phenyl-3-pyrroline were found to be good substrates of MAO-B with Michaelis constants (K_m values) in the micro-molar range. Unlike MPTP, none of the substrates tested were found to be neurotoxic since no striatal dopamine depletion was observed in mice treated with the test compounds.

Acknowledgements:

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Development and optimization of an Analytical Method for the Analysis of Ibuprofen in Dosage Forms.

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Objective

To develop and optimize and HPLC method for the *in vitro* assessment of ibuprofen dosage forms.

Method

An isocratic HPLC method using UV detection was developed using a modular HPLC system, comprised of Spectraseries® pump, Linear® UV detector, a WISP® 710B autosampler and a Spectraphysics® integrator. Separation was achieved on a Phenomenex® Hypersil C_{18} 5 µm, 4.6 mm i.d. X 15.0 cm column with a mobile phase comprising of acetronitrile: 25 mM phosphate buffer (PH 4.29) 50:50. The eluent was monitored at 265 nm. Ketoprofen was used as the internal standard. The effects of mobile phase pH, buffer molarity, composition and organic modifier on retention time were evaluated in order to establish optimal separation conditions for the analysis of ibuprofen. The method will be validated using USP and ICH guidelines and accuracy, precision, linearity, and the limits of detection (LOD) and quantification (LOQ) will be determined.

Results

The impact of mobile phase buffer pH on retention time varied with the organic modifier used (*viz.,* acetonitrile or methanol) to prepare the mobile phase. Mobile phases in which acetonitrile was used revealed retention times of 1.6 and 1.4 minutes for ibuprofen and ketoprofen, respectively at pH =7.2 and retention times of 8.4 and 4.4 minutes were observed for the drugs at pH=3.5.Optimal retention times were obtained using a mobile phase of pH=4.3. In contrast, when methanol was used as the modifier the retention times were 49 and 10.8 minutes for ibuprofen and ketoprofen, respectively at pH=3.6 and 7.2 and 3.2 minutes at pH=7.2. In general, retention times decreased with an increasing pH and decreased with increasing organic modifier content. As the buffer molarity increased from 0.04 M to 0.05 M the retention times increased from approximately 7 to 14 minutes for ibuprofen and 3 to 6 minutes for ketoprofen.

Conclusions

The impact of mobile phase pH, buffer molarity and organic modifier on the retention times of ibuprofen and ketoprofen has been evaluated and an increase in pH was found to decrease retention times, whereas an increase in molarity increased retention times for both compounds. Methanol was selected as the organic modifier of choice due to cost and a better peak shape. A mobile phase comprising of methanol:phosphate buffer (0.025 M, pH=4.29) (50:50), was considered optimal and was used for the validation studies.

Acknowledgements

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Influence of Chemical Modification on the Efficacy of a Sulphadiazine Cream

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Introduction:

Silver sulfadiazine (SSD) is the most widely used topical antimicrobial agent. Its clinical advantages, includes excellent antimicrobial spectrum of activity, low toxicity and minimal pain on application to burn wounds. However, due to precipitation reactions and microbial resistance to SSD a zinc-sulphadiazine (ZSD) preparation was formulated.

Methods:

A zinc sulphate and silver sulphate solutions were used to prepare a ZSD and SSD cream *in situ* during the manufacturing of the cream base. These two products were compared to a commercially available SSD cream (Silbecor®). The *in vitro* antimicrobial activity of these products was compared to a commercially available preparation.

Results:

The formulated ZSD preparation was found to be more active against *Pseudomonas aeruginosa* and *Staphylococcus aureus* than both the commercially available and SSD cream. However, ZSD showed no activity against *Escherichia coli* or *Aspergillus niger* and thus this aspect of the formulation needs to be further investigated.

Evaluating anticholinergic / antipsychotic prescribing patterns at different levels in the South African Healthcare System.

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Purpose:

Anticholinergic drugs are frequently prescribed during antipsychotic therapy to counteract the extrapyramidal adverse effects experienced with treatment. The aim of this study was to determine antipsychotic / anticholinergic prescribing patterns in two types of public healthcare facilities.

Methods:

Data was collected at the Psychiatric unit of a tertiary hospital (TH), as well as a primary health care clinic (PHC). Outpatient medical charts for a randomly chosen month were reviewed. These included new patients as well as patients currently receiving treatment. Patients with pre-existing movement disorders were excluded.

Results:

While older generation antipsychotics were most frequently prescribed at both the TH and the PHC, a wider variety of antipsychotics were used at the TH. Antipsychotics prescribed most often at the TH included haloperidol (26.6%), flupentixol (11.6%) and chlorpromazine (11.6%). Chlorpromazine was the antipsychotic mostly prescribed in the PHC (30.7 %). Anticholinergic medication was more often prescribed at the PHC (76.7%), when compared to the TH (32.2%). While both biperidin and orphenadrine were prescribed at the PHC, only orphenadrine was prescribed in the TH. At the TH, anticholinergic medication was most often prescribed to patients receiving treatment with a single antipsychotic drug (25.6%), 13.1% of these being patients receiving depot formulations of antipsychotics, as opposed to the PHC where anticholinergic medication was most often prescribed to patients being treated with a combination of two antipsychotic drugs (40%). More patients were treated with a combination of antipsychotic drugs at the primary health care clinic (46%) compared to the TH (11.1%).

Conclusions:

This study observed distinct differences in prescribing patterns of antipsychotic medication as well as antipsychotic/anticholinergic co-prescribing at different levels of the health system. Anticholinergic medication was used more often in the PHC, when compared to the TH. This might be attributed to anticholinergic medication being used as "prophylaxis" against extrapyramidal symptoms in the PHC (where a resident is available only once a month), while in the tertiary hospital (with psychiatrists available to monitor patients and take immediate action upon observation of extrapyramidal symptoms) it is only indicated for treatment of extrapyramidal effects. Using of anticholinergics as "prophylaxis" is in contravention of EDL guidelines where extrapyramidal symptoms are treated 1) by changing the antipsychotic drug, 2) by decreasing the dose of the antipsychotic drug, and 3) by controlling extrapyramidal symptoms with an anticholinergic drug. By administering anticholinergic drugs as "prophylaxis", there is an increase in treatment cost, as well as adverse effects experienced by the patient.

Elastic properties of the aorta and the endothelial function in ACE Inhibitor treatment in hypertensive patients

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Purpose:

Increased arterial stiffness and endothelial dysfunction are associated with end-organ damage and are independent predictors of cardiovascular risk. Assessment of pulse wave velocity in conjunction with endothelin-1 level can provide additional useful information on the efficacy of antihypertensive therapies. The purpose of the study was to assess the elastic properties of the arteries and the endothelial function in newly diagnosed hypertensive patients (n=44), before and after 9 months treatment with an angiotensin converting enzyme (ACE) inhibitor- perindopril 4mg.

Methods:

PWV and endothelin-1 determination was measured non-invasively (Powerlab 4 SP system). Aortic and peripheral pulses were recorded (infrared plethysmo-dopler sensors). Endothelin-1 was measured using an I^{125} immuno-assay radioactive ligand system (the amount of radioactive ligand bound by the antibody is inversely proportional to the concentration of the added non- radioactive ligand).

Results:

Aortic elasticity in black hypertensive patients has improved with perindopril treatment, beyond that seen with BP-lowering alone.There was a significant decrease in the aortic PWV (a slower PWV means better elasticity) from 11.8m/s to 8.9m/s. The arterial elasticity became comparable with the arterial elasticity of the healthy volunteers. The endothelin-1 level at the baseline (5.9 ±3.26 pmol/l) was found to be the highest in the hypertensive patients and was higher than in the patients with diabetes, hyperlipidaemia and obesity (4.3 ± 2.4 pmol/l). There was a significant increase in the endothelin-1 level at the 3 months of the treatment but after 9 months there was a decrease of the level to that comparable with the baseline value.

It would seem that perindopril could restore endothelial integrity by inhibition of endothelin-1 production. Since the changes in the endothelin-1 level are directly dependent on the function of the vascular endothelium a longer follow-up is required to determine if ACE inhibitors can significantly decrease the endothelin-1 level. The present study clearly shows that the deterioration of the arterial stiffness and the endothelin-1 level may help in the evaluation of the individual risk in hypertensive patients and in the choice of the treatment.

Power law size scaling of copolymer particles employing GPC-MALLS

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Purpose:

Analysis of light scattering data concerning dissolved polymers could reveal the bulk conformation of a polymer chain. However, monodisperse samples are required to perform accurate calculations. In reality, certain methods of polymer synthesis produce polydisperse samples, thus precluding accurate bulk conformation studies. Additionally, uncertainties at the high and low ends of the light scattering peaks arise from apparatus and column limitations. Elimination of these uncertainties could render a monodisperse segment in the scattering peak that could be fitted with a power law regression equation to study the conformation.

Methods:

Synthesis of copolymers: The synthesis will be disclosed elsewhere and briefly employed a free-radical addition mechanism. Different monomers in various ratios were reacted under appropriate conditions for 120 minutes.

Sample preparation: The reaction was quenched after expiration of the reaction period and precipitated with MeOH. The samples were agitated vigorously with a Vortex® shaker followed by centrifugation for 10 minutes at 3 500 r.p.m. Washing and precipitation was repeated two times. Bulk solvent evaporation was performed in a fume cupboard for 24 hours with subsequent grinding of the material. Finally the ground polymer was transferred to a vacuum oven operated at 25 °C, achieving a vacuum of 13 mbar, for an additional 24 hours.

GPC-MALLS: The MALLS was carefully calibrated and diodes normalised according to the prescribed procedures, followed by alignment of the MALLS and concentration detector (RI detector). The appropriate GPC columns were connected in series to an isocratic HPLC system with THF as elution liquid. Flow rate was maintained at 1 $ml.min^{-1}$ and apparatus temperature at 30 °C. Samples were prepared at a concentration of 3 $mg.cm^{-3}$ and 100 µl of this was injected. Samples included an internal standard for on-line determination of an essential weight-calculation parameter, dn/dc.

Results and Conclusion:

Power law scaling of a double-logarithmic plot of *z*-average root mean square radius of gyration versus weight-average molecular weight produced a superior fit of data compared to the data treatment by the MALLS software package or with the inclusion of uncertainties.

The limitations of the synthesis method (samples of polydispersity ranging 1.5 to 2.1) and MALLS detection limit (20 nm) could be circumvented by finding a monodisperse sample segment, extending application of the GPC-MALLS setup.

Application of this data treatment could be used to gain more reliable insight regarding bulk chain conformation.

The cytotoxic effects of novel synthetic biaryls and aromatic esters.

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Introduction:

Over the past few decades, a major effort has been made to develop anti-cancer drugs through both empiric screening and rational design of new compounds as many different types of cancers are still untreatable. These attempts are made to reduce severe adverse drug reactions of existing cancer chemotherapeutic agents as well as reducing the development of drug resistance.

Research on biaryl and aromatic ester compounds have shown that they posses a wide range of therapeutic activities, including antifungal, anti-inflammatory, antirheumatic, antitumour and antihypertensive actions. The synthetic compounds used in this study have never been tested against cancer cells and as a result, their potential cytotoxic activity is still unknown.

The aim of the study is to screen novel biaryl and aromatic ester derivatives for cytotoxic activity.

Materials and Methods:

All compounds were synthesized using standard organic chemistry techniques. Breast (MCF-7), colon (HT-29), and leukemic (HL-60, K562) cancer cell lines were used in the study. Cell lines were maintained in culture using standard culture techniques. In order to determine the cytotoxic activity of these compounds, the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay was used. Results are expressed as IC_{50} values, as determined by the Enzfitter programme.

Results:

The aromatic ester class proved to be cytotoxic on Graham's, K562 and HL-60 cell lines with IC_{50} values ranging from 37-80 μM. The biaryl class of compounds proved to be cytotoxic on all cell lines tested with IC_{50} values ranging from 40-80 μM. The cytotoxic effects of these synthetic compounds suggest potential for its application in cancer therapy as promising lead compounds.

Isolation and Semi-Synthesis of *Sceletium* Alkaloids

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Purpose:

The *Sceletium* plant has been reported to contain psychoactive alkaloids, specifically mesembrine, mesembrenone, mesembrenol and some other related alkaloids. *Sceletium* is marketed as dried plant powder and as phyto-pharmaceutical dosage forms. *Sceletium* products and plant material marketed through health shops and on the internet are associated with unjustified claims which relate to therapeutic efficacy and, in addition, may be of dubious quality. Since relevant alkaloids are not commercially available for use as markers for the analysis and quality control of *Sceletium* plant material and products containing *Sceletium,* the primary purpose of this research was to isolate and characterise such compounds for use as reference standards for quantitative analysis.

Methods:

Mesembrine, mesembrenone, Δ^7-mesembrenone were isolated by solvent extraction and chromatography from dried plant material. Mesembranol and epimesembranol were synthesised by hydrogenation of the isolated mesembrine using platinum(IV) oxide as catalyst and then further purified by semi-preparative column chromatography. All compounds were subjected to analysis by ^{1}H, ^{13}C and 2-D NMR and LC-MS. Mesembrine was converted to hydrochloride crystals and mesembranol was isolated as crystals from the hydrogenation reaction mass. The compounds were analysed and characterised by X-ray crystallography.

Results:

The 2-D NMR spectral data confirmed the structure of the compounds and LC-MS data confirmed the mass of the compounds. The crystallised mesembrine hydrochloride and mesembranol were subjected to X-ray crystallography which confirmed the relative configuration of the compounds.

Conclusion:

The isolated markers were characterised by spectral data for structural confirmation and were qualified for use as reference standards for the analytical method development and subsequent quality control procedures for *Sceletium* plant material and its dosage forms.

Acknowlegments:

A research grant (I.K.) and doctoral bursary (S.P.) from the NRF are gratefully acknowledged.

Development of a Silicone Moulded Compartmentalised Tray: An Approach to Enhance Drug Uniformity in the Preparation of Polymeric Films

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Purpose:

Mucoadhesive films are being widely investigated for novel drug delivery. An extensive search of the literature and patent applications indicate a difficulty with achieving uniform drug distribution in films. The disadvantages with current patent applications such as those of Yang et al (U.S Pat. No. 60/443,741) and Zerbe et al (U.S.Pat. No. 5,948,430) for enhancing drug uniformity are that they require additional pharmaceutical excipients and sophisticated drying equipment leading to increased costs. Therefore, the aim of this study was to compare the drug uniformity of chitosan films prepared in a specially designed silicone moulded compartmentalised tray containing teflon coated perspex inserts as compared to when casted conventionally onto a teflon coated perspex tray.

Methods:

Chitosan films containing drug (15 mg Propranolol HCl per 3cm^2 film) were prepared by casting the drug containing polymeric solution onto: (a) a teflon coated perspex tray (TCPT) and (b) into individual wells of a silicone moulded tray (SMT) containing teflon coated perspex inserts. Both trays were placed in an incubator at 37^{0}C for 24 hours to allow for solvent evaporation. Films (3x1cm^2 pieces) were characterised in terms of drug content (UV Spectroscopy, λ max=289nm), mucoadhesion (Texture Analyser XT2*i)*, drug release (Shaking Water Bath), film thickness (Electronic Digital Micrometer) and surface morphology (Scanning Electron Microscopy).

Results:

Films prepared using the TCPT demonstrated a non-uniform drug distribution of 87.73±58.18% that clearly did not meet compendial specifications. However, films prepared using the SMT significantly enhanced drug uniformity (96.67±1.16%). Further, the mucoadhesion and thickness of films from the SMT were more uniform (113±20 mN and 0.13±0.02mm) as compared to those from the TCPT tray (367±174 mN and 0.21± 0.095mm). Calculation of similarity factors also indicated more reproducible drug release for films from the SMT (f_2>100) than those of TCPT (f_2<100). The morphology of films from the SMT was similar in surface homogeneity to those from the TCPT. Inter batch reproducibility of films prepared in the SMT was also confirmed.

Conclusion:

A specifically designed silicone moulded tray with teflon coated perspex inserts generated homogenous films with uniform drug content, mucoadhesion, drug release, thickness and morphology.

Synthesis, characterization and preliminary evaluation of hydrophilic polymeric nanoparticles and scaffolds for controlled drug delivery

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Purpose:

The study was aimed at developing polymeric nanoparticles and scaffolds intended for implantation into the brain for the treatment of Parkinson's Disease.

Methods:

Nanoparticles were formed using an emulsification-diffusion method. Briefly 1.5g cellulose acetate phthalate (CAP) was dissolved in 100mL acetone and left to saturate with an equal volume of deionised water for an hour. PVA (40mL, 5% w/v) was added to the system and agitated for 15min. Diffusion of the organic phase to the continuous phase was induced by the addition of water (60mL). Excess solvent was evaporated using a Rotavap, the resulting solution was centrifuged and the sediment was lyophilized for 24-hours leading to the formation of nanoparticles.

A freeze-dry method was employed to develop the calcium-alginate scaffolds. Calcium gluconate (0.4%w/v) was added to alginate (2%w/v) and agitated for 15 minutes. This homogenous mixture was placed in moulds and lyophilized for 24 hours. The lyophilized scaffolds were placed in crosslinking solutions: 2%w/v each of barium chloride, calcium chloride and zinc sulphate for an hour and thereafter lyophilized for a further 24 hours. The resulting scaffolds were removed from the moulds, washed with deionised water to leach out the remaining salts and air-dried prior to analysis.

Fourier Transform Infrared (FTIR) Analysis was carried out on both the polymer (CAP) and the nanoparticles so as to determine differences in chemical structure.

Microscopy and Size Imaging was undertaken for the measurement of nanoparticles size and scaffold morphology.

Textural Analysis (TA): Hydrated samples were evaluated for changes in matrix resilience, fracture energy and deformability moduli.

Results:

FTIR analysis demonstrated that the overall native polymeric (CAP) structure was maintained however slight changes were noted. These changes show that surface interaction has taken place in the formation of the nanoparticles. Results from microscope images of the nanoparticles showed that the particle size ranged from 500nm-0.3μm. TA resilience studies revealed that hydrated barium alginate scaffolds demonstrated greater resilience (62%) as compared to hydrated calcium alginate (29.5%) and zinc alginate scaffolds (26%).

A multi-layered double-disk polymeric device for phase-controlled drug delivery

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Purpose

To formulate and evaluate a multi-layered double-disk polymeric device for phase-controlled chronotherapeutic drug delivery.

Methods

Preparation of the device: Hydroxyethylcellulose (HEC) and polyethylene oxide (PEO) polymer grades were compressed into robust compacts using a Beckman Hydraulic Press (4-8 tons). Two theophylline-loaded disks were suspended between three alternating heterogeneous polymeric layers.

Textural Profile Analysis: A Texture Analyzer was used to determine the Indentation Hardness which was represented by a conversion to the Brinell Hardness number (BHN).

In vitro release studies: Studies were performed using the USP (XXV) rotating paddle method in a six-station dissolution apparatus comprising 900mL phosphate buffered saline (PBS)(37 °C) at a pH range of 3 - 6.8. At pre-determined intervals dissolution media were sampled and drug content was analyzed with UV spectrophotometry at the wavelength maximum.

Fourier Transform Infrared (FTIR) Analysis: FTIR was performed on native polymers and compressed samples using a Nicolet Impact 400D apparatus to elucidate any polymeric structural changes during compression.

Results

Robust matrices were produced upon compression of HEC, PEO and the drug-loaded double disks. Textural analysis confirmed BHN values to range from 2.071 - 2.949 N/mm^2 which demonstrates desirable compressibility characteristics. HEC and PEO were used as a retentive mechanism in achieving a significant lag phase of between 3-5 hours prior to drug release. Drug release occurred in a biphasic release pattern with an initial lag-phase and a subsequent exponential release phase to completion. This biphasic release ranged from 7-26% at $t_{12hours}$ followed by 19-75% at $t_{24hours}$.

The multi-layered double-disk polymeric device was successfully designed for phase-controlled drug delivery, which demonstrates desirable release kinetics for chronotherapeutic disorders.

A novel multi-unit gastrofloatable device for delivery of 'Narrow Absorption Window' (NAW) bioactives

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Purpose

The purpose of this study was to develop a novel Multi-unit Gastrofloatable Device (MGD) for prolonged local drug delivery to the upper gastrointestinal tract employing a lyophilized, buoyant, polysphere system.

Methodology

Preparation of the MGD: Ionotrophic gelation with subsequent lyophilization at a -60 ^{0}C condensation phase and a sublimation time of 24 hours at 25mtorr was employed to formulate the MGD. Variable concentrations of sodium alginate, pectin and PLGA (0.25% $^{w}/_{v}$ -2% $^{w}/_{v}$) as a matrix consolidator were used in accordance with a Box-Behnken statistical design. Model compound riboflavin was dispersed in a 2:1 ratio (polymer: drug).

Drug encapsulation efficiency (DEE): Samples of 50mg MGD's were dissolved in PBS (pH 6.8). Dissolution of MGD's was facilitated by triturating them prior to buffer addition. Drug content was determined by UV spectrophotometry.

In vitro release studies: A USP(XXV) rotating paddle method was utilized for release studies. MGD's were immersed in HCl buffer media (pH 1.5; 37ºC) and PBS (pH 6.8; 37ºC) over a 24 hour period. Samples of 5mL were taken every 2 hours and analyzed by UV spectrophotometry.

Determination of buoyancy: Buoyancy lag time and duration of buoyancy was determined in HCl buffer media (pH 1.5; 37ºC) and PBS (pH 6.8; 37ºC) for comparative purposes. The time interval between the introduction of the MGD's into the dissolution medium and its buoyancy to the top of the medium was taken as the buoyancy lag time and the duration of system floatation was visually observed.

Results

The MGD's remained buoyant in HCl buffer (pH 1.5) and PBS buffer (pH 7.4) for prolonged periods of time ($t>48$ hours and $t\geq24$ hours respectively). The duration of floatability in the respective buffer media were independent of formulation variables. The MGD's were able to release riboflavin in two phases with an initial up-curving zero-order release phase (65% at t_{4hours}) followed by a sustained lag phase (40% at $t_{24hours}$). DEE studies demonstrated encapsulation efficiencies between 70-90%.

The study proves that the MGD may be suitable for application as a gastroretentive system to improve the oral bioavailability of Narrow Absorption Window (NAW) bioactives that are characterized by site-specific absorption in the upper GIT.

The role of Acetyl-L-carnitine on the long-chain fatty acid metabolism in MPTP treated rats

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Background and purpose:

Very long-chain fatty acids (VLCFAs) play an integral role in several physiological processes and are essential components of phospholipids in membranes. Fatty acid elongation in the cytoplasm (endoplasmic reticulum) and in the peroxisomes utilizes malonyl-CoA as a carbon source, whereas mitochondrial elongation uses acetylcarnitine or acetyl-CoA. Fatty acids are involved in the biosynthesis of docosahexaenoic acid (DHA, 22:6*n*-3) and the formation of acetylcarnitine. MPTP causes a decrease of acetyl-CoA production due to the inhibition of the acyl-CoA dehydrogenase enzymes, ETF, or ETF-QO and causes neurological damage which leads to clinical symptoms similar to Parkinson's disease and a metabolic profile similar to GA II, suggesting that it may be possible to induce a deficiency similar to GA II. We argued that if the neurological symptoms are the result of deceased acetyl-CoA production, which could prevent fatty acid elongation, the problem could possibly be rectified by supplementation with acetyl-L-carnitine (ALCAR). ALCAR supplementation had already been shown to prevent the development of the clinical symptoms associated with MPTP treatment, but it has not been known if acetylcarnitine will increase the fatty acid elongation.

Methods:

Very long-chain fatty acid analysis: Rats were treated with ALCAR and their serum analyzed for certain metabolites, using the GC/MS. The fatty acid concentrations were expressed in consequential ratios (C24:C22 and C26:C22), which provide a more sensitive criterion than concentrations per se to indicate the possible defective enzymes involved in the fatty acid elongation pathway.

Acyl- and acetylcarnitine analysis: Acetyl- and acylcarnitine concentrations were determined in ALCAR treated rats before and after a single treatment with the GA II inducing chemical, MPTP. LC/MSMS was used for these analyses.

Results:

ALCAR did not influence the VLCFA biosynthesis under normal circumstances, but it did activate VLCFA biosynthesis when their concentrations were reduced. ALCAR treatment can however, be considered as safe in the treatment of VLCFA and neurodegenerative defects, since it had little/no effect on the VLCFA biosynthesis. ALCAR promoted the formation of some acylcarnitines (especially glutarylcarnitine), conjugates of the GA II metabolites, after MPTP treatment, suggesting that ALCAR might play an important role in the detoxification of GA II metabolites.

Antioxidant Properties of *Lippia Javanica* (Burm. f) Spreng

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Purpose:

Medicinal plants have become the focus of intense study recently in terms of their traditional uses, which are often supported by their actual pharmacological effects. With the increasing acceptance of traditional medicine as an alternative form of health care, the screening of medicinal plants for bioactive compounds has become important. The aim of this study was to do a preliminary screening to determine whether the selected plants exhibited antioxidant activity in certain assays and to isolate bioactive compounds from species exhibiting activity.

Methods:

Guided by a literature survey and taxonomic information, various plants were selected and collected form the botanical garden and the wild. The dried and crushed leaves of the plants were extracted using petroleum ether, dichloromethane, ethyl acetate and ethanol. Subsequent screening of the crude extracts of 21 plants, using the FRAP-assay (Ferric Reducing Antioxidant Power) showed *Lippia javanica* to possess the best activity and was selected for further study. Superoxide anion generation was determined using the nitroblue tetrazolium assay. Brain homogenates containing 1mM KCN and varying concentrations (1.25, 2.5 and 5mg/ml) of *L. javanica* extracts were tested for possible free radical scavenging activity. Absorbance values were converted to concentration nitroblue diformazan (µmoles/mg protein) for comparative purposes. The protein content was determined using the Lowry-method and the results were expressed in mg protein/ml homogenate as an average of two experiments. A one-way analysis of variance test was used to evaluate any significant differences between the varying concentrations of the extracts and the control and/or toxin.

Results:

During antioxidant screening, several plants exhibited activity with the FRAP-assay, one of these being *L. javanica* of which the ethanol extract exhibited a FRAP value of 9502.13±148.03µM FRAP, which was the highest value. *In vitro* results of the nitroblue tetrazolium assay demonstrated that extracts of *L. javanica* significantly curbed the 1mM KCN induced superoxide anion generation in a dose dependant manner. The petroleum ether extract had the best activity, but there was no significant difference between the 2.5 and 5mg/ml concentrations.

Conclusions:

All of the extracts significantly reduced superoxide anion generation at all concentrations used. The results of this study show that the extracts of *L. javanica* are effective in scavenging free radicals and that these extracts have the potential to be neuroprotective.

Metallothionein expression in tissues of rotenone-treated rats

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Purpose

Mitochondrial NADH:ubiquinone oxidoreductase (complex I) carries out a number of well defined functions required for cell physiology. Deficiencies of complex I lead to multi-system disorders that include several well-known phenotypes such as type 2 diabetes mellitus and Alzheimer's disease as well as less known phenotypes such as MELAS, Leigh syndrome and MERRF. It was recently found that ROS sensitive proteins known as metallothioneins (MTs), are over-expressed in complex I deficient cell lines and that these proteins have a protective effect against ROS related pathologies. This study investigated the expression of different MT isoforms in rotenone-treated Sprague Dawley rats, an *in vivo* model that has been used to study cellular biological responses of mitochondrial complex I deficiency.

Methods

The hypothesis of this study states that a rotenone-induced complex I deficiency would lead to an increase of MT mRNA expression *in vivo.* The specific aim was to determine the relative mRNA expression levels of the three main MT isoforms in different tissues of rotenone-treated rats using real-time PCR. In this study the differential expression of MT-1, MT-2 and the brain specific isoform, MT-3 in brain, liver, heart- and skeletal muscle tissues of rotenone-treated Sprague Dawley rats is described.

Results

The results indicate that MT-1 expression is significantly increased in the liver as well as, but to a lesser extent, in the brain and heart muscle. MT-1 expression in skeletal muscle was not detected. In contrast, significant increases in expression were observed for MT-2 in all the tissue types with an approximate two-fold increase at the highest rotenone dosage in liver, brain and heart muscle. Skeletal muscle had the smallest increase. For MT-3, no detectable levels of expression could be observed in skeletal and heart muscle. Surprisingly, levels of expression occurred in the liver which slightly (43%), but significantly increased at the highest rotenone dose. As expected, much higher relative levels of MT-3 expression was observed in brain tissue with a more pronounced increase (almost two-fold) at the highest rotenone dose. In accordance with the hypothesis, the *in vivo* data generated from this study supports the published *in vitro* findings which showed that a rotenone-induced complex I deficiency results in enhanced MT expression. This suggests that MTs may play a protective role in diseases resulting from complex I deficiencies.

Anti-oxidant effects of novel gold compounds

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Auranofin (AF), the orally administered gold-based anti-arthritic agent, emerged as a clinically useful therapeutic drug for the treatment of rheumatoid arthritis in the late 1970's.

The toxicity of anti-rheumatic gold compounds has limited their use, yet both the toxicological and therapeutic actions of these drugs remain unclear. To improve the bioavailability, pharmacokinetics and to obtain a safer profile, three derivatives of auranofin viz. Asa-fin, Mpta-fin and Pta-fin, with varying degrees of lipophillicity have been designed and synthesized. Reactive oxygen species (ROS) play an important role amplifying the inflammatory process and in tissue injury. It has been documented that AF inhibits many aspects of immune cell function, including superoxide and hydrogen peroxide production.

The in vitro anti-oxidant efficacy of the gold compound AF and the three novel AF derivatives, were compared by studying their effects on the generation of ROS using N-formymethionyl-leucyl-phenylalanine (FMLP) and phorbol myristate acetate (PMA) stimulated human neutrophils. The production of ROS was measured using a luminol enhanced chemiluminescence and a flow cytometry procedure to determine superoxide release with Hydroethidine, hydrogen peroxide release with Dihydrorhodamine 123 and nitric oxide release with2'7'-Dichloroflourescin diacetate.

AF, Asa-fin and Mpta-fin showed a biphasic effect on the hydrogen peroxide produced by the FMLP stimulated neutrophils on the chemiluminometer. Addition of low concentrations of AF, Asa-fin and Mpta-fin ($\leq$ 0.5 μM) enhanced, while higher concentrations (0.5-12.5 μM) inhibited hydrogen peroxide release. Pta-fin had no effect on the hydrogen peroxide produced.

Concentrations of AF and Asa-fin ($\geq$ 12.5μM) decreased the release of hydrogen peroxide and superoxide released by the PMA stimulated neutrophils on the flow cytometer. Mpta-fin and Pta-fin exerted no effect on the hydrogen peroxide and the superoxide produced by the PMA stimulated human neutrophils. All the drugs had no effect on nitric oxide released by the PMA stimulated human neutrophils.

These findings suggest that the gold compounds AF, Asa-fin and Mpta-fin inhibit respiratory bursts and the generation of inflammatory reaction products by neutrophils. Further testing could prove these compounds to be promising anti-inflammatory drugs.

Prescribing Patterns of Antiretroviral Drugs in a Section of the Private Health Care Sector in South Africa

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Purpose:

The general objective of this study was to investigate the prescribing patterns and cost of antiretroviral drugs (ARV), in the private health care sector in South Africa by using a medicine claims database.

Method:

A quantitative, retrospective drug utilisation review was performed on data from 2001, 2002 and 2004. Each year was devided into three four-month study periods, namely January to April, May to August, and September to December.

Results:

Antiretroviral drugs represented 0.38 per cent (n=1 475 380) for 2001, 0.72 per cent (n=2 076 236) for 2002, and 1.68 per cent (n=2 595 254) for 2004 of all studied prescriptions. The total cost of the ARV drugs represented 1.31 per cent (R379 708 489) for 2001, 3.03 per cent (R601 350 325) for 2002, and increased to 5.25 per cent (R661 223 146) for 2004. All ARV medicine items claimed during 2001 (n=9 796) and 2002 (n=35 271) were innovator products.

During 2004, 94.77 per cent (n=96 609) of ARV medicine items (n=101 938) claimed were innovator medicine items. Only 5.23 per cent (n=5 329) of all the ARV medicine items (n=101 938) claimed during 2004 were generic products. The average cost per ARV medicine items for 2004 increased from R317.93±190.80 for the period January to April to R369.20±219.50 for the period May to August, and decreased to R324.79±212.48 for the period September to December and resulted in a cost saving of R41 044.35 for the period May to August versus September to December for the ARV medicine items.

Conclusion:

Both the ***prevalence*** and ***cost*** of drug therapy increased from 2001 to 2002. The prevalence increased from 2002 to 2004, but the cost of drug therapy decreased during 2004. An increase in prevalence could be due to an increase of HIV/AIDS infections in people in the private sector or due to the availability of ARV medicine on medical aid plans, or an increase in medical scheme members. The decrease in the cost of drug therapy is probably a result of the implementation of the new pricing regulations in May 2004.

The Impact of Therapeutic Education on Hypertensive Patients' Levels of Adherence to Therapy.

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Purpose

The burden of non-communicable diseases such as hypertension in South Africa continues to increase, whilst resources available are insufficient to meet this demand. The control of hypertension is far from optimal, the major reason being poor adherence to anti-hypertensive therapy. One of the main reasons for poor adherence to therapy by patients is their lack of pertinent health-related information. The aim of this project was to educate selected participants about hypertension and its therapy in order to investigate the impact of such an intervention on the participants' adherence to their medication.

Methodology

45 hypertensive patients from the Rhodes University support staff were involved in the study. Patient Therapeutic Education was in the form of talks and discussions involving all the participants as one group or as individuals. The participants were also given written information, about hypertension and its therapy, in English and isiXhosa. Adherence levels were measured, before and after the educational intervention using pill counts, self-reports and prescription refill records. The participants' levels of knowledge about hypertension and its therapy, as well as their blood pressure, were also measured before and after the intervention. The post-intervention measurements commenced three weeks after the intervention which lasted five months.

Results

There were significant increases in the participants' levels of knowledge about hypertension and its therapy. There were also increases in the levels of adherence, but these were not statistically significant. The participants' blood pressure readings showed significant increases after the intervention. This was probably because behaviour change takes time and the post-intervention measurements might have been started before significant changes in adherence had occurred. These increases in adherence would have resulted in decreases in blood pressure. There was a Christmas break during the pre-intervention period where participants had no interaction with health care providers and the amount of physical exercises they undertook was reduced. This, coupled with unhealthy eating habits during the festive season, would have further contributed to the increases in participants' blood pressure.

Conclusion

Patient education programmes can be utilised to increase patients' adherence to therapy for chronic conditions.

Acknowledgements

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In Vitro Dissolution and Optimization of Melatonin-loaded PLGA Implants for use in Neurodegenerative Disorders

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Purpose:

Melatonin is a good candidate for use in neurodegenerative disorders due to its anti-oxidative and anti-inflammatory properties. However, it has a very short $t_{1/2}$ of ≈45mins and is promptly metabolized to 6-sulphatoxy melatonin (6-STMT) after oral administration. Due to the first pass metabolism of melatonin, it is not available in therapeutic amounts to cross the blood brain barrier. The objective of this study was to develop a novel brain implant based on an alpha-OH polyester, PLGA to be used for the delivery of melatonin in a neurodegenerative brain via a stereotaxic procedure.

Methods:

Variants of melatonin-loaded implants were constructed by salting-out and cross-linking techniques with a combination of chemical inducers such as acetone and ionic salts in accordance with a Box-Behnken design. The influence of the salting-out and cross-linking agents on the release of melatonin from the PLGA scaffolds was investigated. Differential drug-release studies were performed by immersing each implant in a 100 mL PBS at pH 7.4. The studies were done in triplicates at 37°C in a rotating shaker bath at 50rpm. At various time intervals over 30 days samples were drawn to determine the amount of drug released. Melatonin assays were undertaken employing UV-spectroscopy (278.2nm). Cumulative drug released was profiled as a function of time. Drug entrapment efficiencies (DEE) were evaluated for each formulation and the MDTs were also calculated. The formulations were optimization for DEE and the rate of drug release.

Results:

The implants demonstrated DEE between 46% and 90% and a mean dissolution time at 30 days (MDT_{30}) of 6 to 26. The fractional drug release (M_t/M_∞), and the drug release kinetics were calculated from the power law $M_t/M_\infty=k_0t$, where k, the kinetic constant was found to be k_0 of 0.004 to 0.038. The optimized implant formulation demonstrated a DEE of 89% and a 30-day zero-order kinetics *in vitro* melatonin release.

The Influence of Barium and Calcium on the Hydrational and Physicomechanical Properties of Nicotine-Loaded Alginate-Hydroxyethylcellulose Gelispheres

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Purpose:

The study aimed to develop a novel reinforced crosslinked multi-polymeric drug delivery device to provide sustained and prolonged delivery of newly researched neuroprotective agents. Nicotine was employed as a model neuroprotectant drug for incorporation into the developed device. Textural analysis of the developed gelispheres in their unhydrated and hydrated states enabled elucidation of mechanisms of interaction between the constituent polymers (Alginate, Hydroxyethylcellulose and Polyacrylic acid) and crosslinking agents (Calcium and Barium).

Methods:

Formulation of Gelispheres: Ionotropic gelation was employed to formulate the gelispheres in accordance with a Plackett-Burman Statistical Design. The polymer solution consisted of a dispersion comprising of Alginate and Hydroxyethylcellulose (HEC) into which $1\%^{w}/_{v}$ Nicotine was incorporated. The polymer dispersion was titrated dropwise into the gently vortexed crosslinking solution composed of Polyacrylic acid and binary combinations of $BaCl_2$ and $CaCl_2$. A 30 minute curing time was employed for the formulated gelispheres subsequent to which they were dried under ambient conditions in an extractor for 72 hours.

Textural Analysis: A Texture Analyzer (TA.*XTplus,* Stable Micro Systems, UK) was employed to conduct textural analysis of the unhydrated gelispheres. Matrix degradation was evaluated on gelispheres that were immersed in 100mL phosphate buffered saline (PBS) pH 7.4 maintained at 37°C. Samples (N=5) were extracted at predetermined intervals over a period of 48 hours and evaluated for changes in matrix resilience, deformability moduli and fracture energy.

Results:

The concentration of Alginate in the gelisphere matrix combined with the concentration of $BaCl_2$ employed in the crosslinking solution had the most significant impact on the degradation dynamics of the gelispheres. Higher concentrations of both components resulted in robust matrices that demonstrated the capacity to retain high fracture energies and low porosity (low resilience). Thus it can be concluded that modulation of these factors can result in the development of a novel drug delivery system that has the capacity to significantly delay drug release kinetics.

Baseline studies to initiate Pharmacy and Therapeutics Committees in twelve Primary Health Care centers in Karnataka, India

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Purpose:

India's health care system has improved considerably in the last 3 decades. However, the rational use of medicines is one aspect that requires attention. This requires the mutual beneficial interaction of health professionals including doctors and pharmacists. One method of achieving this is through the implementation of Pharmacy and Therapeutics Committee (PTC). PTC is a relatively new concept in India. A training program for the medical officers, pharmacists and nurses from 12 Primary Health Care centers (PHCs) in Karnataka, India was undertaken to introduce the concept of PTCs and was used to identify the activities as well as assess the functions that can be initiated at these PHCs.

Method:

36 health professionals (doctors, pharmacist and nurses) from 12 PHCs discussed within their groups and reported on the functions and activities that could be initiated at these 12 PHCs. This study involved recording the Process, Impact and Outcome indicators of the PTCs.

Results:

The health care professionals were very positive in reporting their consensus regarding Process indicators like PTCs having a defined place in the organizational structure, making decisions on availability and use of medicines, meeting more than 3 times a year, allowing peer evaluation of PTC decisions, and promoting educational activities. Impact indicators like documenting percentage overrun on drug budget were also accepted. The indicators that were rejected by a few groups involved documenting number of cases of antibiotic resistant microorganisms per annum, documenting mortality rate per annum due to adverse drug reactions and policy on drug promotion by pharmaceutical industry.

Conclusion:

A high level of commitment and participation in PTCs by the healthcare professionals is essential. PTCs should be able to play a greater role to improve the health and economic outcomes of hospital care resulting from drug use.

Despite the lack of PTCs in the Karnataka hospitals, it is nevertheless recommended that PTCs should be established in all healthcare units. This will require both policy direction and institutional support.

Investigation into the Prescribing Patterns and Cost of Antidiabetic Medicine in South Africa

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Purpose:

The purpose of this study was to investigate the prescribing patterns and cost of antidiabetic medicine in South Africa.

Methods:

A retrospective drug utilisation review was conducted on 1-year (2004) prescription data for patients receiving antidiabetic medicine (oral and insulins) claimed through a national medicine claims database. Descriptive statistics were used in the analysis, including averages, frequencies, and effect-sizes. Prescribed daily dosages (PDDs) and cost-prevalence ratios were also calculated. Data were analysed using the Statistical Analysis System® SAS 9.1®.

Results:

Antidiabetic agents accounted for almost 2.7% (N = 5 305 882) and 4.5% (N = R661 223 146.00) respectively of the total prevalence and cost of all medicine items. The average cost of antidiabetic medicine on the database decreased with approximately 30% from the beginning of the study period to the end thereof [i.e. R 245.42 (SD = 313.35) during January to April 2004, *vs.* R 172.79 (SD = 235.33) during the September to December 2004].

The usage of insulin reported in this study were 18.9% (n = 143 447). The three most frequently prescribed classes of insulin (insulin lispro; soluble insulin and isophane; and soluble insulin aspartame and protamine) together accounted for 63% of all the insulin prescribed, and 67% of the total cost of prescribed insulin. The average cost of the insulin were R 668.26 (SD = 336.40).

Oral antidiabetic medicine accounted for 81% and 39% respectively of the total prevalence and cost of all antidiabetic products prescribed, with an average cost of R 99.77 (SD = 99.04). Metformin was the most frequently prescribed oral antidiabetic medicine, with an average cost of R58.42 (SD=31.78). A trend towards combination therapy away from monotherapy was also observed, as about 46.1% (n = 116 318) of prescriptions issued during the study period were for one oral drug only. Almost 39% (n = 62 717) of the "combination therapy" prescriptions was for a sulfonylurea in combination with a biguanide plus at least one other antidiabetic product.

PDDs calculated for acarbose 50mg, metformin 500mg, chlorpropamide 250mg, glimeparide 4mg and the combination product "metformin/glibenclamide 250/1.25mg" were 141.1mg/day, 1199.5mg/day, 503.2mg/day, 4.3mg/day and 367.5/1.84mg/day respectively.

Conclusion:

The prescribing prevalence and cost of antidiabetic medicine contributed to the economic burden placed on the private health care system of South Africa as these medicines has shown to be relatively expensive on the database (cost to prevalence ratio – 1:0.6). The decrease in the average cost of antidiabetic medicine products from the beginning of the study period till the end thereof could possibly be attributed to the implementation of new pricing regulations in South Africa in 2004; and to the utilisation of cheaper generic equivalents. With a few exceptions, PDDs calculated for oral antidiabetic medicines were more or less in line with recommended treatment guidelines, indicating that there is not an over or under utilisation of antidiabetic medicine and that there is good adherence to the recommended treatment algorithms developed for the treatment of diabetes.

Preparation and thermal stability of polymorphic forms of stavudine

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Purpose:

The goal of this study is to prepare the three polymorphic forms of stavudine (d4T), and to confirm the thermal stability of these polymorphs.

Methods:

The polymorphic crystal forms of stavudine were prepared by recrystallization of the same stavudine raw material in a series of analytical grade solvents. An array of physico-chemical analyses, specifically DSC, TGA, TM, IR spectroscopy, KF water determination and XRPD were performed on the prepared crystals in order to characterize the crystal forms. The thermal stability of two of the prepared polymorphs and of a mixture of polymorphs were determined by means of VTXRPD.

Results:

Polymorphic form I and II, as well as the hydrate (form III), were prepared by the recrystallization of stavudine raw material. Form I was prepared using ethanol, methanol, n-propanol, ACN, and acetone as solvents. Form II was prepared using n-butanol, 2-butanol, chloroform, benzene and ethyl acetate as solvents, whereas the hydrate was prepared using THF and water as solvents. The DSC thermograms of all three polymorphs show a sharp endotherm between 167°C – 174°C, indicating the melting of stavudine. Form III has a small additional exotherm/endotherm between 132°C – 140°C, indicating dehydration of the hydrate. TM analysis illustrates the dehydration taking place between 130°C – 140°C. The theoretical weight loss of form III [$(d4T)_3 \cdot H_2O$] after dehydration is 2.61%. The KF and TG analysis of the hydrate prepared from THF shows a moisture content of 2.42% and a weight loss of 1.89%, and the hydrate prepared from water shows a moisture content of 2.91% and a weight loss of 3.05%. Three different IR spectra were obtained for form I, II and III, but these spectra aren't unique enough in order to make an accurate distinction between the polymorphs. According to the XRPD analyses each polymorph has the following unique diffraction angles (°2θ): form I – 19.1°, form II – 11.2° and 18.6°, and form III – 6.5°, 7.3° and 15.4°. The raw material and a few crystals prepared have diffraction angles of both form I and II and are classified as mixtures of the two. Form II, the suspected mixture, and the hydrate were subjected to increasing temperatures, with increments of 10°C between each diffractogram, from 25°C – 160°C during VTXRPD analysis. The diffractograms of form II and the mixture shows no significant change in appearance or diffraction angles, whereas the hydrate converts to form I as it dehydrates.

Conclusion:

The polymorphic forms of stavudine can be prepared, and XRPD is most effective in distinguishing between these forms. Form III desolvates and converts to form I, but contrary to reports by Gandhi *et al*., form II remains stable with increasing temperatures.

References:

R.B. Gandhi, J.B. Bogardus, D.E. Bugay, R.K. Perrone and M.A. Kaplan, Pharmaceutical relationships of three solid state forms of stavudine. Int. J. Pharm. 201, 221-237 (2001).

The formulation, manufacture and evaluation of capsules containing freeze-dried aqueous extracts of *Leonotis leonorus* or *Mentha longifolia*.

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Introduction:

Leonotis leonorus and *Mentha longifolia* are two herbs commonly used in South Africa, mostly in oral liquid dosage forms. Several disadvantages are associated with the traditional dosage forms which can perhaps be remedied by using an appropriate oral solid dosage form, provided the actual plant material in the latter still resemble, as closely as possible, the traditionally used material and provide products of suitable pharmaceutical quality. In this study the suitability of capsules containing the freeze-dried aqueous extract of the plants was investigated.

Methods:

Fresh leaves of *L. leonorus and M. longifolia* collected in Montagu and Kirstenbosch Garden in the Western Cape Province of South Africa, respectively, were washed with water and dried at 60℃ until a constant weight. Traditional decoctions of the dried leaves were made, filtered, supernatant freeze-dried, and each dried extracts evaluated, in a pre-formulation study, for its organoleptic and physicochemical (e.g. particle size and shape, powder density, flowability, solubility, etc) properties and levels of flavonoid marker compounds (using a validated HPLC assay). Based on the pre-formulation study results the formulation of the capsules was decided and capsules manufactured. Thereafter the manufactured capsules were pharmaceutically evaluated for weight and content uniformity, moisture content, microbial contamination, dissolution profile and stability using conventional pharmaceutical methods.

Results:

The freeze-dried aqueous extracts of *L. leonorus* and *M. longifolia* produced were moderately fine powders with irregular particle shapes, were sparingly soluble in water, but highly wettable, had good flow properties, on average contained 4.89 ± 0.25% and 8.40 ± 0.14% moisture for *L. leonorus* and *M. longifolia,* respectively, had microbial contamination counts well within the specifications and were suitable plant raw materials for hard capsule manufacture. The capsules produced were physically elegant and acceptable, on average contained 165.3 ± 1.629 µg luteolin and 382.1 ± 10.77 µg apigenin (n = 6) in the *L. leonorus* and *M. longifolia* capsules, respectively, and met the pharmacopoeia specifications for content and weight uniformity (RSD of 1.19±1.78% and 1.58±1.24%, respectively), microbial contamination levels and rapid dissolution. The capsules were however very unstable even when stored, outside a container, at room temperature and humidity conditions, had practically no shelf-life (t_{90} = 3.67 weeks and 5.39 for *L. leonorus* and *M. longifolia* capsules, respectively), and significantly different dissolution profiles (f_2 = 25.06 and 44.61 for *L. leonorus* and *M. longifolia* capsules, respectively), after 12 weeks storage.

Discussion and Conclusion:

The results showed that the aqueous extracts *L. leonorus* and *M. longifolia* were suitable as raw materials of the plants as far as manufacture of capsules were concerned, but the stability and shelf-life of these extract-containing capsules were unacceptable. As such these capsules were thus not adequate replacements for the traditional decoction forms of these two commonly used medicinal plants.

Percutaneous Diffusion of Ketoprofen across Human Skin by Dermal Microdialysis

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Purpose:

Clinical trials are currently the most widely employed method of assessing the bioavailability and bioequivalence of many topical dosage formulations. Dermal microdialysis has the potential to address the gap in available skin sampling techniques and facilitate the generation of concentration-time profiles at the target site. The purpose of this investigation is to assess *in vitro* recovery, variability and to establish an *in vivo* concentration-time profile for ketoprofen.

Methods:

Linear probes for use in microdialysis were manufactured in the laboratory employing single fibres from haemodialysis cylinders inserted in flexible grade nylon tubing and fixed with cyanoacrylate glue. The probes were prepared with a 30 mm accessible dialysis membrane length and stabilized by an internal stainless steel guide wire. The probes $(n = 3)$ were placed in a cell containing a well-stirred solution of 0.2 M phosphate buffer (pH 7.4) at five different concentrations of ketoprofen (0.5, 1, 2, 5 and 10 µg/ml) and perfused with drug-free buffer at 0.5 µl/min to measure the *in vitro* recovery of ketoprofen. To establish an *in vivo* concentration-time profile, three chambers containing 2.5% w/v ketoprofen in 100% ethanol were glued to the skin. The probes were inserted into the dermis with an average depth of 0.613 ± 0.084 mm, verified with an ultrasound instrument (Dermoscan C), and perfused with normal saline at 0.5 µl/min. Samples were collected every hour for 3 hours after an equilibration period of 1 h had elapsed and then analysed by HPLC.

Results:

A 0.69% intra probe-to-probe variation and an *in vitro* recovery of 92.8 ± 0.3% were observed. There was no statistically significant difference (ANOVA, $p > 0.05$) noted between the probes and within the concentrations used. Similar *in vivo* concentration-time profiles were observed with the three dermal probes. A maximum concentration of 0.48 ± 0.07 µg/ml was noted at 1 h.

Conclusion:

The linear dermal probes were reproducible and thus unlikely to contribute significantly to any possible variability that may arise during *in vivo* experiments. The high *in vitro* recovery suggests that ketoprofen is not likely to have binding problems on the membrane as observed by many medicinal compounds. From the *in vivo* results, dermal microdialysis sampling can be used to determine the dermal bioavailability of ketoprofen and importantly, has good potential for use to determine the bioequivalence of ketoprofen formulations.

Acknowledgement:

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Prescribing Patterns of Anti-migraine Drugs in a Primary Care Patient Population

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Background:

Research shows that migraine affects between 5% and 15% of males and 13.5% and 31% of females in South Africa. Few studies have been published on the prescribing patterns of anti-migraine drugs in South Africa.

Objectives:

The aim of the study was to investigate the prescribing of drugs for the prophylaxis and treatment of migraine in a primary care patient population in South Africa.

Methods:

Computerised prescription records for 2004 were obtained from a South African medical aid administrator serving various private medical aid schemes. The total database contained 1 202 072 records of 37 805 patients. Outcome measures were prescribing frequency and cost.

Results:

A total of 1 311 products for the treatment of migraine at a cost of R206 036 were prescribed to 578 patients in 2004. Most patients (62.98%) were female. The average age of patients was 40.54 (SD = 14.40) years, with more than half of the patients (55.36%) between 30 and 49 years of age. Female patients received on average 2.60 anti-migraine products over the one-year period, compared to 1.70 products for males. Approximately half of the products were for the prophylaxis of migraine of which clonidine and flunarizine were the most frequently prescribed, while 47.02% of the products were for the treatment of migraine. Of the products specifically prescribed for the treatment of migraine, ergotamine was the most frequently prescribed (45.78%), followed by zolmitriptan (22.73%) and rizatriptan (16.72%). The selective serotonin ($5HT_1$)-receptor agonists (triptans) accounted for 25.50% of all anti-migraine drugs. No significant differences in the prescribing of the triptans between females and males were observed ($\chi^2 = 7.13$; $df = 4$; $P > 0.1$). Tablets were the preferred dosage form (81.34% of all products). Combination analgesics, non-selective COX inhibitors and decongestant products containing analgesic combinations were the other most often prescribed drug classes to this patient population.

Conclusions:

Migraine affects primarily the economically active sector of the community and pharmacists can play an important role in managing and counselling migraine sufferers. The high prescribing rate of combination analgesics to this patient population was a cause for concern since most of these products contain meprobamate and codeine, both of which have addictive potential.

Prescribing Patterns of Non-steroidal Anti-inflammatory Drugs

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Background:

Non-steroidal anti-inflammatory drugs (NSAIDs) are effective in controlling inflammatory conditions, but have been identified as the best recognised cause of iatrogenic disease in the United Kingdom, with their most widely recognised adverse effects being on the upper gastrointestinal (GI) tract. Evidence-based strategies to minimise GI risk and improve overall prescribing habits exist and should be applied in practice.

Objectives:

The objective of the study was to investigate the prescribing patterns and cost of NSAIDs in a South African private sector patient population, and to investigate the prescribing of GI drugs to patients who were prescribed NSAIDs.

Methods:

Data for 2005 were obtained from a South African medical aid administrator serving various private medical aid schemes. The total database contained 1 402 891 records for medicine, consultations and procedures. All medicine records for NSAIDs were extracted from the database using the MIMS classification system. Patients who were prescribed GI drugs were identified. Main outcome measures were prescribing frequency, cost and potential identification of iatrogenic disease.

Results:

A total of 48 947 NSAIDs were prescribed to 20 172 patients during 2005, at a total cost of R1 628 884.80. Patients received an average of 2.43 (SD = 2.48) NSAID products over the year at an average cost of R33.28 per prescription item. The average age of patients was 38.08 (SD = 16.13) years. More NSAIDs were prescribed to male patients (54.31%). COX inhibitors accounted for 96.80% of prescribing frequency and 86.69% of cost, Specific cyclo-oxygenase-2 inhibitors (COXIBs) for 2.18% of frequency and 12.50% of cost, and Selective COX2 inhibitors for 1.02% of frequency and 0.82% of cost. Patients between the ages of 30 and 49 years were prescribed 50.75% of the NSAIDs, but prescribing to this age group accounted for only 36.97% of the total cost. The older age groups accounted for a disproportionately higher percentage of cost. The most frequently prescribed active ingredient was diclofenac, accounting for more than half (56.88%) of the total number of items prescribed and for 33.42% of the total cost. Ibuprofen and piroxicam were the second and third most frequently prescribed active ingredients. The most frequently prescribed trade name product was Adco-diclofenac® 75 mg injection (3 501 items). The trade name product that accounted for the highest overall cost was Arthrotec® 75 mg tablets. Nearly half (45.62%) of the patients who were prescribed a NSAID also received one or more drugs acting on the GI tract (52.02% of these patients were males).

Conclusions:

NSAID prescribing was in agreement with the results of previous studies. The high prescribing rate of GI drugs to the patient population was, however, a cause for concern since it is possible that these products are used to counteract the adverse effects of the NSAIDs.

Bioavailability of an L-carnitine – magnesium fixed-dose combination product in healthy male volunteers

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Purpose:

A bioavailability study of Carnimag®, containing 100mg L-carnitine base, 100mg magnesium chloride, 185mg magnesium oxide and 48mg crystallized water, was conducted in fed male healthy volunteers to investigate the absorption of L-carnitine, its acyl esters and magnesium following single and multiple doses.

Methods:

A single centre, open label, multiple-dose bioavailability study was conducted in fed, healthy male volunteers. A study diet, without excess L-carnitine or magnesium, was started 7 days before the first administration of Carnimag® and continued until completion of the study. Volunteers were admitted from Day –2 (-36h) until Day 1(0h) for the baseline pharmacokinetic (PK) profile. Volunteers received two (2) Carnimag® capsules daily for seven days (Day 1 to Day 7) and were again admitted on Day 6 (132h) to Day 8(162h) for the steady-state PK profile. Blood and urine samples were collected at specific times during the PK profiles for the analysis of magnesium and L-carnitine by LC-MS/MS. Paired t-Tests were performed to assess the statistical significance of any changes between baseline and steady state in the 24-hour urinary excretion of magnesium and L-carnitine, the urinary concentrations of free L-carnitine, acetylcarnitine, total acylcarnitine, urinary acylcarnitine/free L-carnitine ratio and plasma L-carnitine and total acylcarnitine area under the plasma concentration- time curve (AUC).

Results:

Twenty-four (24) volunteers completed the study. The dose of two Carnimag® capsules daily for seven days was well tolerated. The changes from baseline in urine concentrations from baseline to Day 7 are tabulated.

Table 1. Change from baseline to Day 7 in urinary concentrations of 24h-magnesium, 24h-Free L-carnitine, Acetylcarnitine, Total acylcarnitine and Acylcarnitine/Free L-carnitine ratio

	24h-Magnesium [µg/mmol creatinine]	24h-Free L-Carnitine [mmol/mol creatinine]	Acetylcarnitine [mmol/mol creatinine]	Total acylcarnitine [mmol/mol creatinine]	Acylcarnitine/ Free L-carnitine ratio
N	25	25	25	25	25
Mean	7969.3487	0.7752	0.5015	0.798	0.1009
SD	20739.4379	0.5676	0.5044	0.9211	0.2713
p-value[1]	0.0666	<0.0001	<0.0001	0.0002	0.0753

Conclusions:

The urine concentrations of L-carnitine significantly increased following single and multiple daily doses of two Carnimag® capsules, indicating that the active constituents of the capsules were absorbed. Although there was a significant increase in the urinary excretion of magnesium following the first dose of two Carnimag® capsules (p=0.0395), the additional increase at Day 7 was not statistically significant (p=0.0666). Additional studies are planned to evaluate the multi-compartmental and population pharmacokinetics of L-carnitine.

The applicability of sphericaly agglomerated chitosan formulations

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Purpose:

Chitin is the most abundant natural amino polysaccharide and its annual production equals that of cellulose, a well known powder utilized in the tablet manufacturing industry. The improvement of the micromeritic properties of chitosan with the least amount of effort, and to achieve matrix type drug release additionally from a chitosan formulation through an alternative granulation technique could be of great advantage.

Methods:

Scanning electron microscope (SEM) photographic images: Agglomerate and powder samples were visually assessed. Samples were positioned on a double-sided conductive carbon tape to a sampling tray and dusted with an inert gas. Samples were consequently sputter-coated with a gold/palladium (80:20) to form a layer of approximately 28 nm on the surface of the samples under a vacuum superior than 0.06 Torr.

Tablet evaluation: Evaluation of crushing strength, diameter and thickness was determined for 10 tablets of each formulation using a Pharma Test® (model PTB-311) tablet test unit. For weight variation, twenty tablets of each batch were dusted and weighed on a Precisa® analytical balance. For friability, ten tablets of each formulation were tested on a Roche® friabilator for a duration of ten minutes at 50 rpm.

Drug Release Study: Dissolution tests were performed in a six station dissolution apparatus. A thermostat regulated the temperature of the medium at 37±.05 ˚C. Dissolution studies were performed in 900 cm^3 of 0.1 M HCl (pH ~ 1.20) and 900 cm^3 Sörensen buffer (pH ~ 4.5). The standardised USP basket-method was used in all studies and baskets were rotated at a constant speed of 50 rpm. Samples were removed at specified intervals and then analysed by UV spectroscopy.

Results:

The SEM micrographs indicated that it was possible to incorporate propranolol hydrochloride (p-HCl) in the agglomerates without any detrimental effect on micrometric properties. SEM images revealed the manner in which p-HCl adhered to chitosan. Spherically agglomerated chitosan was compressed successfully and used in tablet formulations without any tableting excipients other than Kollidon® K25, an excipient that proved essential in the agglomeration step. No glidants were necessary, as the powder flowed freely into the tablet die. The mathematical comparisons of some of the dissolution profiles of the p-HCl tablets and Inderal® LA in simulated gastric media presented acceptable f2-values and good area under graph correlations.

The antioxidant properties of *Plumbago auriculata*

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Purpose:

Plants are known to be a source for compounds with antioxidant properties such as, flavanoids, anthocyanins and carotenoids. These compounds have the ability to scavenge free radicals and are therefore necessary to prevent oxidative stress and related diseases such as, neurodegenerative diseases; Parkinson's disease, Alzheimer's disease, Huntington's disease and amyotrophic lateral sclerosis.

For the purpose of this study, *Plumbago auriculata* extracts were selected to be subjected to an *in vitro* assay to determine the antioxidant properties there of, from an initial screening of a selection of plants.

Methods:

Several plant species were screened for antioxidant properties with the oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays. *P. auriculata* was chosen from the list for further studies and extracted by Soxhlet extraction, using four solvents, petroleum ether, dichloromethane, ethyl acetate and ethanol, in order of increasing polarity. To eliminate the effect, the green colour of the extracts had on the assay, chlorophyll a and b were degraded to pheophytin a and b (lighter green to yellow colour) through ultra-violet irradiation. The extracts were then screened for lipid peroxidation reduction in rat brain homogenates with the slightly modified thiobarbituric acid (TBA) assay of Ottino & Duncan (1996) and correlated to antioxidant activity. The assay relies on the assessment of lipid peroxidation on the bases of the complex formation between malondialdehyde (MDA), an end product of lipid peroxidation, and TBA, generating a pink colour to be measured spectrophotometrically at 532nm.

Results:

The experimental data showed the extracts of *P. auriculata* to have antioxidant activity with the values found to be, dichloromethane (0.0090 nmol MDA/mg tissue), petroleum ether (0.0080 nmol MDA/mg tissue), ethyl acetate (0.006 nmol MDA/mg tissue) and ethanol (0.0058 nmol MDA/mg tissue) in comparison to Trolox, (0.0002 nmol MDA/mg tissue), a known antioxidant.

Conclusion:

P. auriculata extracts have been successfully screened for antioxidant activity and are therefore suitable for further investigation into the compounds responsible for these properties.

Zinc and Vitamins A and D in Ulcerative Colitis

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Aims:

Ulcerative colitis is a systemic disease of which inflammation of the bowel is the primary manifestation. The study aimed to evaluate whether zinc in combination with vitamins A and D would: (1) alleviate both physical and psychiatric symptoms; (2) elevate plasma zinc levels; and (3) show cost benefits.

Method:

In a single-blind pilot study, 20 ulcerative colitis patients had their psychiatric symptoms rated at 3-monthly intervals on the Hamilton Depression Rating Scale (Ham-D) and Sheehan Disability Scale (SDS). Their standard daily treatment regimen was substituted with:

a) 10 patients: 15mg zinc plus 4750 IU vit A and 400 IU vit D3 in cod-liver oil;
b) 10 patients: 15mg zinc plus 5000 IU vit A and 40 IU vit D3 as dry powder.

Ethics do not allow for a placebo group. Relapses were treated with standard therapy. Each visit included: monitoring of blood zinc, copper, vitamins A and D; stool chart diaries, endoscopy, and bowel biopsies (blinded investigator). Patient's treatment charts underwent cost-analysis for the year prior to the study and the year of the study.

Results:

Both the Ham-D and SDS demonstrated a significant improvement in depressive symptoms and quality of life, in keeping with positive changes in laboratory and other clinical parameters. This included symptomatic improvement of polyarthralgia (in all 4 patients), and resolution of pyoderma gangrenosum (in the one patient with this lesion). The cost-analysis of the patient's treatment charts for the year prior to this study amounted to R147 746.49, and R32 291.58 for the year under study (ie an approximately 80% cost reduction or 21,86% of former costs).

Conclusions:

Results indicate that treatment with zinc and vitamins A and D: (a) is an effective treatment modality for this illness and that the degree of psychiatric morbidity is underestimated; (b) raises plasma zinc levels; (c) shows cost benefits.

The Pharmacological Interaction of Commercial Essential Oils in Combination with Conventional Antimicrobials

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Purpose:

The emergence of multi-drug resistant strains due to incorrect use and overuse of existing antimicrobials is becoming a formidable threat in the fight against disease. Alternative therapies to the standard treatments with single agents are being sought. This study aims to investigate the *in vitro* pharmacological interactions between essential oils and conventional antimicrobials when used in combination.

Methods:

Popular commercial oils (thyme, tea tree, rosemary and peppermint) of known antimicrobial efficacy were combined in various ratios with ciprofloxacin and tested against *Staphylococcus aureus* and *Klebsiella pneumoniae.* Similarly, the essential oils were combined with amphotericin B and tested against *Candida albicans.* Isobolograms graphically display the interactions that the inhibitors have on microbial growth when combined in various ratios. Investigations of the synergistic, antagonistic and additive profiles are presented.

Results:

Interactions of the essential oils when combined with ciprofloxacin against *Staphylococcus aureus* indicate mainly antagonistic profiles. When tested against *Klebsiella pneumoniae* antagonism, synergy and additive profiles are noted, depending on the combined ratio. The interaction of thyme, tea tree, rosemary and peppermint essential oils with amphotericin B indicate mainly antagonistic profiles when tested against *Candida albicans.* The predominantly antagonistic interactions noted may warrant caution when combining natural therapies with conventional antimicrobial treatment regimens.

The effect of multiple doses of *Tribulus Terrestris* on serum LH and testosterone levels in healthy males

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Introduction and aim:

Tribulus Terrestris is a herbal preparation freely available and marketed as a food supplement. Very little scientific data has been published on the effects of Tribulus Terrestris and most information is obtained from internet sources and on labels of supplement products. Advertisements state that the use of Tribulus Terrestris increases LH and therefore boosts the production of testosterone. The aim of this study was to measure the effect of Tribulus Terrestris on serum LH and testosterone levels in healthy young males.

Methodology:

Tribulus Terrestris was administered to 6 healthy males at a dose of 400mg at 12hour intervals for 7 days. Blood samples were collected before administration (day 0) and then daily for 7 days. All samples were collected on 08h00 in the morning. Each subject was his own control. The serum was used to measure LH and testosterone concentrations with immuno assay methods (AXSYM, Abbott).

Results:

All LH and testosterone levels were within normal range. Although there was considerable biological variation, no increase in serum LH or testosterone was found over the 7 day period when the measured concentrations were compared to baseline levels.

Conclusion:

The claim that the use of Tribulus Terrestris will boosts the production of LH and testosterone could not be proven under the conditions used in this study. Athletes using this product should not expect to obtain an anabolic effect over a short period.

Antimalarial activity of indigenous South African medicinal plants

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Purpose:

The increase in drug resistance and decreased efficacy of classical antimalarials is of great concern and the lethal implications it has for the people of sub-Saharan Africa demands urgent attention.Traditional South African medicinal plants, such as *Pelargonium*, *Agathosma* and *Hermannia* are widely used to treat fever and flu-like symptoms associated with malaria. Traditional remedies have been an important source of new compounds; however the plants used in the South African traditional healthcare system have not been adequately investigated, including the three ethnomedicinal indigenous genera included in this study.

Methods:

The investigated plants, namely *Pelargonium*, *Agathosma* and *Hermannia* were collected from natural populations at various localities in South Africa. Aerial plant parts were air dried, pulverised and the 50 extracts prepared with acetone or methanol:dichloromethane. The 21 *Pelargonium*, 17 *Agathosma* and 12 *Hermannia* extracts were tested for antimalarial activity against a chloroquine-resistant *Plasmodium falciparum* strain using the [^{3}H]-hypoxanthine incorporation assay. Toxicity profiles were determined using the tetrazolium cell proliferation (MTT) assay against a human kidney epithelial cell line.

Results:

Both the *Pelargonium* and *Agathosma* species had promising activity, with *P. panduriforme, P. citronellum, P. radens* and *P. quercifolium* being the most active (IC_{50} range: 1.34±0.29 to 2.66±0.36µg/ml), while *A. pungens, A. ovata* and *A. roodebergensis* displayed promising activity (IC_{50} range: 3.61±0.27 to 5.18±0.15µg/ml). Of the 17 *Hermannia* species, only *H. trifurca* had activity below 20µg/ml. Due to the favourable toxicity profile of select species, HPLC analyses were performed and revealed a vast chemical diversity among the active *Pelargonium* and *Agathosma* species, with flavones and flavonols predominating in all species. The combined effect of the plant-derived antimalarial agent, quinine and the more active plant extracts yielded variable interactions, with the tested *Agathosma* and *Hermannia* species interacting in a synergistic manner; whilst the select *Pelargonium* species interacted in an additive or antagonistic manner.

Development and Validation of an *In Vitro* Test Method for the Assessment of Clobetasol 17-Propionate Release from Topical Formulations

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Purpose:

A key element of the FDA SUPAC-SS guidance document [1] is the requirement that an *in vitro* release test method be used to determine if the diffusional rate of release of a drug from a formulation is the same following any post approval formulation changes, as prior to the change. The objective of these studies was to develop and validate a reliable, reproducible and discriminatory *in vitro* release test method for use in formulation development studies to assess product quality and ensure batch-to-batch consistency of topical formulations manufactured to contain 0.05% w/w clobetasol 17-propionate (CP).

Methods:

A glass Franz diffusion cell system (n=6) (Crown Glass Company Inc., New Jersey, USA), was used for these studies. The diffusion cell system was equipped with a hot water circulator (Grant Instruments Ltd, Cambridge, England) with the temperature set at 32° ± 0.5˚C. Method development studies included an evaluation of receptor media with appropriate sink characteristics, membranes with minimal reactivity with CP, formulation components and the effect of different magnetic stirrer bars. The method was validated by investigating the *in vitro* release of CP from extemperaneous cream formulations that varied in CP concentration and intrinsic viscosity. A specific and sensitive validated HPLC method with UV detection at 240 nm was used to analyze samples and to determine the amount of drug released per unit area. The cumulative amount of CP released over a 72 hour time period versus time was plotted.

Results:

The *in vitro* release of CP from 0.05% w/w cream formulations was best achieved using a receptor medium consisting of a binary mixture of water:propylene glycol (50:50) and a nitrocellulose membrane (Millipore, Bedford, MA, USA) with an average pore size of 0.025 µm and magnetic stirrer bars (2x2mm, PermeGear, Inc, Bethlehem). The results of the validation study indicate that, as predicted by Higuchi's equation, Q *vs.* $t^{1/2}$ increases as the concentration of CP in the formulation increases, but decreases with an increase in the viscosity of the formulation. These results show that this *in vitro* release testing method is able to detect the effects of changes in a formulation on the release rate of CP from formulations in which CP is suspended and/or dissolved. It can therefore be used in conjunction with traditional quality control tests to determine the quality and consistency of CP topical formulations and associated release characteristics during product development studies.

References:

1. Guidance for Industry: SUPAC-SS Nonsterile Semisolid Dosage Forms. Scale-Up and Postapproval Changes: Chemistry, Manufacturing and Controls: In Vitro Release Testing and In Vivo Bioequivalence Documentation. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, May 1997.

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Investigation of neuroprotective effects of Nevirapine and Efavirenz

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Purpose:

Impairment of cognitive functions associated with damage in the cerebral cortex, basal ganglia, and hippocampus following HIV-infection in the Central Nervous System, is associated with neuronal damage due to oxidative stress as implicated in AIDS Dementia. In the past few years, highly active antiretroviral therapy (HAART) has significantly reduced the incidence of this Dementia by inhibiting viral replication in the central nervous system. Thus the present study aims to elucidate and characterize the manner in which Nevirapine (NVP) and Efavirenz (EFV) afford neuroprotection.

Methods:

Male rats of the Wistar strain weighing between 200-250g were used. Lipid peroxidation was performed using the thiobarbituric acid (TBA) assay to assess levels of oxidation stress induced by these drugs in the presence of Quinolinic acid (QA) and Iron (Fe^{2+}).

Results:

The results show that both drugs reduce lipid peroxidation more effectively at higher concentrations.

Conclusion:

These drugs offer protection against lipid peroxidation induced by QA and Fe and thus likely to reduce the severity of AIDS dementia.

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