The SA Pharmacology & Toxicology Congress
2 – 5 October 2007
Buffelspoort Holiday Resort
Marikana, North West Province
Programme
Tuesday 2/10/2007

Arrive at venue

12:00 > Registration and Checking in

14:00 - 15:00 SAPS EXCO Meeting

15:00 - 17:00 IUPHAR BID Meeting

17:00 - 18:00 Company symposia  
Chair: Jacques Snyman

Dr Reddy’s Laboratories  
Ranbaxy  
Jansen-Cilag  
Cipla Medpro  
Lundbeck  
Servier

18:00 Cocktail

Opening of Congress: Jacques Snyman  
Welcome address:  
President of SAPS Douglas Oliver  
President of TOXSA Mary Gulumian

Presentation by Sanofi Aventis
Wednesday 3/10/2007

7:30 - 8:30  Breakfast
8:00 - 8:30  Registration

8:30 - 10:30  Session 1: Drug development
Chair: Connie Medlen

8:30 - 9:20  **Evan Siegel.** Preclinical testing of new drugs
9:20 - 9:40  **G. Kemp.** The application of PET in drug development
9:45 - 10:00 **P. van der Bijl.** Microbicides: the challenge of female-controlled HIV protection
10:00 - 10:30 **G. Maartens.** The role of clinical pharmacology in supporting antiretroviral scale-up

10:30 - 11:00  TEA

11:00 - 12:30  Session 2  Antiretroviral drug research
Chairs: Gary Maartens/Wim du Plooy

11:00 - 11:15  **P. Mugabo.** Nevirapine pharmacokinetics in premature infants; preliminary results
11:15 - 11:30  **T. Kredo.** Initial experience with an antiretroviral therapeutic drug monitoring service
11:30 - 11:45  **L. Brown.** Transport of nevirapine across intestinal epithelial cells: interactions with tuberculosis drugs and traditional medicines
11:45 - 12:00  **K. Cohen.** The effect of rifampicin on nevirapine plasma concentrations in South African adults with HIV-associated tuberculosis
12:00 - 12:15  **N. Katende-kyenda.** Prelavence of drug-drug interactions (DDIs) between antiretroviral agents in different age groups in a setting of the private health care sector in South Africa for the year 2006
12:15 - 12:30  **J-S. van der Walt.** Nonlinear mixed effect models for lamivudine and zidovudine in healthy male volunteers

12:30 - 13:20  LUNCH

13:20 - 13:30  Company symposium
Chair: Tiaan Brink

**Pfizer**

13:30 - 14:00  Poster session I  (Young researcher competition posters)
Chair Wim du Plooy
14:00 -15:00  Session 3: Reactive metabolites and drug interaction studies
Chair: Tiaan Brink

14:00 - 14:30  B Harvey. Reactive nitrogen and oxygen intermediates and oxidative stress in neuroleptic-induced tardive dyskinesia: Implications for prevention and treatment.

14:30 - 14:45  L. Cassim. Melatonin counters the 5-fluorouracil-induced morphological alterations and decrease in volume of rat hippocampal cells

14:45 - 15:00  O. Meissner-Kirch. One of the problems with traditional medicines: interactions with prescription drugs

15:00 - 15:30  TEA

15:30 - 17:00  Session 4: Drug development workshop/ Panel discussion
Chair Jacques Snyman

15:30 - 16:00  L. Katsoulis. Changing Paradigms in Drug Development

16:00 - 17:00  Panel discussion: Connie Medlen, Evan Siegel, Lynn Katsoulis

17:00 - 17:45  Pharmacology AGM

18:00  Braai
Thursday 4/10/2007

7:30 - 8:30  Breakfast
8:00 - 8:30  Registration

8:30 - 10:30  **Session 5: Toxins and herbal remedies I**
Chair: Mary Gulumian

8:30 - 9:10  *Kai Sovolainen.* Fumonisin B1: Harmful Effects and Cellular Mechanisms Behind Its Toxicity
9:10 - 9:40  *V. Sewram.* Medicinal Plant Usage and Public Health Research: An Epidemiological Perspective
9:40 - 10:00  *DR Katerere.* Investigating the potential mutagenicity of traditional African herbal medicines
10:00 - 10:20  *E. Elgorashi.* Mutagenic constituents of *Helichrysum simillimum* DC
10:20 - 10:35  *V. Maphosa.* Toxicity evaluation of an aqueous extract of *Leonotis leonurus* (L) in rats

10:35 - 11:00  **TEA**

11:00 - 12:30  **Session 6: Toxins and herbal remedies II**
Chair: Vanessa Steenkamp

11:00 - 11:30  *Jens Dahlmann.* Use of LC/MS/MS to speed up your toxin screening applications
11:30 - 12:00  *J. Seier.* Toxicity testing of traditional herbals
12:00 - 12:15  *C. Wium.* Plant poisonings: The Tygerberg experience
12:15 - 12:30  *GJ Amabeoku.* Anticonvulsant activity of *Nylandtia spinosa* L. Dumont (Polygalaceae) aqueous leaf extract in mice

12:30 - 13:10  **LUNCH**

13:10 - 13:30  **Company symposia**
Chair: Duncan Cromarty

13:10 - 13:20  Beckman Coulter
13:20 - 13:30  BiocomBiotech

13:30 - 14:00  **Poster session II**
Chair: Ronel Kahler-Venter
14:00 - 15:00  **Session 7: Toxins and herbal remedies III**  
Chair: David Katerere  

14:00 - 14:15  *VJ Maharaj*. Bioprospecting. Adding value to South Africa’s biodiversity  
14:15 - 14:30  *ES Bizimenyera*. *Peltophorum africanum* Sond (Fabaceae) extracts have a potential role in medicine  
14:30 - 14:45  *P.I. Aziba*. Antispasmodic, analgesic and sedative effects of *Mormodica involucrata* methanolic extracts in rodents  
14:45 - 15:00  *N Chellan*. A toxicological assessment of *Athrixia Phylicoides* aqueous extract following chronic ingestion in a rat model  
15:00 - 15:15  *N. Pillay*. Antimalarial properties of South African medicinal plants  
15:15 - 15:30  TEA  

15:30 - 16:30  **Session 8: Young Scientists’ Competition**  

**Parallel session A**  

**SAPS session** Chair: Douglas Oliver  

15:30 - 15:35  *G.F. Sibandze*. Pharmacological properties of Swazi medicinal plants  
15:35 - 15:40  *I.O. Ishola*. Evaluation of antinociceptive activities of the aqueous root extract of *Alchornea cordifolia* in mice  
15:40 - 15:45  *T. Hanly*. The women’s Health Initiative study: Impact on the prescribing of hormone replacement therapy in a defined South African population  
15:45 - 15:50  *G. Zheve*. Investigating the antioxidant and iron-binding properties of non-nucleoside reverse transcriptase inhibitors, efavirenz and nevirapine in HIV-induced neurodegeneration  
15:50 - 15:55  *C. Toua*. Behavioural and pharmacological evaluation of social isolation rearing in rats: Relevance for schizophrenia  
15:55 - 16:00  *B van Niekerk*. The effects of ozone exposure on markers of cellular resilience in cultured human epithelial HeLa cells  
16:00 - 16:05  *W. Hochfeld*. Utilization of the Roche amplichip for pre-prescription genotyping  
16:05 - 16:10  *P. Naude*. The anti-inflammatory properties of brown coal derived potassium humate in a graft-versus-host reaction  
16:10 - 16:15  *L. Potgieter*. The modulating effects of selected antidepressants and related drugs on markers of cellular resilience in cultured human neuroblastoma cells  
16:15 - 16:20  *J. Gandy*. The anti-allergic properties of potassium humate
Parallel session B

**TOXSA session**  Chair: Mary Gulumian

15:30 - 15:35  *P. Komane.* An investigation into the surface activity of airborne particles in the gold mining environment

15:35 - 15:40  *K. Makinson.* Validation of biomarkers for improved assessment of exposure and early effect from exposure to crystalline silica

15:40 - 15:45  *RA Street.* Wild garlic (*Tulbaghia violacea*): a heavy metal accumulator?

15:45 - 15:50  *L. Khoali.* Intracellular calcium levels in relation to lipid peroxidation in U937 phagocytic cells exposed to crystalline silica

15:50 - 15:55  *N. Matiwane.* Comet assay parameters for use in assessment of genotoxic effects

15:55 - 16:00  *N. Mbatha.* Evaluation of intracellular GSH in silica-induced apoptosis

16:00 - 16:05  *M. Leshwedi.* The anti-inflammatory properties of *Salacia leptoclada* and *Warburgia salutaris*: Their possible use as therapeutic agents in silica-induced cellular injury

16:05 - 16:10  *LJ McGaw.* Ethnoveterinary plant extracts: relationships between antibacterial activity and cytotoxicity

16:10 - 16:15  *EO Iwalewa.* Mechanisms of the toxic effect of *Harungana madagascariensis* stem bark extracts in-vivo and in-vitro

16:15 - 16:20  *CA Pallant.* Antimicrobial activity of Venda medicinal plants used in respiratory tract infections

16:20 - 16:25  *A Thorburn.* Phytochemical analysis and antimicrobial activity of *Piper capense*

16:30 - 17:30  **Session 9: Toxicology**  Chair: S Hietkamp

16:30 - 17:00  *T Naude.* Effects of tropane alkaloids of *Datura stramonium* and *D. ferox* (“stinkblaar”) containing respectively both L-hyoscyamine and scopolamine (hyoscine) and only scopolamine in humans and various animal *spp.*

17:00 - 17:30  *F Reyders.* Overview of the 2007 Pet Food Toxicosis Crisis

17:30 - 18:15  **TOXSA AGM**

19:00  **Dinner/Awards**
Friday 5/10/2007

7:30 - 8:30  Breakfast
8:00 - 8:30  Registration

8:30 - 10:30  **Session 10: Analytical technology in pharmacology and toxicology**
Chairs: Duncan Cromarty/ Ronel Kahler-Venter

9:00 - 9:30  **Jens Dahlmann.** Supporting confidence in metabolite identification
9:30 - 9:45  **A.D. Cromarty.** Quantitative determination of methotrexate in just 1µl plasma
9:45 - 10:00  **AD van Eyk.** The Effect of Human Vaginal Mucosa Barrier Damage on the Diffusion of Permeant Molecules
10:00 - 10:15  **JM. Van Zyl.** Comparative permeation of drug compounds across bronchial tissue
10:15 - 10:30  **HI. Seifart.** In vitro transcorneal diffusion and penetration of antimicrobial agents and their potential use in ophthalmological therapy

10:30 - 11:00  **TEA**

11:00 - 13:00  **Session 11: Antimalarials and antimicrobials**
Chair: Ivan Havlik

11:00 - 11:15  **N. Malangu.** Acute poisoning at selected hospitals in South Africa
11:15 - 11:30  **T Ndlovu.** Isolation and characterization of the major compounds from *Pentanastia prunelloides*
11:30 - 11:45  **M Molefe.** Food safety and the impact of antimicrobial resistance on regulatory policies in veterinary medicine and stock remedies
11:45 - 12:00  **K Obikeze.** Cardiovascular effects of a compound isolated from *Leonotis leonurus*
12:00 - 12:15  **C. Lategan.** Isolation and characterization of novel antiplasmodial compounds from *Siphonochilus aethiopicus*
12:15 - 12:30  **L. Wiesner.** Antimalarial study of compounds isolated from *Xerophyta* species
12:30 - 12:45  **E. van den Heever.** Impact of nitrogen fertilization on the growth and antifungal activity of *Tulbaghia violacea*

**Close of proceedings**
Invited Speakers
Dr. Siegel founded Ground Zero Pharmaceuticals Inc. in 1999 from his consulting firm, Ground Zero Strategics Ltd. GZP provides services in regulatory affairs and product development across many therapeutic areas, in the management of medical product portfolios, technology assessment, and arranging partnering and joint ventures for intellectual property in pharmaceuticals, biologics and medical devices. GZP have offices in Irvine, California, and Melbourne, Australia, with Associates throughout the US, Australia, Canada and Sweden.

Dr. Siegel served as Consultant and later Chief Executive Officer of OXO Chemie Inc. and was Director of Regulatory Consulting Services and Principal Regulatory Scientist for Quintiles Inc.

Siegel has held positions as a Toxicology Reviewer at the US Food and Drug Administration and was Supervising Toxicologist and Chief of Special Services for the Food and Drug Branch, California Department of Health Services.

He has served in regulatory affairs and executive positions in both the pharmaceutical industry (Astra, Syntex, Medical Science Systems and OXO Chemie) and trade association environments. Siegel is an Adjunct Professor in the Centre for Integrated Preclinical Drug Development, University of Queensland, Australia.

He holds a Master and PhD in Virology and Molecular Biology from the Waksman Institute of Microbiology.

Has had numerous awards bestowed on him, including:

- NIH Graduate Fellowships, 1970-1975
- Cancer Research Training Grants 1972-1975
- Busch Equipment Grants, Waksman Institute of Microbiology, 1972-1975
- NIH Postdoctoral Fellowships, Medical Genetics, 1974-1977
- University of North Carolina School of Medicine Postdoctoral Medical Genetics Training Grants, 1974-1977
- 1984 Outstanding Young Men of America
Kai SAVOLAINEN

Abbreviated Curriculum Vitae:

Kai Savolainen was born in Copenhagen in 1950. He graduated from the Medical School of the University of Helsinki in 1976, and completed his Dr. Sci. degree in medicine in 1981, also in the University of Helsinki. He also obtained a PhD in Toxicology from the University of Kansas while working there as a Fogarty postdoctoral fellow during 1985-87.

He was a Diplomate of American Board of Toxicology 1986-1995, and he has been after a 1995 EUROTOX Registered toxicologist.

He served in several positions at the National Public Health Institute of Finland during 1982-1995, and was Head of the Department of Toxicology during 1992-95. He was Professor of Chemical Environmental Hygiene at the University of Kuopio in 1995, and then Professor and Chairman of the Department of Pharmacology and Toxicology of the University of Kuopio 1996-98 when he joined the Finnish Institute of Occupational Health and served as the Director of the Department of Industrial Hygiene and Toxicology during 1998-2005. He was member of the Executive Group of the Institute during 2003-2005, and Chair of the Institute Research Committee during 1999-2004. Currently he is responsible for the 'New Technologies and Risk' Research at the Institute, and is the leader of the Research Programme on Safe Nanotechnologies and Safety of Engineered Nanoparticles.

He has published over 400 scientific papers, among them more than 170 peer reviewed papers. He has mentored 14 PhD students and 10 MS students, and acted several times as a PhD thesis opponent or examiner. Kai Savolainen has served in numerous national and international scientific expert groups within European Union and beyond, and he has been invited to give more than 50 invited talks in international congresses. Kai Savolainen leads several large international, mainly European Union, funded research consortia with a focus on the safety of engineered nanoparticles.

Kai Savolainen has been active in national and international scientific organizations. He was the president of the Finnish Society of Toxicology 1992-93, President of the International Neurotoxicology Association 1999-2000, Member of the Governing Board of International Council for Laboratory Animal Science. He has served as the member of the Executive Committee of the International Union of Toxicology (IUTOX) 1989-98, of those 1992-98 as the Secretary. He is currently the President of IUTOX. He was also the President of the 10th International Congress of Toxicology organized in Tampere, in Finland in 2004.
Jens DAHLMANN
Darmstadt, Germany
jens.dahlmann@eur.appliedbiosystems.com

A Product Specialist Mass Spectrometry with a wide range of experience in the analytical chemistry. Able to work on own initiative and as part of a team. Proven skills involving demonstration, operation of Applied Biosystems/MDS Sciex mass spectrometers. Experienced in trouble shooting of instrumentation as well as solving customers application problems. High degree of international experiences with Applied Biosystems customers. Dedicated to maintaining high quality standards of the PSM Support Center especially in co-operation with the EMT market.

EXPERIENCE:
Sep. 1999 – May 2004  Doctoral student, University of Jena
Member of the research group of Prof. Dr. Bernd Luckas. Responsibilities and achievements:

- Structure elucidation of natural compounds from freshwater as well as from the marine environment.
- Analysis of food contaminants and environmental samples according to regulatory law.
- Operation of various analytical devices such as LC/MS and LC/(MS/MS equipment from Applied Biosystems/MDS Sciex.
- Implementation of analytical methods and offering analytical support (on-site) for foreign countries such as Vietnam, China, Canada, and Sweden in the framework of international projects funded by the E.U. and the BMBF (Bundesministerium für Bildung und Forschung).
- Leading the ISO (International Organisation for Standardisation) working group for microcystin analysis, ISO 20179.

Product Specialist Mass Spectrometry, Applied Biosystems, Darmstadt, Germany

Member of the PSM Support Central Europe. Responsibilities:

- Provision of telephone, in-house and on-site technical support.
- Support sales in the achievement of the orders plan by provision of demonstrations, sample analysis and technical information.
- Training of customers and on-site and in-house.
- Numerous pre-sales visits in EMT countries in form of seminar tours, conference participations, and customer visits.
- Editorial work within various ISO groups (International Organisation for Standardisation)

INTERESTS:
Cycling, trekking, travel, business and law, viniculture
ABSTRACTS
WEDNESDAY 3 October 2007

Session 1: Drug development

Preclinical testing of new drugs

Evan Siegel

Plenary lecture discussing the process of drug development

The use of Positron Emission Tomography in Medical Research.

Gerdus Kemp\textsuperscript{1,2}, CE Medlen\textsuperscript{2}

1. PET Labs Pharmaceuticals, Little Company of Mary, Pretoria.
2. Department of Pharmacology, University of Pretoria.

Amazing discoveries\textsuperscript{1} are occurring daily in biology as we try to find a route to the genetic core of cells, determining in what way organs systems behave or fail during disease. This search for molecular errors and its diagnosis is creating a new vision for molecular medicine. Positron emission tomography (PET) is a diagnostic method that creates high resolution, three dimensional tomographic images of the distribution of positron emitting radionuclides linked to molecules, antibodies, etc. in the human body.

PET Labs houses a commercial radiopharmaceutical manufacturing and distribution center and is home to the first hospital based Siemens-CTI cyclotron in Africa that support both the commercial and research and development programs. The current strategy in molecular imaging is to identify a target molecule in a specific organ or its disease state in a living organism, develop a high-affinity probe for the molecule, and ultimately use the probe to detect the distribution and pharmacodynamics of the molecule.

The aim of this collaboration between PET Labs and the Department of Pharmacology is to achieve leadership in research and education in areas critical for the international competitiveness of the medical and pharmaceutical industry in South Africa.

References:

Increasingly women are the face of AIDS and in sub-Saharan Africa, 60% of individuals infected by HIV are women. Surveys performed in six African countries have shown that women between the ages of 15 and 24 are 2½ times more likely to be infected with the virus than their male counterparts. These trends are also seen in countries such as India and the Russian Federation. Often AIDS also disproportionately affects women in industrialised countries, eg in the USA AIDS is the leading cause of death for African-American women aged 25 - 34. Biological vulnerability together with a woman’s lack of control over her own sexuality increases the risk of infection with HIV. Microbicides are anti-infective agents suitable for self-administration before sexual intercourse. Formulations under development include gels, suppositories, films and slow-release microbicide-loaded intravaginal rings that can be left in position for some weeks, or even longer, to provide continuous protection. By developing safe and even partially effective topical microbicides, the power of protection against becoming infected by HIV will be placed in the hands of women themselves. Many candidate microbicides are in the development pipeline, representing a wide range of chemical entities. These include polyanionic glycoconjugates, nanobodies, virus-cell fusion inhibitors (peptides), NNRTIs, NRTIs, CCR5 blocking peptides, protease inhibitors and inhibitors of mannose receptors, each with their own unique mechanism of action. In this regard, the European Microbicides Project (EMPRO), a consortium of 35 principal investigators from various institutions and companies in Europe and Africa, is supporting the development and identification of promising anti-HIV microbicides. Advantages and disadvantages of the various categories of microbicides, the role of the pharmaceutical industry, availability to users, factors that determine the willingness to use these agents and criteria for successful microbicides will be discussed.
Rapid scale up of antiretroviral therapy is occurring in Southern Africa, which has the world’s largest HIV burden. Clinical pharmacologists have played an important role in supporting this scale up. In the public sector there is a limited armamentarium of antiretroviral (ARV) agents, some of which are seldom used in industrialised countries as they are toxic. Pharmaco-epidemiological studies have identified troublesome ARV agents in our population and suggested strategies for minimising toxicity. Some of these strategies have been adopted as policy. Pharmacokinetics has an important role to play as there is little data from African populations. The safe management of drug interactions with ARVs (particularly involving rifampicin, anticonvulsants, herbal medicines and antimalarials) is of critical importance. Some data is emerging to assist clinicians managing interactions with rifampicin. Pharmacoeconomic studies can evaluate different ARV strategies using more expensive but less toxic agents. Finally, adherence is the key to ARV success, and data from a series of studies have shown the value of pharmacy refill as a tool for measuring adherence.
Session 2: Antiretroviral drug research

Nevirapine Pharmacokinetics in Premature Infants; Preliminary Results.

Pierre Mugabo1, Mark Cotton2, Johan Smith3, J.L.Van Zyl4, Helene Rabie2, Peter Smith5, Mark Mirochnick6, Ilse Els3, Wilhelm Steyn7, David Hall7.

(1) Department of Pharmacology, University of the Western Cape (UWC); (2) Children's Infectious Diseases Clinical Research Unit, University of Stellenbosch (US); (3) Neonatal Services, US; (4) High Risk Neuro – Developmental Follow – up Clinic, US; (5) Department of Pharmacology; University of Cape Town; (6) Department of Paediatrics, Boston University School of Medicine, USA; (7) Department of Obstetrics and Gynaecology, US. Email address: pmugabo@uwc.ac.za

Purpose: To determine if premature infants (PI) whose HIV-positive mothers (HPM) received NVP during labour and those whose HPM missed NVP dose during labour and received NVP dose just at birth require a single dose of NVP at 48-72 hrs after birth in order to maintain NVP therapeutic plasma concentrations. To determine the following NVP pharmacokinetic (PK) parameters: maximum plasma concentrations (Cmax), time to the maximum plasma concentrations (Tmax), area under the plasma concentrations-time curve (AUC), volume of distribution (VD), clearance (Cl), half life (T1/2) and elimination constant (Ke). To find out NVP dosing regimen required to maintain plasma concentrations (PC) above 100ng/ml throughout the first week of life in both groups of PI. To determine mother to child HIV transmission (MTCHT) rate at 4-6weeks after birth by HIV – RNA - PCR.

Methods: After informed consent, PI born before 37 weeks of gestation has been completed were involved in the study. Blood samples were collected just after birth, then 1, 2, 4,6,8,14,21 and 28 days after birth. PC of NVP were determined by Liquid Chromatography Mass Spectrometry. AUC was calculated using a “Graph Pad Prism 4” software. VD was obtained using the formula VD=D/C0. Cl was estimated from the ratio of the dose administered to the observed AUC. T1/2 was calculated using the formula “T1/2= 0.693 x VD/Cl”. Ke was obtained by dividing the Cl by the VD. HIV-RNA-PCR test was done at 28 days after birth. The study approved by UWC and US ethics committees.

Results: Results from 21 PI (11male and 10 female) are reported. The mean (±SD) gestational age (GA) and birth weight (BW) are respectively 27.5 ±5.48 weeks (range 19-38) and 2.25±656.96kg (range 1.02-3.36). Cmax were achieved within 24 hrs after birth in both groups of PI. Cmax, AUC, VD, Cl, T1/2 and Ke are respectively 1653.2ng/ml, 185270hr x ng/ml, 125ml, 1.07ml/hr, 90hr and 0.008 in PI whose mothers received NVP during labour and 2530.3ng/ml, 304278 hr x ng/ml, 90.1ml, 0.8ml/hr, 90.1hr, and 0.006 in PI whose mother missed NVP during labour. PC exceeding 100ng/ml (10 times the in vitro IC50) are achieved over 11 days in both groups of neonates (NN). T1/2 is prolonged in both groups of PI compared with the median T1/2 which was 64.9 hrs (range 35.4 – 330.7 hrs) in the US and Ugandan studies conducted in full term NN. No correlation was found between the BW and T1/2 and the GA and T1/2. The estimate for proportion of HIV (+) based on 1 HIV(-) and 15 HIV(-): 1/16=0.0625 = 6.25%, 95% confidence interval estimate for proportion of HIV (+) is (0.0016, .3023) or (0.16%, 30.23%). According to these results, the MTCHT rate is 6.25%. PI whose mothers missed NVP dose during labour, and received a dose at birth do not require an additional dose at 48-72 hrs after birth to maintain therapeutic concentrations.

Acknowledgement: Medical Research Council for financial support of the study.
Initial experience with an antiretroviral therapeutic drug monitoring service

Tamara Kredo*, Karen Cohen, Rory Leisegang, Peter Smith, Gary Maartens
Division of Clinical Pharmacology, Department of Medicine, Health Sciences Faculty, University of Cape Town, Groote Schuur Hospital, Cape Town, Observatory 7925,*Correspondence: tamara.kredo@uct.ac.za

Purpose:
Therapeutic drug monitoring (TDM) of antiretrovirals (ARVs) is a tool for optimizing efficacy and minimizing toxicity of combined antiretroviral therapy (cART). Our laboratory has recently started a therapeutic drug monitoring service for antiretrovirals. The non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs) are candidates for TDM as they fulfill the necessary criteria: there are known dose-response relationships, there are established target therapeutic ranges, there is known significant inter-patient variability and there are reliable, specific assays available. Although routine ARV TDM is not supported by current data, its use in specific clinical situations where pharmacokinetics and dynamics are less predictable may be warranted (e.g. pregnancy, paediatrics, drug-interactions, drug intolerance and virological failure). We review all ARV TDM requests since the commencement of the service in October 2006.

Methods:
A structured TDM form was created to guide requesting clinicians to provide necessary information for interpretation of the results. Data on demographics, current ARV and concomitants medication were collected. The reasons for request were solicited, including paediatrics, drug interactions, virological failure, suspected toxicity, hepatic or renal dysfunction. Date and time of blood sampling and timing of last ARV dose was recorded. ARV plasma concentrations were measured using validated liquid chromatography mass spectrometry methods. The sample results were categorized as low, adequate or high according to known therapeutic indices. The data were prospectively entered into an Access database. Descriptive statistics were generated using Stata9.2.

Results:
Sixty two percent of requests were from tertiary institutions, 17.9% from secondary hospitals and 17.9% from primary care clinics. The majority of requests were for lopinavir/ritonavir (LPV/r) (69.6%) followed by efavirenz (EFV) 19.6%, nevirapine (NVP) 8.9% and ritonavir RTV 1.8%.

Paediatric results: Sixty percent of all requests were for paediatric patients, 93% of whom were below the age of 2 years. Median age of all children was 330 days (IQR 288-530 days). There were CD4% data for 60% of requests. The mean CD4% was 35.4% (SD 11.6%). Eighty-eight percent of paediatrics had only one request, 8.8% two requests and 3.3% were sampled three times. LPV/r was the dominant concentration requested, 94.1%, followed by RTV and EFV at 2.9% each. When providing reasons for requests, ‘paediatric patient’ was the reason in 85.3%, followed by tuberculosis queries (12.5%), virological failure (5.9%), potential drug interactions(5.9%) and toxicity (2.9%).

Adults: 39.3% of all requests were for adult patients. The median age was 36.6 years (33.6-39.5 years). There were CD4 count data for 80% of requests, with mean CD4 count of 77 cells/mm$^3$ (IQR 36-367 cells/mm$^3$). Sixty-eight percent of adults had only one request, 27.3% two requests and 4.5% were sampled three times. EFV was the dominant concentration requested, 45.4%, followed by LPV/r and NVP at 22.7% and 31.8% respectively. Concern regarding drug-interactions was the reason for request in 59.1% of adults, followed by rifampicin queries (28.6%), virological failure (22.7%), toxicity (27.3%) and pregnancy (4.5%).

Overall results: LPV/r was most frequently requested, 69.6%, EFV was next most frequent, 19.6% followed by NVP, 8.9% and RTV, 1.8%. Overall reasons for requests included concern regarding interaction with rifampicin in 42.9%; potential drug interactions in 26.8%, of which 42.9% were due to rifampicin interaction. Virological failure, drug toxicity and pregnancy were reasons in 12.5%, 12.5% and 1.8% respectively. Concentrations were described as low, adequate or high in 35.7%, 46.4%, and 17.9% of requests respectively. EFV concentrations were low in 36.4% of requests and high in 18.2% of samples. LPV/r concentrations were low in 33.3% of requests and high in 29%. The median EFV concentration was 1.3mcg/mL (IQR 0.3 – 3.4mcg/mL). LPV/r median was 2.1mcg/mL (IQR 0.1 – 4.9mcg/mL). NVP median concentration was 2mcg/mL (IQR 1.8 – 4.4 mcg/mL).

Conclusions
The recent introduction of an ARV TDM service has allowed Two-compartment disposition models including transit compartment absorption model were developed for 3TC and AZT using healthy volunteer data. Characterisation of absorption obtained from these models may improve population PK modelling especially with sparsely sampled study designs without concentration-time data during the absorption phase.
Transport of nevirapine across intestinal epithelial cells: interactions with tuberculosis drugs and traditional medicines

Liesl Brown¹,²*, Odette Heyneke¹, Deon Brown³, Piet van Wyk¹, Sias Hamman³
¹Department of Pharmacology and Therapeutics, ²School of Pharmacy, University of Limpopo; ³Tshwane University of Technology, *Corresponding author: lbrown@medunsa.ac.za

OBJECTIVES:
(a) To determine the effect of rifampicin, extracts of two traditional medicinal plants, Hypoxis hemerocallidea and Sutherlandia frutescens and a constituent of S. frutescens (i.e. L-canavanine) on the transport of nevirapine across Caco-2 intestinal epithelial cells. (b) To investigate the role of the efflux transporter, P-glycoprotein (P-gp), in the intestinal transport of nevirapine.

METHODS:
Caco-2 cell monolayers were grown on transwell filters. The optimum concentration of nevirapine where P-gp efflux effects occurred was determined. Nevirapine transport in the apical to basolateral (AP-BL) and basolateral to apical (BL-AP) directions was determined in the absence (NVP 1) and presence of verapamil (NVP 2), rifampicin (NVP 3), extracts of H. hemerocallidea (2 g/100 ml) (NVP 4), S. frutescens (1 g/100 ml) (NVP 5) and a solution of L-canavanine (NVP 6) from which the apparent permeability coefficients (P_app) were calculated. The cumulative transport (% of initial concentration) and P_app values of the different experimental groups were statistically compared by means of a one-way repeated analysis of variance (ANOVA).

RESULTS:
Nevirapine alone (NVP 1, 5 mM) gets effluxed in the BL-AP direction (P < 0.05) and is therefore a substrate for P-gp. The transport studies involving NVP 2-6 experimental groups indicate inhibition of P-gp. The results suggest possible drug-drug and herbal-drug interactions that may have clinical significance when nevirapine is co-administrated with these compounds with possible increased absorption of nevirapine.

CONCLUSIONS:
Co-administration of rifampicin and extracts of traditional medicinal plants influence the intestinal absorption of nevirapine by means of P-gp efflux inhibition.
The effect of rifampicin on nevirapine plasma concentrations in South African adults with HIV-associated tuberculosis

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Background
Nevirapine-containing antiretroviral therapy and rifampicin-based antitubercular therapy are commonly co-administered in Africa, where nevirapine is often the only available non-nucleoside reverse transcriptase inhibitor. Rifampicin induces the metabolism of nevirapine, but the extent of the reduction in nevirapine concentrations has varied widely in previous studies. We describe the steady state pharmacokinetics of nevirapine during and after antitubercular therapy in South African patients.

Methods
16 participants were admitted for intensive pharmacokinetic blood sampling while taking rifampicin-based antitubercular therapy, and again 10 days or more after completion of antitubercular therapy. Nevirapine was administered throughout at standard doses of 200mg 12 hourly. Rifampicin was dosed at 600mg 5 days a week in those weighing ≥55 kg and 450 mg for those <55 kg. Plasma concentrations of nevirapine, its 12-hydroxy metabolite and rifampicin were quantified by validated Liquid Chromatography Mass Spectrometry methods.

Results
Geometric mean ratios for nevirapine pharmacokinetic parameters during versus after antitubercular therapy were 0.61 (90% confidence interval (CI) 0.49-0.79) for Cmax; 0.64 (90% CI 0.52-0.80) for area under the curve up to 12 hours (AUC0-12); and 0.68 (90% CI 0.53-0.86) for Cmin. Nevirapine Cmin was sub-therapeutic (<3mg/L) in 6 patients during antitubercular therapy (1 of whom developed virological failure) and in none afterwards. There was no correlation between rifampicin concentrations and the degree of nevirapine induction assessed by the proportional change in nevirapine concentrations between the 2 admissions. The ratio of nevirapine AUC0-12 to the AUC0-12 of its 12-hydroxy metabolite was significantly lower in the presence of antitubercular therapy, consistent with induced metabolism.

Conclusion
Nevirapine concentrations were significantly decreased by concomitant rifampicin-based antitubercular therapy and a high proportion of patients had sub-therapeutic plasma concentrations. Further study in African patients is required to determine the implications for treatment outcomes.
Prevalence of Drug-Drug interactions (DDIs) between Antiretroviral Agents in different age groups in a setting of the Private Health Care Sector in South Africa for the year 2006

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Purpose:

The aim of this study was to determine the prevalence of DDIs between antiretroviral (ARV) drugs in different age-groups in a section of the private primary healthcare sector in South Africa.

Methods:

A quantitative, retrospective drug utilisation review was performed on 47,085 ARV prescriptions claimed through a medicine claims database during 2006. The DDIs found were classified according to a clinical significance rating as described by Tatro (2005).

Results:

An average of 5.23 ± 3.86 ARV prescriptions (n = 47,085) per patient were claimed during 2006 for 8,999 HIV patients (3.27% of the total number of patients, N = 275,424). ARV prescriptions represented 4.73% of the total number of prescriptions claimed during the study period (N = 993,804). HIV patients received an average of 2.36 ± 0.61 ARVs per prescription. Only 4.95% of the prescriptions had only one ARV medicine item, 56% had two, 37% had three, 1.75% had four and < 1% had more than four ARV medicine items.

A total number of 960 DDIs were identified, of these, 1.88% were for patients <=6 year, 4.27% for patients > 6 years and <= 12 years, 0.63% for patients > 12 and <= 19 years, 32.40% for patients < 19 years and <= 40 years, 60.21% for patients < 40 years and <= 60 years and 0.63% for patients > 60 years having the highest number of DDIs and patients older than 60 years the lowest. The majority of DDIs between the ARVs presented in significance levels 2 and 4 (Moderate and Major/moderate). The most important interactions were between: indinavir and ritonavir (n = 196); efavirenz and lopinavir/ritonavir (n = 80) and efavirenz and indinavir (n = 64) all interacting at clinical significance level 2.

Conclusion:

The results of this study emphasize the importance of using drug utilisation study as an identification tool to provide insight into the prescribing and utilization patterns of antiretroviral drugs, in order to provide optimal therapy for patients infected with HIV.
Nonlinear mixed effect models for lamivudine and zidovudine in healthy male volunteers

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Purpose:
Lamivudine (3TC) and zidovudine (AZT) are potent selective inhibitors of HIV reverse transcriptase and, due to their synergistic effect, are available as a 3TC/AZT fixed dose combination (FDC) tablet for use in combined antiretroviral therapy (cART). Both drugs are rapidly absorbed and previous population pharmacokinetics (PK) evaluations were limited by insufficient data to describe the absorption process. Here, concentration-time data from healthy volunteers were evaluated to characterize 3TC and AZT absorption processes.

Methods:
Log transformed concentration-time data following a single dose of 3TC 150mg/AZT 300mg in 25 healthy male volunteers were modeled using population analysis in NONMEM VI. A range of absorption models including transit compartment absorption model in combination with one, two and three compartment disposition model was evaluated. Population PK parameters were estimated using first-order conditional estimation with interaction method. Model fit was assessed using parameter imprecision, goodness-of-fit plots and posterior predictive checks. In model comparisons objective function value (OFV) differences, which are goodness-of-fit statistics (lower the better) were also considered.

Results:
The transit compartment model linked with first order absorption described the absorption process substantially better compared to other absorption models tested, for both 3TC and AZT. Absorption parameter estimates (mean (95% confidence interval)), i.e. absorption rate constant (Kₐ), mean transit time and number of transit compartments, were 0.332 (0.295-0.369) h⁻¹, 0.549 (0.461-0.637) h and 7.04 (4.14-9.94) for 3TC and 2.34 (1.68-3.0), 0.395 (0.304-0.486) and 6.95 (3.3-10.6) for AZT, respectively. Two-compartment disposition models fitted both 3TC and AZT data best according to selection criteria. Although 3-compartment models had significantly lower OFVs (p=0.01), the posterior predictive checks revealed more model misspecifications.

Conclusions
Two-compartment disposition models including transit compartment absorption model were developed for 3TC and AZT using healthy volunteer data. Characterisation of absorption obtained from these models may improve population PK modelling especially with sparsely sampled study designs without concentration-time data during the absorption phase.
Poster session I

Student Award Competition Posters for SAPS and TOXSA
Session 3: Reactive metabolites and drug interaction studies

Reactive nitrogen and oxygen intermediates and oxidative stress in neuroleptic-induced tardive dyskinesia: Implications for prevention and treatment

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The dopamine supersensitivity hypothesis remains an important construct in explaining the aetiology of neuroleptic induced tardive dyskinesia (TD). Despite advances in the treatment of schizophrenia and the lower tendency of new generation agents to evoke motor dysfunction, TD remains a clinical concern. Long-term neuroleptic administration alters dopaminergic turnover, which has been proposed to lead to increased formation of reactive oxygen species (ROS) and the induction of oxidative stress. The striatum, a critical brain region involved in regulating movement, is highly susceptible to oxidative stress. Clinical and pre-clinical studies in TD have demonstrated increased free radicals and decreased superoxide dismutase in the disorder as well as increased synthesis of ROS by haloperidol, while lipid peroxidation has been found to be significantly higher in patients treated with older antipsychotics compared to newer atypical agents.

Antioxidants have possible therapeutic value in treating TD. However, under certain conditions of redox status, antioxidants may act as pro-oxidants. We have investigated the biological and pharmacological role of nitric oxide, superoxide and antioxidant treatment in an animal model of TD, looking specifically at important factors associated with the development of TD, including withdrawal of the neuroleptic, advancing age, status of cellular markers of oxidative stress and reversal of the bio-behavioural manifestations of TD with an atypical neuroleptic. This paper will review this work, providing support for the role of oxidative stress in TD, and will attempt to suggest new avenues of treatment, possible drawbacks and also discuss its prevention.
Melatonin counters the 5-fluorouracil-induced morphological alterations and decrease in volume of rat hippocampal cells

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Introduction 5-Fluorouracil (5-FU) is a fluorinated pyrimidine antimetabolite that inhibits thymidylate synthase, thereby blocking DNA synthesis and exerting an antineoplastic effect. Due to this inhibition of DNA synthesis, 5-FU is associated with a wide range of toxicities, particularly to the immune, gastrointestinal and haematopoietic systems. It has recently been reported that standard doses of 5-FU induce the phenomenon of “chemobrain” in breast cancer patients, a phenomenon characterised by concentration and memory problems. Patients furthermore exhibit difficulty with spatial memory tasks, which are co-ordinated from the hippocampus. The extent of this cognitive impairment is mild to moderate, and due to the acute administration of 5-FU; long-term therapy may involve a degree of cognitive recovery, through neuroplasticity mechanisms.

Aims of the study Having previously reported that melatonin, a powerful antioxidant and pineal hormone, attenuates 5-FU-induced decreases in serotonin, dopamine and norepinephrine levels, we therefore decided to investigate the ability of melatonin to counteract 5-FU-induced neurotoxicity. An investigation into the effects of 5-FU and melatonin on hippocampal volume and histology may be of benefit in preventing, or reducing the impact of cognitive impairment experienced by patients on 5-FU therapy.

Method Male Wistar rats were treated for five days with either 1 mg/kg 5-FU, melatonin, a combination of these, or 0.9% saline. On the sixth day, the animals were killed and the brains frozen. Cryostat-cut sections of the hippocampus were stained with the Nissl stain and visualised. Stereology software was used to measure the volumes of the dentate gyrus (DG), CA1-2 and CA3 regions.

Results and discussion 5-FU significantly decreases the volume of the CA1-2 and CA3 regions in rat hippocampus, which may be a mechanism by which this agent induces cognitive impairment and possibly depression, the latter being associated with a decrease in hippocampal volume. This agent also causes a significant loss of the morphological integrity of these regions of the hippocampus, with evidence of cell lysis occurring, possibly due to 5-FU-induced lipid peroxidation of cell membranes. The closely-packed, band-like appearance of all the hippocampal regions analysed is also disrupted. Co-administration of the antioxidant melatonin is able to counter the 5-FU-induced decrease in hippocampal volume, particularly in the CA3 region, and also prevents the structural damage associated with this agent.

Conclusions The stereological and pictorial results presented confirm that 5-FU induces hippocampal toxicity, in particular to the CA3 region, and that the co-administration of melatonin is beneficial in alleviating the severity of this damage. These findings support the use of melatonin co-therapy to decrease the toxicity of 5-FU and improve the quality of life of patients treated with this drug.
One of the Problems with Traditional Medicines: Interactions with Prescription Drugs

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Background
Traditional medicines are still widely used in South Africa. As affordable and accessible herbal remedies, their use is encouraged by Government and WHO. Worldwide, their popularity is increasing. While consumers generally regard them as natural and therefore safe, they may have serious adverse effects as well as significant interactions with conventional medicines.

Method and Objective
The present study was undertaken to search the literature for reported actual and potential interactions of traditional medicines with commonly used prescription drugs in order to establish guidelines for the safe use of traditional health care.

Results
Herbal medicines as part of complementary/alternative medicine (CAM) are increasing in popularity. This applies to South Africa as well as the international community. Parallel to their escalating use grows the volume of literature on interactions with prescription drugs. Less known and less well described are adverse effects and interactions of African traditional with conventional medicines, although research in this field is increasing. A table of commonly used indigenous plant medicines and their interactions with relevant conventional drugs is provided.

Discussion
Traditional medicines, usually in the form of herbal medicines, have several problems. These relate mainly quality, safety and efficacy. Of growing concern is their interaction with prescription drugs. While the traditional healer relies on his knowledge of herbal therapies, the medical doctor has no training in plant medicine. In addition, he is often not aware that his patient is taking herbal preparations concomitantly.

Conclusion and Recommendations
Herbal medicines pose a potential for serious interactions. The patient should know that herbal preparations are not necessarily safe and should inform his doctor of their use. The doctor should take a comprehensive medication history which includes the use of traditional medicines. The medical curriculum should provide some knowledge in phytotherapy. Researchers should include the issue of interactions with conventional drugs when studying the efficacy and toxicity of medicinal plants. In this way, the safe use of two coexisting health care systems is promoted.
Session 4: Drug Development Workshop/Panel discussion

Changing Paradigms in Drug Development

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The cost of developing new drugs has increased substantially over the last decade yet the rate at which new drugs receive marketing approval has decreased. The rising costs of commercialising new molecular entities (NME) and the increasing demands on pharmaceutical companies to meet the public’s demand and business objectives has caused the drug development industry to re-evaluate the conventional drug development process. Significant advances have recently been made in the statistical methods used to analyse data and the technology used to collect clinical data which have improved the efficiency and accuracy of data collection which in turn has increased the measurement of responses to NME. The improved tools have enabled drug developers to deviate from the conventional phased drug development process and are expected to lead to an improvement in the rate at which NMEs obtain regulatory approval. A biphasic system, an exploratory and confirmatory phase, rather than the conventional 3 phase process appears to be a superior process to determine the safety and efficacy of new drugs or biologics, particularly those with a narrow therapeutic index.

The presentation will include a comparison of the goals in the various stages of the conventional and new drug development processes and some examples of new technologies being used to collect data from patients that are increasing the efficiency and accuracy of data collection in clinical trials. The discussion will also include the deficiencies facing the commercial drug development industry in South Africa and opportunities for the development of commercial services that can be provided by the various academic institutions in South Africa.

Panel discussion: Drug development
Fumonisin B₁: Harmful Effects and Cellular Mechanisms Behind Its Toxicity

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Fumonisin B₁ (FB₁) is a mycotoxin produced by the fungus *Fusarium verticillioides*, which commonly infects corn and other agricultural products. *Fusarium* species can also be found in moisture-damaged buildings, and therefore there may also be human exposure to *Fusarium* mycotoxins, including FB₁. FB₁ bears a clear structural similarity to the sphingolipids, and due to this similarity, it is known to affect the metabolism of sphingolipids by inhibiting the enzyme ceramide synthase. It is neuro-, hepato- and nephrotoxic in animals, and it is classified as possibly carcinogenic to humans. The cellular mechanisms behind FB₁-induced toxicity include the induction of oxidative stress, apoptosis and cytotoxicity, as well as alterations in cytokine expression. The effects of FB₁ on different parameters vary markedly depending on what types of cells are studied or what species they originate from. These aspects are important to consider when evaluating the toxicity potential of FB₁.
The purported health benefits and aggressive marketing of numerous indigenous products has prompted increased concerns about the quality, safety and efficacy of these products. Given that herbal products are usually complex mixtures of multiple active ingredients and that they are not subject to the same regulatory requirements as pharmaceuticals, it is not surprising that little is known about the biological effects of such matrices in humans. Nevertheless, because a substantial portion of the public perceives these products as safe, it is likely that many adverse effects associated with traditional medicines and other dietary supplement use have not been recognized or reported.

Pharmacologic studies are indeed necessary for the long-term goal of identifying the active ingredients in plants, however these will not be forthcoming rapidly enough to meet the acute public health needs for knowledge on efficacy and safety since these products are being widely consumed at various dosages. Currently there is lack of data on adverse effects, drug-herb interactions, evidence of efficacy, absence of information on active constituents, variation between and within products, and lack of monitoring of contamination and adulteration. The analysis of the effects of medicinal plants needs to also consider the concurrent use of multiple products, fluctuations in use, seasonality of symptoms, and the confounding factors that could lead to misinterpretation of effect.

Well-conducted and well-designed epidemiological studies can help overcome the knowledge deficits implicit in these concerns with the aim of minimizing bias and to allow external generalizability of the findings. It is possible through an epidemiologic cohort to study multiple outcomes as well as the ability to closely quantify patterns of medicinal plant use over time. As with other exposures, cohort study designs also eliminate any risk of recall bias in the reporting of prior exposures. Case-control studies can also be successfully conducted to measure associations between risk of an outcome and the exposure to plants. Such studies differ in their point of departure, with selection of subjects based on the presence or absence of an outcome and not the presence or absence of the medicinal herb use. It is then possible to compare the proportion of users in the outcome groups and estimate the relative risk of that outcome as a function of medicinal plant usage. A third study design, which is very effective for the study of herb-drug interactions is the case-only design. In this design, individuals with a disease are selected, and differences in proportions of dual exposures are measured.

A case-control study has been successfully undertaken in the former Transkei region of the Eastern Cape Province to measure the association between wild plant exposure and the risk of developing oesophageal cancer. Numerous plants were identified as high risk and the strength of the epidemiologic evidence was further supported by laboratory-based investigations. The rationale for epidemiologic research within the context of herbal medicines usage and the results of the above study will be discussed in further detail.
Investigating the potential mutagenicity of traditional African herbal medicines

DR Katerere, SS Ntuli, KM Thembo & HF Vismer

Traditional herbal medicine (commonly known as *muthi* in Southern Africa) has a long history of use world-wide. The trade in medicinal herbs tops US$60 million annually in South Africa. There are two major problems associated with the use of African traditional medicines: first, the conditions under which the herbs are stored and traded have long been a cause for concern; second, while acute toxicity is well documented, there are very few studies which have looked at long-term toxicity.

In this study, we investigated the contamination with mycotoxigenic fungi and two classes of mycotoxins, fumonisins and aflatoxins of several prepared traditional medicines bought anonymously in major cities of South Africa and Botswana. We then tested the potential mutagenicity of the medicines using the AMES assay on two *Salmonella typhimurium* strains.

Of the 17 samples, 12 showed fungal contamination *Fusarium, Aspergillus, Mucor* and *Penicillium* species. Thirteen (13) samples had detectable levels of fumonisins and none was contaminated with aflatoxins. The AMES assays showed several of the herbal products to, in fact, exert an anti-mutagenic effect against both TA-98 and TA-100 in the presence and absence of metabolic activation.

Further work will focus on following the supply chain of the herbal trade in South Africa, and on following up on the botanical identity of mutagenic herbal products.
Mutagenic Constituents of Helichrysum simillium DC.

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Purpose:
Frequently used plants in traditional medicine are assumed safe, due to their long history of use and considered to have no side effects because they are natural. However, recent investigations have revealed that many plants used as food or in traditional medicine have mutagenic effects in *in vitro* assays. This raises concern about the potential hazards resulting from the long-term use of such plants. South African plants, used frequently in traditional medicine, were evaluated recently in our laboratory for potential mutagenic effects using the *Salmonella* /microsome mutagenicity assay against *Salmonella typhimurium* TA98 and TA 100 bacterial strains. The 90% methanolic extracts of *Helichrysum simillium* was one of a few extracts that showed mutagenicity with and without metabolic activation. The objective of this study was to isolate the active mutagenic constituents from the extracts of *H. simillium*.

Methods:
*Extraction:* Dried and powdered plant material was extracted sequentially with dichloromethane and 90% methanol.
*Mutagenicity assay:* A mutagenicity test was carried out using the *Salmonella* /microsome assay based on the plate-incorporation procedure with *Salmonella typhimurium* tester strain TA 98. The assay was performed according to Maron and Ames (1983).

Results:
A bioassay-guided fractionation of the active constituent(s) is currently underway. The results will be discussed.

References:
Toxicity evaluation of an aqueous extract of Leonotis leonurus (L) in rats

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Purpose Leonotis leonurus (L) R.Br. (Lamiaceae) is a plant that is used by resource poor farmers in the Eastern Cape to treat helmintiasis in livestock. Use of plants in the ethnoveterinary treatment of livestock ailments has become a common practice among rural livestock farmers due to high costs of conventional drugs. If properly evaluated, these plants may form an alternative cost effective strategy in the management of animal diseases. In this study, \textit{L. leonurus} was evaluated in rats for its potential toxicity.

Methods Preparation of aqueous extract of Leonotis leonurus: Leaves were dried and pulverized into fine powder. A known quantity of the powder (300g) was mixed with distilled water (3000ml) and boiled for 5 min at room temperature, after which the mixture was filtered initially using cotton wool, and again under reduced pressure on a (Whatman no 1, 125cm diameter) filter paper. The resultant filtrate was lyophilized for 48hrs, yielding 53g of extract powder. The extract was then suspended in distilled water before administration.

Animals: Wistar rats of either sex, weighing between 150 to 300g were used. They were reared under standard conditions at the animal House of the Faculty of Science where temperatures were maintained at room temperature. Animals were kept in cages, fed rat cubes and allowed free access to fresh water in bottles \textit{ad libitum}. They were deprived of food, but not water for 16hrs prior administration of the test suspension.

Toxicity tests: Aqueous extract of \textit{L. leonurus}, was administered orally to female rats in doses of 200, 400, 800, 1600, and 3200mg/kg in acute toxicity, and the control group received 2ml/kg of the distilled water. In subacute toxicity, male rats received doses of 400, 800 and 1600mg/kg) of extract daily for a period of 14 days, with the control group receiving 3ml/kg of distilled water. Haematology, chemistry and histopathology tests were conducted on the blood and organs. Weights and general changes were also noted.

Results In acute toxicity test, the extract caused death in animals receiving 3200mg/kg doses. In the sub-acute test the extract at 400 did not cause significant changes in red blood cell counts (RCC), packed cell volume (PCV), haemoglobin (HB) concentration, and mean corpuscular volume (MCV). At doses of 800 and 1600mg/kg, the extract caused significant decrease in the levels of RBC, PCV, number of platelets as well as white blood cells and its differentials. Biochemical parameters were affected, whereby the extract caused a significant decrease in the levels of creatinine and alkaline phosphate (ALP). Changes were also noted in the body weights but no significant changes were observed in the levels of electrolytes (sodium, potassium and chloride). The animals showed a starry hair coat, respiratory distress, and mortalities were recorded. The extract also caused various histopathological changes in various internal organs. The study concluded that farmers need to exercise caution when using for \textit{L. leonurus} for medicinal purposes, especially if used in high doses.
Session 6: Toxins and herbal remedies II

Use of LC/MS/MS to speed up your toxin screening applications

J. Dahlman

Toxicity testing of traditional herbal medicines at the MRC Primate Unit: challenges and limitations

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Many SA research institutions study the use and health application of traditional herbal remedies, and there is an increasing interest in commercial drug development based on such natural remedies. In either case it is clear that toxicity testing has to form part of the strategy, since it is an important component of any drug development pipeline. Pre-clinical safety testing has become a sophisticated science, and strategies are designed to provide toxicogenetic data, safety information, and to inform the next level of pharmacological testing.

In nations with well developed pharmaceutical industries, pre-clinical safety testing is often conducted by commercial companies, dedicated to that activity. However, in South Africa, and true to most developing countries, the capacity for animal research, including testing, is mostly in academic institutions. The Primate Unit of the Technology and Business Development Directorate of the MRC is such a facility, and is increasingly approached by local organisations to conduct toxicity testing with traditional medicines. While engaging in this activity it soon became clear that working with this material is quite different to testing pure compounds. The challenges and limitations are particularly in the areas of availability of information to inform the testing strategy, dosage, administration, palatability and, last but not least, funding. Many characteristics and properties of the plant material make it difficult, and, at times impossible, to comply with conventionally recommended strategies and international standards. In our presentation we describe how the Primate Unit approaches toxicity testing of milled whole plant materials in a vervet monkey model, and how we deal with above challenges.

With our methods we have been able to get vervet monkeys to voluntarily consume on average 19 times the therapeutic dose (range 9 to 66 times) and demonstrate toxicity in two out of eight traditional herbal medicines tested by us so far.
Objective:
The objective of this study was to evaluate the spectrum of potentially poisonous plant exposures dealt with by the Tygerberg Poison Information Centre.

Methods:
Telephonic plant-related consultations dealt with by the Centre from 1993 – 2005 were analyzed.

Results:
During the 13 year study period, the Centre dealt with 39813 consultations of exposures to poisonous substances, of which 1012 were plant-related (3%). 800 of these were patient-related. Of the 800 plant exposures, 768 (96%) were in humans and 32 (4%) in animals. The breakdown of human exposures was as follows: 544 (68%) in children, 149 (19%) in adults and 75 (9%) in adolescents. All exposures in children were accidental compared to 16% in adolescents and 66% in adults.
The most commonly encountered plant exposures were: Calcium-oxalate crystal containing plants (132: 17%), seringa tree berries (Melia azedarach) (117: 15%), Datura stramonium (61: 8%), Oleander (47: 6%), and Brugmansia species (37: 5%). Plant dermatitis cases amounted to 71 (9%). Plants in this category included several Euphorbia, Smodingium and Peucedanum species. 40% of all exposures were due to miscellaneous plant species. Four deaths were recorded. One case purportedly resulted from ingestion of the Indian Bean (Abrus precatorius). Identification of this plant, however, was not confirmed. The other three cases were due to unidentified roots and bulbs used in traditional medicines. Other causes of death in these cases could not be excluded.

With the exception of plants containing atropine-like alkaloids, the incidence of systemic effects after plant ingestions were relatively low (47: 13%), mostly gastro-intestinal in nature. It is important to note that most exposures in the adolescent group were intentional, and all of these were due to plants containing atropine-like alkaloids (Datura stramonium and several Brugmansia species). 63 (84%) adolescents presented with systemic symptoms and signs of atropine poisoning. In children the most common plant ingestions were seringa tree berries (Melia azedarach) and the leaves of plants containing calcium-oxalate crystals, e.g. Dieffenbachia amoena (dumb cane), Alocasia macrorrhiza (elephant’s ear) and Monstera deliciosa (delicious monster). Symptoms and signs of plant toxicity were highest in those plants containing calcium-oxalate crystals (50%) followed by seringa berries (10%). Although oral exposures to Nerium oleander (oleander) and Ricinus communis (castor oil plant) occurred, no symptoms and signs of systemic poisoning were recorded.

Conclusion:
Compared to drug overdose and exposures to household and agricultural poisonous chemicals, the incidence of plant exposures and/or poisonings are low. Fortunately the amount of plant material ingested by children is small and, therefore, the incidence of serious systemic toxicity in this age group is low. Ingestion of plants containing atropine-alkaloids is often associated with symptoms and signs of systemic toxicity. This type of poisoning is prominent in the adolescent age group. Plant dermatitis is a prominent entity in adult exposures.
Anticonvulsant Activity of *Nylandtia spinosa* L. Dumont (Polygalaceae) Aqueous Leaf Extract in Mice

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**Purpose:**
*Nylandtia spinosa* L. Dumont, formerly known as *Mundia* spinosa DC. belongs to the family, Polygalaceae. It is a rounded, thorny shrub with oblong and subsessile leaves, and is upto 1 m in height. It is found in the Western Cape but mainly in Namaqualand, Western Karoo and Eastern Cape. Infusion of the leaves has been used to control and/or manage children epileptic fit (Personal Communication). The claim of therapeutic success of the plant has not been subjected to scientific scrutiny. This study investigated the anticonvulsant activity of the aqueous leaf extract of the plant in mice as well as the safety profile and the chemical compositions.

**Methods:**
*Preparation of Aqueous Extract:* Leaves were collected from Kirstenbosch National Botanical Garden, Cape Town (NS 01), and standard extraction method was used to obtain the crude aqueous leaf extract of *Nylandtia spinosa*.

*Assessment of anticonvulsant activity:* Modified method of Vellucci and Webster (1984) was used for the assessment. Male albino mice weighing 18-30 g were used in groups of eight per dose of plant extract or standard anticonvulsant agents, phenobarbitone, diazepam and phenytoin. The convulsant agents used to induce seizures in mice include pentylenetetrazole (PTZ), bicuculline, picrotoxin and N-methyl-DL-aspartic acid (NMDLA). The effect of the plant extract or standard anticonvulsant drugs on the seizures induced by the convulsant agents were studied.

*Acute Toxicity Study:* The method of Lorke (1983) was used. LD$_{50}$ was calculated for the plant extract given both orally and intraperitoneally.

*Phytochemical Analysis:* Standard phytochemical tests and protocols were used to screen the dried powder of the leaves for various chemical components.

**Results:**
*N. spinosa* protected mice against PTZ-induced seizures and significantly delayed the onset of both bicuculline- and picrotoxin-induced seizures but did not affect NMDLA-induced seizures. Similarly, phenobarbitone and diazepam protected mice against seizures induced by all the convulsant agents with the exception of NMDLA seizures. The data obtained indicate that *N. spinosa* has anticonvulsant activity. The plant is also shown to be safe both orally and intraperitoneally and contain alkaloids, flavonoids, tannins, saponins, reducing sugars and cardiac glycosides.

**References:**
Scientific research on traditional medicines can lead to new pharmaceutical products, typically herbal formulations or new chemical entities. The Bioprospecting research group of CSIR, Biosciences focuses on the discovery and development of herbal remedies and prescription drugs based on South Africa’s traditional medicinal plants as well as its rich biodiversity. Traditional Healers currently provide CSIR scientists with indigenous knowledge (IK) that stimulates research that can eventually lead to discovery and development of new herbal remedies. The rights of these providers of IK, to share in future financial benefits that might be derived from commercial exploitation of any such products, is protected through a Memorandum of Understanding (September 1999) and a Benefit Sharing Agreement (February 2003) signed between the CSIR and the Traditional Healers Committee. An insightful and strategic look at CSIR’s results so far reveals a very interesting and valuable data set which immediately provides scientific evidence of efficacy required for the validation of the claims of traditional healers.

South Africa’s rich plant biodiversity is estimated to consist of approximately 24 000 indigenous plant species of which, CSIR Biosciences has collected approximately 11 000 and prepared an estimated 32 000 corresponding plant extracts. These form part of CSIR’s database of extracts. CSIR have received and captured more than 250 claims for cures based on medicinal plants and completed desktop/literature studies on at least 50% of these for the purpose of determining what prior research is already in the public domain, establishing the therapeutic area and identifying possible biological assays. At least 72 claims for cures were identified for which the therapeutic concepts were established for different diseases e.g. asthma, arthritis, malaria, HIV. Samples for at least 32 claims were tested for efficacy in suitable biological assays of which 15 demonstrated positive results and are in further development. This has provided the scientific evidence demonstrating efficacy of these traditional claims, and clearly provides data for the process required for further development into validated herbal treatments or prescription drugs. While 17 claims did not give a positive result in the chosen biological assays, they cannot be ruled out as alternative biological assays need to be sought and the possibility of novel mechanisms of action investigated. This supports the holistic approach to testing of traditional medicines rather than a reductionist one. Currently CSIR has 15 leads in development for therapeutic areas including mosquito repellency, asthma and allergies, arthritis, anti-inflammatory, benign prostatic hyperplasia, malaria, HIV and erectile dysfunction.

Research results on selected leads currently under development are discussed.
**Peltophorum africanum** Sond (Fabaceae) extracts have a potential role in medicine

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**Introduction:** There is an increasing interest in ethnomedicinal and ethnoveterinary practices, especially as it relates to the use of medicinal plants for treating various ailments. About 80% of people in the developing world depend on phytomedicine for primary healthcare. Despite this, however, most medical and veterinary professionals distrust herbal medicines due to lack of scientific evidence of efficacy and safety. Hence, there is need for their validation, before herbal medicines gain wider acceptance and use. Pastoralists and rural farmers use extracts of *Peltophorum africanum* (a medicinal plant widely spread in southern Africa and other tropical regions), to treat diarrhoea, dysentery, pain, infertility, and to promote well-being and resistance to diseases in cattle.

**Methodology:** To evaluate these ethnobotanical leads, dried leaves, bark and root from mature *P. africanum* trees were extracted with acetone. Minimum inhibitory concentrations (MIC) were determined for *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*. Thin layer chromatograms were sprayed with 0.2% 2, 2-diphenyl-1-picryl hydrazyl (DPPH) for qualitative screening for antioxidants. Quantification of antioxidant activity was determined and compared with that of L-ascorbic acid and Trolox (6-hydroxy-2, 5,7,8-tetranethylchromane-2-carboxylic acid). Anthelminthic activity was evaluated *in vitro* by effects of extracts on the egg hatching and larval development of parasitic nematodes *Haemonchus contortus* and *Trichostrongylus colubriformis*. In *vivo* efficacy and safety of acetone extracts at doses up to 750 mg kg\(^{-1}\) was evaluated in lambs artificially infected with *Haemonchus contortus* and *Trichostrongylus colubriformis*.

**Results:** The extracts showed substantial activity against both Gram-positive and Gram-negative bacteria, with Minimum Inhibitory Concentration (MIC) values of 0.08 mg ml\(^{-1}\) for *Staphylococcus aureus* and 0.16 mg ml\(^{-1}\) for *Pseudomonas aeruginosa*. The acetone extracts of the bark, and root of *P. africanum* showed higher antioxidant activity than L-ascorbic acid (Vitamin-C) and Trolox (6-hydroxy-2, 5,7,8-tetramethylchromane-2-carboxylic acid), a synthetic vitamin-E analogue, and much higher than a standardized *Ginkgo biloba* extract (EGb 761). The respective EC\(_{50}\) for the *P. africanum* root and bark extracts, L-ascorbic acid, and EGb761 were 3.82 µg ml\(^{-1}\), 4.37 µg ml\(^{-1}\), 5.04 µg ml\(^{-1}\), and 40.72 µg ml\(^{-1}\). The standardised extract of *Ginkgo biloba* (EGb 761) is widely employed for its significant benefit in neurological disorders. The extracts inhibited egg hatchability and larval development (from L\(_1\) to infective stage L\(_3\)) of both *Haemonchus contortus* and *Trichostrongylus colubriformis* (both parasitic nematodes of ruminants) at concentrations of 0.1-1.0 mg ml\(^{-1}\). The plant extracts, at the concentration of 5-25 mg ml\(^{-1}\) completely lysed larval forms (L\(_1\)) and eggs of the nematodes. In all assays, the root extracts had higher antibacterial, antioxidant and anthelminthic activity than the bark and leaf. Acetone extracts at the doses tested showed no efficacy on faecal egg and adult worm count reductions in sheep. No signs of toxicity were observed in these sheep. Bergenin, a compound that was isolated from the root extract had antibacterial, and antioxidant activities.

**Conclusions:** *P. africanum* extracts have therefore, potential for treatment of infection-related diseases by either directly inhibiting bacterial growth or by stimulating the immune system of the host. Antioxidants may have neuro-protective (preventing apoptosis), as well as neuro-regenerative roles. Due to the high antioxidant activity of its extracts, *P. africanum* has prospects in the management or control of neurodegenerative diseases and human immunodeficiency virus (HIV) infection (AIDS). Thus there is great potential of *P. africanum* extracts in medicine. More work is needed to examine the anthelminthic efficacy of *P. africanum* in animals. Work is ongoing to devise better methods of plant extraction easily adaptable to rural communities for sustainable exploitation of the plant.
Antimalarial Properties of South African Medicinal Plants

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Introduction:
The emergence and spread of drug resistant parasites is a major contributing factor to the escalating prevalence and distribution of malaria, especially in sub-Saharan Africa. The urgent need for novel, affordable antimalarial treatments and the fact that plants have historically proven to be a major source of antimalarial agents led to the establishment of a multidisciplinary consortium to scientifically investigate South African medicinal plants for the treatment of malaria.

Methodology:
Extracts of 134 plant taxa, selected semi-quantitatively using weighted criteria, were tested for in vitro activity against Plasmodium falciparum using the parasite lactate dehydrogenase (pLDH) assay. Active extracts were subjected to bioassay-guided fractionation. Active compounds were characterized by NMR spectroscopy and mass spectrometry. Compounds were evaluated for cytotoxicity against a Chinese Hamster Ovarian (CHO) cell line using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay.

Results:
Several plant species and genera were shown to possess in vitro antiplasmodial activity for the first time. An example, Vernonia staehelinoides is discussed. The active ingredients were found to have limited selectivity (ratio of cytotoxicity vs. antiplasmodial activity) but led to the identification of simplified scaffolds for chemical modification with the view of exploring structure-activity relationships. Thus a rational rather than random approach to the selection of antimalarial screening candidates may provide promising sources of potential antimalarial lead compounds.

References:

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Antispasmodic, Analgesic and Sedative Effects of *Mormodica Involucrata* Methanolic Extract in Rodents

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**Purpose:**
In Swaziland, a very small country in Southern Africa, herbal medicine is very well accepted as alternative medicine because of the socio-cultural affinity of the people. The rich biodiversity of the flora in the Kingdom is exploited very well in traditional practise.

The need to give scientific backing to dosages, side effects of these plants in herbal medicine is being subjected to extensive research. For the purpose of this study *Mormodica Involucrata* has been investigated, for receptor effect, analgesic and sedative properties in Rodents.

**Method:**

Fresh leaves (100g) were macerated in methanol for 24h solvent elimination was carried out under reduced pressure, which yielded a light greenish semi-solid compound (extract). For the receptor studies, the isolated rat stomach strip (RSS) was used. The excised muscle was placed in organ bath containing Tyrode solution. Circular muscle strip contractions was monitored through a force displacement transducer (7010 Ugo Basil) under resting tension 0.5g. Contractile responses were recorded isometrically on a two channel recorder (Gemini 7070). In the analgesic studies, experimented pain was induced into mice (20-30g) intraperitoneally (i.p) using 0.4ml of 1% (v/v) acetic acid for the test, and saline for the control study. Various doses of the extract were used in these studies.

**Results:**
At concentrations of 150 and 300mg/ml, the extract significantly inhibited acetylcholine induced contractions of (RSS) by 15 +1.0% and 25 +1.5% respectively (p<0.05 n=10). The extract action was reversed by mevinphos 10⁻⁹M, an organo phosphate anticholinesterase. The extract at 55-75mg/kg inhibited acetic acid-induced writhing, and sleep in mice, at higher concentration of the extract in the range 350-480mg/kg caused death within 1h of I.p administration in 20% and 100%, respectively (p<0.05 n=10 per treatment). These results show *mormodica involucrata* possesses antispasmodic, analgesic and sedative effects in rodents.
A Toxicological Assessment of *Athrixia Phyllicoides* Aqueous Extract Following Chronic Ingestion in a Rat Model.

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*Athrixia phyllicoides* is an aromatic, indigenous shrub found in the mountainous and grassland areas of the eastern parts of South Africa. Leaves and stalks of this shrub are used to brew “bush tea” which the indigenous communities drink as a beverage. Surveys have shown that the consumption of bush tea is wide-spread and commercialization of the extract holds economic and developmental potential, provided that the safety of the herbal tea is established. McGaw et al (2007) showed that aqueous extracts of *Athrixia phyllicoides* was not toxic in brine shrimp and cell line cytotoxicity assays. However these results need to be verified in a mammalian model. The conjecture presented is that daily ingestion of high doses of an aqueous *Athrixia phyllicoides* extract by Wistar rats, over a 3 month period, will provide biochemical and histopathological evidence of potential toxicity, if any.

Plant material used in this study included leaves and fine twigs of *Athrixia phyllicoides* received from and identified by Prof. Jana Olivier (UNISA). The extract was prepared on a pilot-scale by boiling the plant material (1.4 kg; not milled) with water (20 L) in a steam pot for 10 min. The extract was filtered by decanting through a fine synthetic polymer mesh. This process was repeated 5 times and the extracts pooled. The pooled extract was concentrated using reverse osmosis and freeze-dried. For this study, three-month-old Wistar rats (40 male and 40 female) were individually caged on corn cob bedding with free access to food and water. Animals were randomised into four groups (10 male and 10 females per group) receiving either no dried extract (control group), 30 mg/kg, 90 mg/kg or 180 mg/kg dried extract daily (30 mg/kg = x10 human consumption of 1 cup of “bush tea”). The extract, at the precise individual dose, was given to the rats in raspberry flavoured gelatine cubes. Rats were weighed once a week in order to adjust doses. Food intake, urine and stools (e.g. consistency, colour) were monitored throughout. Twenty-four hour metabolic profiles of the animals were assessed using metabolic cages every 30 days. After three months (90 days) of extract ingestion, blood was collected and the rats were killed. Liver, kidney, spleen, lung, heart, pancreas, ovaries or testis and samples of the entire gastro-intestinal tract were collected and fixed for histological evaluation in 10% phosphate buffered formalin. All tissues were processed into paraffin wax and haematoxylin and eosin stained slides were produced. Histopathological assessments of the tissues were done by an independent veterinary pathologist.

None of the animals in the study showed external signs of ill-effects and no animals died during the course of the study. In comparison with the control group, no significant differences were found in food and water intake or in body mass. Twenty-four hour metabolic profiles showed no significant difference in faecal mass and urine secretion between groups. Mean alkaline phosphatase, creatinine and urea serum levels were within the normal ranges for all the groups. No signs of liver, renal or gastro-intestinal toxicity could be found in any of the groups after chronic ingestion of the extract. Histology of the other tissues sampled (spleen, lung, heart, pancreas, ovaries or testis) showed no specific histological changes within or between groups.

This study showed no evidence of toxicity following daily ingestion of high levels of aqueous *Athrixia phyllicoides* in the Wistar rat.

Session 8: Young Scientists Award Competition

Parallel sessions

SAPS and TOXSA student presentations/posters
Pharmacological Properties of Swazi Medicinal Plants

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Purpose:
Traditional medicine is widely used as a source of primary healthcare for more than 80% of the population of Swaziland. Traditional healers use various plants and combinations thereof to manage different ailments. With the increasing prevalence of antibiotic resistant micro-organisms, novel drugs could be sourced from traditionally used phytomedicines. In consultation with Swazi traditional healers, fifteen Swazi plants were selected and screened for their antimicrobial, anti-oxidant and toxicity profiles.

Methods:
The plants were collected from the Manzini region in Swaziland, air-dried at room temperature and extracted with dichloromethane/methanol (1:1). Ultra Performance Liquid Chromatography was done to determine chemical profiles of the extracts. Antimicrobial activity was determined using the minimum inhibitory concentrations assay against Staphylococcus aureus (ATCC 25923), Staphylococcus epidermidis (ATCC 2223), Escherichia coli (ATCC 25922), Klebsiella pneumoniae (NCTC 9633) and Candida albicans (ATCC 10231). The DPPH (2,2-diphenyl-–picryl-hydrazil) scavenging activity and ferrous metal chelating activity were correlated to the total phenolic content of the plant extracts. Toxicity profiles of the extracts were tested on human kidney epithelial cells using the tetrazolium-based MTT viability assay.

Results:
A number of the plant extracts (51.52%) displayed good antimicrobial activity with MIC values ranging from 0.0013 to 1.00mg/ml. The fruit of Ozoroa sphaerocarpa and the leaves of Dichrostachys cinerea were the most active especially against S. epidermidis. The synergistic interaction observed between Syzygium cordatum and O. sphaerocarpa against E. coli supports the rationale by traditional healers to use these plants in combination. D. cinerea (leaves) was the most active free radical scavenger (IC₅₀: 5.89 ± 0.39µg/ml) as compared to ascorbic acid (IC₅₀: 5.61 ± 1.13µg/ml). For approximately 70% of the plants, there was a correlation between DPPH scavenging and ferrous metal chelating activity. The toxicity profile of the plant extracts indicted that about 40 % of the extracts had IC₅₀ values greater than 100µg/ml.
Evaluation of antinociceptive activities of the aqueous root extract of Alchornea cordifolia in mice

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ABSTRACT
This study presents the result of the antinociceptive profiles of an aqueous root extract of Alchornea cordifolia in mice.

The antinociceptive activity of the oral doses of 100-400mg/kg of Alchornea cordifolia was assessed using the acetic acid-induced mouse writing reflex, formalin-induced paw licking, tail clip and hot plate models of pain.

The hot plate model of pain was used to assess the effect of A. cordifolia on nociceptive response induced by thermal stimuli in mice. The acetic acid-induce mouse writing, formalin-induced paw licking and tail clip tests were further utilized to screen the analgesic property of the extract.

The analgesic tests showed that A. cordifolia possess both central and peripheral analgesic activity, as shown by its ability to significantly (P<0.05) inhibit nociceptive response associated with both phases.

The ability of the oral of the extract (100-400mg/kg) to prolonged the reaction time of the animals to noxious heat in hot plate and tail clip models of pain in a dose dependent manner suggest a central analgesic effect. However, the formalin test result showed that the extract (100-400mg/kg, p.o) increased pain threshold in a dose dependent manner in both phases but the effect is more prominent against the inflammatory phase (second phase).

In the mouse writing reflex test, the extract (100-400mg/kg, p.o) significantly inhibited writhes in mice in a dose dependent manner.

Acute toxicity studies of oral doses of aqueous root extract of Alchornea cordifolia in mice revealed that it has a wide margin of safety as it well tolerated by the animals.

The results of the study suggest an analgesic property demonstrated by the aqueous root extract of Alchornea cordifolia both centrally and peripherally.

Keyword: Alchornea cordifolia, antinociceptive, acute toxicity.
The Women’s Health Initiative Study: Impact on the Prescribing of Hormone Replacement Therapy in a Defined South African Population

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Background:

The Women’s Health Initiative (WHI) study is the largest, prospective longitudinal study to date investigating the effect of hormone replacement therapy (HRT) in healthy postmenopausal women. The controversy surrounding the results of the study, however, has led to much uncertainty regarding the prescribing of HRT.

Aim:

The primary aim of the study was to investigate the impact of the WHI study on the prescribing of HRT in a defined South African population and to establish whether patient therapy was appropriately individualised as a result of the WHI study.

Methods:

A retrospective drug utilisation review (DUR) using claims data from a South African medical scheme was conducted to identify HRT-related prescribing patterns in the defined study populations. This included an investigation into patient characteristics, prescribing patterns and disease state prevalence in the sub-groups of patients either initiating or discontinuing HRT in the six-month period post-WHI publication.

Results:

Patients initiating HRT post-WHI publication were generally found to be in the younger menopausal age categories (40 to 49 years). These patients were more likely to have been initiated on HRT types other than those investigated in the WHI study and were at a higher risk for disease states for which HRT use is beneficial. Patients discontinuing HRT post-WHI publication were generally found to be in the older menopausal age categories (60 to 69 years), were more likely to have been combined HRT users (although not necessarily the type investigated in the WHI study) and were at a higher risk for disease states for which HRT use is considered harmful.

Conclusion:

It can be concluded that HRT was appropriately individualised according to recommendations based on the results of the WHI study in the defined populations of this study.
Investigating the antioxidant and iron-binding properties of non-nucleoside reverse transcriptase inhibitors, efavirenz and nevirapine in HIV-induced neurodegeneration.

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Purpose:
Oxidative stress-induced neuronal death implicated in the pathogenesis of AIDS dementia complex (ADC) is due to the release of damaging inflammatory-related agents by HIV-infected microglia in the brain. Such agents include oxidative free radicals and quinolinic acid (QA), which is consistently elevated in the brains of ADC patients. The ability of brain tissue to readily accumulate reactive free iron in neurodegenerative disorders including ADC makes it vulnerable to oxidative damage. Since non-nucleoside reverse transcriptase inhibitors (NNRTIs), efavirenz (EFV) and nevirapine (NVP) have been shown to reduce the frequency and neurological deficits associated with ADC, we investigated the possible mechanisms through which these agents offer such neuroprotection.

Methods:
In this study, the antioxidant activity of EFV and NVP was evaluated by various \textit{in vitro} antioxidant assays, including 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging, superoxide anion radical scavenging, Fe\textsuperscript{2+}-induced lipid peroxidation and Fe\textsuperscript{2+}/Fe\textsuperscript{3+} metal chelating activity, using the ferrozine and electrochemical detection method. Furthermore, the thiobarbituric acid (TBA) and nitroblue tetrazolium (NBT) assays were used to measure the extent of QA-induced lipid peroxidation and superoxide anion generation, in rat hippocampal tissue following treatment with NVP or EFV.

Results:
NVP and EFV exhibit effective DPPH and superoxide radical scavenging and Fe\textsuperscript{2+}/Fe\textsuperscript{3+} binding abilities, hence preventing or delaying oxidative damage of biomolecules through scavenging radicals and binding to the metal ions. In addition, these NNRTIs reduce Fe\textsuperscript{2+} and QA-induced lipid peroxidation and superoxide anion generation \textit{in vivo}. The free radical scavenging and antioxidant properties of these agents may be a mechanism through which these agents offer neuroprotection in ADC. These findings strengthen the argument that these NNRTIs not only have antiviral effects but possess potential neuroprotective properties, which could be exploited as a possible therapeutic approach against HIV-induced neurodegeneration disorders.
Purpose:
Schizophrenia affects approximately 1% of the population. Despite marked improvement in drug treatment, 20% of patients remain treatment resistant while motor side effects are an ongoing problem that hamper compliance and outcome. Animal models enable detailed study of the neurobiology and treatment of a psychiatric illness. Deficits in sensory motor gating, such as altered prepulse inhibition (PPI) of startle, is typical of schizophrenia. We studied PPI following acute challenge with the glutamate NMDA antagonist, dizocilpine (MK-801), and its reversal by atypical and typical neuroleptics. Thereafter we applied this approach to PPI and cortical NMDA receptor binding studies in a neurodevelopmental model of schizophrenia.

Methods:
**PPI testing:** PPI was determined using a San Diego SR-lab startle response system. 120 dB pulses were presented with or without prepulses (76 dB, 80 dB, 84 dB), using a 67dB background noise.

**MK801 model:** Animals received either saline (ip) pre-treatment 30min prior to PPI testing, followed by either a second saline injection (control), or MK-801 (0.25mg/kg ip) 15min prior to PPI testing. Further groups were pre-treated with increasing dosages of either clozapine (5 and 10mg/kg ip) or haloperidol (0.1; 0.2 and 0.5 mg/kg ip) 30min before PPI, followed 15min later by either saline or 0.25 mg/kg MK-801.

**Social isolation model:** Animals were group- or isolation-housed for 8 weeks and assessed for deficits in PPI. Isolation groups were injected with either clozapine or haloperidol in the last 11 days of isolation rearing. In separate groups, social isolation animals were again group-housed in the last 4 weeks and tested for PPI changes.

**Catalepsy:** Catalepsy was routinely assessed using a rat catalepsy box, repeated at 30 min intervals for 240 min after neuroleptic administration.

Results:
Mk-801 evoked significant deficits in PPI that was reversed by 5mg/kg clozapine but not by dosages of haloperidol that did not simultaneously induce catalepsy (0.1; 0.2 mg/kg). Significant deficits in PPI, as well as increased frontal cortex NMDA receptor density, was induced after 8 weeks of social isolation that could not be reversed by re-socialization. Chronic clozapine but not haloperidol treatment blocked PPI deficits following social isolation, although neither altered NMDA receptor density.

Conclusion:
PPI deficits are mediated by altered NMDA receptor density and activity. Social isolation rearing represents a robust animal model with significant face and predictive validity for schizophrenia.
The effects of ozone exposure on markers of cellular resilience in cultured human epithelial HeLa cells

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Purpose:
Ozone is a natural occurring gas with strong oxidizing properties. While its biological effects are associated with toxicity, it has also been used for therapeutic purposes. In biological systems ozone can elicit dose-dependent oxidative stress, which may induce adaptation. The mechanisms for these effects remain illusive. Previous studies in our laboratory indicated that repetitive exposure of cultured human epithelial cells to ozone may induce cytoprotective mechanisms (adaptation), which may include the up-regulation of anti-apoptotic pathways. The aim of the current study was to determine the effects of single and repetitive exposure of human cell lines to ozone on the expression of genes that encode for anti apoptotic (AKT, Bcl2, CREB, NFKB and BDNF) and pro apoptotic (Bax, Caspase 3 and 8) proteins, as well as the corresponding protein expression levels.

Methods:
Cultured human epithelial HeLa cells were exposed to control or ozone-saturated glucose-free Krebs-Henseleit solution. Exposures consisted of 4 x 5-minute exposures every four hours, followed by a 16 hours incubation in normal culture medium and then a 25-minute exposure. Cells were then lysed immediately (0 h) or after 4h or 8h incubation in normal culture medium. The relative expression of genes encoding for pro-apoptotic factors Bax, caspase-3 and caspase 8, and anti-apoptotic factors BDNF, NF-κβ, Akt, CREB and Bcl-2 was then determined with quantitative real-time RT-PCR. In follow-up studies, currently being conducted, the corresponding expression of these proteins will be quantified with Western blot analyses.

Results:
When measuring gene expression immediately after treatment (0h), genes encoding for Bcl-2, CREB, BDNF and caspase-3 were down-regulated by ozone administered 4 x 5 minutes every 4 hours, but these returned to pre-treatment values after 8 hours. However, 8 hours after the treatments, cells treated with ozone for 4 x 5 minutes every 4 hours plus an acute 25-minute exposure 16 hours later, showed an up-regulation of the genes encoding for the anti-apoptotic factor Akt, and a trend towards up-regulation of CREB, while a single 25-minute exposure of the cells to ozone up-regulated the expression of caspase-3 after 8 hours. It needs to be established how these results relate to the up- or down-regulation of pro-apoptotic and anti-apoptotic proteins, as measured by Western blot analyses. The current data therefore suggest an anti-apoptotic mechanism for adaptive effects seen in cultured human epithelial HeLa cells after repetitive exposure to ozone.
Utilization of the Roche Amplichip For Pre-Prescription Genotyping.

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Purpose: CYP2D6 and CYP2C19 are highly polymorphic P450 isoenzymes, responsible for the metabolism of up to 30 percent of commonly prescribed medications.¹ The aim of this study was to investigate the inter-ethnic variations of enzymatic activity and allelic frequency within a demographically representative South African sample group.

Methods: Venous blood samples were collected from 100 patients, recruited from the clinics of Pretoria Academic, Kalafong and Mamelodi hospitals. The Roche Amplichip platform was then used to genotype patients for polymorphisms in CYP2D6 and CYP2C19. Predicted phenotype distribution, haplotype prevalence and allelic frequency was then determined for each respective ethnic group.

Results: CYP2D6- Both Indians and Caucasians showed total distribution for extensive enzymatic expression (100%) vs Coloureds (66%) and Blacks (56%). Intermediate enzymatic expression was highest amongst Blacks (36%) vs. Coloureds (22%), whereas poor enzymatic expression was highest amongst Coloureds (11%) vs. Blacks (5%). CYP2C19- Extensive enzymatic expression was highest amongst the Black population (96%) vs. 90%, 86% and 70% for Coloureds, Caucasians and Indians respectively. All the ethnic groups contained poor metabolizers, with Blacks containing the lowest distribution percentage (4%), and Indians the highest (30%).

The varying expression of these highly polymorphic genes reflects large inter ethnic variations. These variations will have profound clinical significance in the response to CYP2D6 and CYP2C19 substrate drugs, particularly for those with a narrow therapeutic index.
The anti-inflammatory properties of brown coal derived potassium humate in a graft-versus-host reaction.

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It has been documented that potassium humate inhibits contact hypersensitivity in the rat model. In this study the effect of brown coal derived potassium humate (from the Letrobe Valley, Australia) on a graft-versus-host reaction model was investigated. In this model, Sprague-Dawley rats served as lymphocyte donors and BD IX rats, sensitised to the lymphocytes of Sprague-Dawley rats, served as the recipients. Potassium humate was administered daily at a dose of 60 mg/kg bodyweight. Viable lymphocytes, isolated from the spleens of the Sprague-Dawley rats were injected into the right hind paw of the BD IX rats, some of which were pre-treated with cyclophosphamide to induce immunosuppression. The cells were hosted in the right popliteal lymph node. An increase in the size of the popliteal lymph node was calculated by the percentage increase of the right vs. the left popliteal lymph node. Potassium humate significantly inhibited both the rejection reaction of rats that had an intact immune system as well as the graft-versus-host reaction.

To determine whether the above reaction could be repeated in vitro, the effects of potassium humate on lymphocyte proliferation and mixed lymphocyte culture were also investigated. Potassium humate increased lymphocyte proliferation of resting-, phytohaemagglutinin A (PHA)- and pokeweed mitogen (PWM)-stimulated lymphocytes in vitro from concentrations of 20 to 80 µg/ml, in a dose dependant manner. On the other hand potassium humate, at 40 µg/ml, significantly inhibited the supernatant concentrations of the following cytokines i.e. TNF-α, IL-1β, IL-6 and IL-10, which were released by PHA stimulated lymphocytes. Potassium humate had no effect on a mixed human lymphocyte culture.

These results indicated that potassium humate inhibited both the rejection as well as the graft-versus-host reaction in a rat model possibly due to the inhibition of pro-inflammatory cytokines responsible for the initiation of these reactions. The increased lymphocyte proliferation observed, might be due to increased IL-2 production as previously been documented. Interestingly potassium humate decreased the weight loss experienced by cyclophosphamide treated rats.
The modulating effects of selected antidepressants and related drugs on markers of cellular resilience in cultured human neuroblastoma cells

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Purpose:
The neurodegenerative hypothesis of depression postulates that major depression is associated with impaired neuroplasticity in particularly the hippocampus and prefrontal cortex, and that antidepressants may be neuroprotective. Therefore the primary aim of the current study was to determine the effect of selected antidepressant drugs on the survival (mitochondrial activity) of cultured human neuroblastoma cells, before and after glutamate-induced excitotoxicity, as well as to determine the effects of these drugs on pro- and anti-apoptotic pathways.

Methods:
The free fraction of plasma protein-bound drugs were determined in Ham’s F12 medium ± 10% foetal bovine serum (FBS). Samples containing 10% FBS along with a study drug was centrifuged in microcentrifuge tubes with 30 kDa pore size filters to remove plasma albumin. The free and total drug concentrations were determined by means of HPLC analysis.

Cultured human neuroblastoma SH-SY5Y cells were pre-treated for 24 hours in 24-well plates with and without 10 mM glutamate plus a pharmacological (low) and high (≥10x low) concentration of fluoxetine, mirtazapine, tianeptine, imipramine, myo-inositol, gabapentin and lithium. Thereafter mitochondrial activity was determined with the standard MTT cell viability assay.

In follow-up studies, currently being conducted, the effects of the antidepressants are being determined on genes encoding for the pro-apoptotic factors Bax, caspase-3 and caspase-8, and anti-apoptotic factors BDNF, NF-κβ, Akt, CREB and Bcl-2, utilising quantitative real-time RT-PCR. This will be followed by Western blot analyses of the corresponding proteins.

Results:
Results from HPLC analyses indicate that drugs are moderately bound to plasma albumin in culture medium and it is possible to calculate the free drug concentrations.

A 24-hour pre-treatment with pharmacological (low) concentrations of lithium, myo-inositol, imipramine and gabapentin, as well as the high gabapentin pre-treatment concentration, protected against glutamate induced excitotoxicity, as measured with the MTT assay. However, no significant increase in mitochondrial activity was observed with fluoxetine, mirtazapine and tianeptine.

Quantitative real-time RT-PCR and Western blot analysis are being conducted to establish how these results relate to the activation and/or inhibition of pro-apoptotic and anti-apoptotic pathways. The current data supports the hypothesis that antidepressants may be neuroprotective and that this may contribute to the antidepressant action of these drugs.
The anti-allergic properties of potassium humate

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Background:
Although the anti-inflammatory properties of humate derived from peat, sapropeles and mumie have been described, no clinical studies has been done on the anti-inflammatory effects of humate derived from coal. Leonardite humate compared favourably with prednisolone in suppressing contact hypersensitivity in a rat model.

According to a report by the European Agency for the Evaluation of Medicinal Products on toxicity studies (Feb 1999), humic acids extracted from brown coal has no toxic effects on rats in a chronic study at oral dosages as high as 1g/kg BW, whereas the LD50 in rats, after oral administration of humic acids, has been reported to be greater than 11g/kg BW. This report has recently been confirmed by a separate study.

The objective of this study was to establish the safety and therapeutic efficacy of oral potassium humate in reducing the signs and symptoms of hay fever in atopic patients during the grass pollen season.

Method:
In this parallel double-blind placebo controlled phase II study potassium humate was randomly assigned, at a dosage of 1.8g in divided doses/day, to atopic patients (n = 40) presenting with acute symptoms of hay fever. The blood and nasal samples were used to determine the safety and the effects of potassium humate on basophil activation, cytokine levels and eosinophil migration. A skin prick test was used to determine its anti-allergic effects. An in vitro neutrophil adhesion test was used to determine the effects of the product on the adhesion of human neutrophils to ICAM-1expressing baby hamster kidney cells.

Results:
A significant decrease in the skin prick test results (presented elsewhere) and eosinophil counts was observed. No significant differences were observed with regard to neutrophil adhesion nor were there any differences observed with regard to the stimulation of basophils. However decreases were observed in the expression of IL-4, IL-5, IL-8 and IL-1β after treatment, although not reaching statistical significance. The product had no effect on neutrophil adhesion to ICAM-1.

Conclusion:
This study confirmed, without doubt, that this product possesses anti-inflammatory as well as anti-allergic properties possibly due to a decreased recruitment of eosinopils to the site of inflammation.
An Investigation into the Surface Activity of Airborne Particles in the Gold Mining Environment

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Purpose:
Occupational exposure to crystalline silica dust contributes to silicosis, a disabling, irreversible, and fatal lung disease. Crystalline silica has been shown to be more toxic when compared to amorphous silica. The trend in past epidemiological studies has been the establishment of a relationship between total respirable dust and occurrence of a biological reaction without taking into account the surface activity of the particles. With similar exposure to crystalline silica, the risk of silicosis has been shown to be different indicating that there are other factors in addition to its concentration that may determine its toxicity. The aim of this study was therefore to investigate the surface properties of dust collected from various mines and to compare these properties to their in vitro toxicities.

Methods:
Mine dust samples were collected from different mines. Representative bulk dust samples were collected from extraction fans and from occupational areas by sweeping in and around the area. Respirable dust samples were collected on PVC filters inserted in the miner’s personal sampler. The bulk samples were separated into different fractions. The physicochemical properties and the biological activities of the bulk sample and its separated fractions were analysed using X-Ray Diffraction, Scanning Electron Microscopy, Scanning Electron Microscopy-Energy Dispersive X-ray Spectroscopy, X-Ray Photoelectron Spectroscopy, Inductively Coupled Plasma Mass Spectrophotometer, Chemiluminescence, Brunauer-Emmett-Teller, Fluorescence Spectrophotometry, Electron Spin Resonance Spectrometry, Comet Assay and Mossbauer Spectroscopy.

Results:
From the results obtained, it was found that different mine dusts had different levels of α-quartz. The α-quartz content of the bulk sample and its <20µm fraction were also different. Iron and aluminium were high in all the bulk samples. Silicon was found to be low. Different samples possessed various surface areas in both the bulk and its <20µm fraction. The results indicate that mine dusts from various mines have the ability to generate various levels of free radicals. Mine dusts were found to have the greatest ability to elicit oxidative burst. Equal mass of dust samples could peroxidise membrane lipids at different levels.

Conclusion:
The results obtained indicate that physicochemical properties of a mineral particle determine its toxicity. It is therefore very important to take into consideration the surface activity of a mineral particle when determining dose-response relationships.
Validation of biomarkers for improved assessment of exposure and early effect from exposure to crystalline silica

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Background:
This is Phase III of a project to identify, confirm, and operationalise biomarkers for crystalline silica dust exposure that could be used as early indicators for silicosis in environmental surveillance. In this phase, we aim to operationalise the use of two biomarkers, namely erythrocyte Glutathione Peroxidase and Clara Cell Protein 16, for crystalline silica exposure for use in South African gold mines. In order to ensure optimum reproducibility and precision, Standard Operating Procedures (SOPs) need to be developed for the biomarker specimen handling and storage under field conditions and also for optimum laboratory assay settings.

Methods:
Venous blood was collected from four volunteers into three 5ml Vacutainer tubes (two of which contained an anti-coagulant). Separation of the blood samples into fractions of serum (for CC16 assay) and erythrocyte lysate (for GPx assay) was performed using centrifugation.

Effect of storage temperature: The separated fractions were stored at 4°C, -20°C and -80°C.

Effect of delay in sample processing: The collected blood samples were left to stand for 0, 1 and 2 hours prior to separation.

Effect of ambient laboratory temperature: During the separation process, blood samples were kept at 25°C and 35°C.

Following two weeks of storage in a -80°C freezer, the levels of CC16 and GPx were assayed.

Assay of CC16: The Human Clara Cell Protein ELISA Kit (BioVendor, Czech Republic) manufacturers’ instructions were followed.

Assay of GPx: The Glutathione Peroxidase Assay Kit (Cayman Chemical, USA) manufacturers’ instructions were followed.

Results:
Results of the optimization experiments demonstrated that the blood samples should be separated within two hours of venesection. Sample preparation should then be performed at a temperature of 25°C, following which the samples should be stored at -80°C until assay. Standard operating procedures (SOP) for handling, storage and analysis of samples were developed using the results.

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Wild garlic (*Tulbaghia violacea*): a heavy metal accumulator?

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**Purpose:**
Wild garlic (*Tulbaghia violacea*), is a popular medicinal plant with horticultural potential. Cadmium (Cd) content in the soil has drastically increased and is one of the most important metals to consider in terms of food-chain contamination. The aim of this study was to observe the effect of Cd on plant growth and to determine the distribution of the metal in the leaves, bulbs and roots.

**Methods:**
Cadmium concentrations (2 and 5 mg kg⁻¹) were applied to 1-, 2- and 3-year old plants for 6 weeks. Elemental concentration (mg kg⁻¹) was determined using ICP-OES.

**Results:**
Although Cd had little effect on plant growth, it was readily uptaken and translocated. One year old plants translocated more Cd to the leaves than 2- and 3-year old plants, while the older plants stored more Cd in the bulbs than the younger plants. The Cd content in bulbs exceeded the WHO limits of 0.3 mg kg⁻¹ Cd. As the leaves are used as a vegetable, the antagonistic effect of Cd on micronutrient uptake was also evaluated.
Intracellular Calcium Levels in Relation to Lipid peroxidation in U937 phagocytic cells Exposed to Crystalline Silica

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**Purpose:** Intracellular calcium performs a number of second messenger functions in mammalian cells. Changes in intracellular calcium concentrations \([\text{Ca}^{2+}]_{i}\) in the cells may well play a pivotal role in the cell’s reaction to xenobiotics. Intracellular calcium has been implicated in activation of macrophages which play a crucial role in phagocytosis of particles. The purpose of this study was to investigate the role of intracellular calcium in cellular responses triggered by silica and in relation to lipid peroxidation of the cell membranes using the human macrophage-like cell line U937.

**Methods:**

*Cell Cultures and Treatment:* The U937 cells were cultured in the presence of 5nM PMA for 3 days in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum at 37°C in a humidified atmosphere of 5% CO\(_2\).

*Determination of Intracellular Calcium:* The differentiated U937 cells were exposed to 25, 50, 75 and 100 µg/ml of silica for 2, 7 and 24 hours. \([\text{Ca}^{2+}]_{i}\) was measured in these cells using the fluorescent calcium indicator Fura-2 AM. The fluorescence intensities were determined using Cary Eclipse Fluorescence Spectrophotometer at excitation wavelength 340nm and emission wavelength 510 nm.

*Lipid Peroxidation:* Lipid hydroperoxides were measured in differentiated U937 cells exposed to various silica concentrations mentioned above for one hour using Diphenyl-1-pyrenylphosphine (DPPP). The fluorescence intensities were determined by Cary Eclipse Fluorescence Spectrophotometer at an excitation and emission wavelengths of 351nm and 380nm respectively.

**Results:** The \([\text{Ca}^{2+}]_{i}\) showed a dose dependent increase after 2, and 7 hour incubation with silica, with maximum concentrations of calcium at 75 µg/ml silica. There was a dose dependent decrease of \([\text{Ca}^{2+}]_{i}\) after 24 hours of exposure to silica with the highest concentration at 25 µg/ml silica. The dose dependent increase of cytosolic calcium corresponded with the increasing amounts of lipid hydroperoxides in the cells. These results indicate that the peroxidation of membrane lipids may lead to disruption of the membrane integrity leading to an increase in \([\text{Ca}^{2+}]_{i}\).
Comet assay parameters for use in assessment of genotoxic effects

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Background:
Among the various techniques for evaluating genotoxic risks, the Comet assay is increasingly regarded as one of the more valuable approaches. Applicable to either \textit{in vitro} or \textit{in vivo} methodologies, this assay is rapid, sensitive and non-invasive and measures DNA damage at the levels of individual cells. Currently there is no general agreement with respect to which ‘Comet’ parameters to use. A number of publications recommend the use of the ‘Comet’ tail length, relative fluorescence intensity of head and tail (normally expressed as a percentage of DNA in tail), and olive tail moment (the product of the amount of DNA in the tail and the mean distance of migration in the tail). This study was undertaken to examine the correlations among various ‘Comet’ measurements provided by the Komet 5 image-analysis system with different chemical treatments.

Methods:

\textit{Blood Sampling and Cell Preparation:} Blood was collected in BD Vacutainer CPT and mononuclear cells isolated as recommended by the manufacturer. Cell viability was checked by the trypan blue exclusion method and cell concentrations adjusted to 1x10\textsuperscript{6} cells/ml.

\textit{Treatment of cells:} The experiment was performed on control and cells treated with different concentrations of hydrogen peroxide, chlorpyrifos and endosulfan followed by washing in phosphate buffered saline and eventually cell viability determination using the trypan blue exclusion method.

\textit{Comet Assay:} The alkaline version of the Comet assay was used in this study.

Results:
Scoring of DNA damage was done using the Comet assay image analysis software Komet 5. Our results suggest that olive tail moment and \%Tail DNA give good correlations with the dose of genotoxic agents used.
Evaluation of intracellular GSH in silica-induced apoptosis

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Purpose:
Exposure to crystalline silica results in silicosis for which there is no effective clinical treatment. It is shown that inflammation precedes the fibrotic changes of the lung. It is also shown that oxidative stress and apoptosis of inflammatory cells play an important role in the exacerbation of inflammation. The present study has therefore investigated the effect of silica exposure on apoptosis of inflammatory cells and on the production of oxidative stress in these cells assessed as the concentration of intracellular glutathione (GSH).

Methods:

\textit{Culturing of U937 cells:} The cell line U937 was cultured in RPMI-1640 medium containing 10\% heat-inactivated FBS, 100 units/ml penicillin and 100\mu g/ml streptomycin and incubated at 37°\textdegree C and 5\% CO\textsubscript{2} atmosphere. Experiments were performed on 5X10\textsuperscript{5} cells/ml stimulated with 50ng/ml PMA for 3 days to differentiate into macrophages.

\textit{Induction of apoptosis:} PMA-stimulated U937 macrophages were exposed to 10, 25 and 50\mu g/ml silica for 2, 4 and 24 hours. After treatment cells were harvested and assessed flow cytometrically for apoptosis using Annexin V.

\textit{Determination of intracellular GSH by flow cytometry:} Following treatment of U937 cells (5X10\textsuperscript{5} cells/ml) with silica for 30 minutes, 4 and 24 hours; cells were harvested and washed with PBS. Cells were then stained with 500\mu l of ice-cold 40\mu M mercury orange for 5 minutes on ice, washed once with cold PBS and resuspended again in 500\mu l PBS. Analysis was done using a flow cytometer.

Results:
Silica-induced apoptosis resulted in a significant decrease in intracellular GSH. GSH depletion was greater under apoptotic conditions at 24 hours. In contrast, non-apoptotic control cells retained intracellular GSH. The results showed a close correlation between the onset of apoptosis and GSH depletion in U937 cells. These findings provide strong evidence that silica-induced apoptosis results in GSH depletion which might be responsible for the activation of specific apoptotic signalling.
The anti-inflammatory properties of Salacia leptoclada and Warburgia salutaris. Their possible use as therapeutic agents in silica-induced cellular injury.

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The plants Salacia leptoclada and Warburgia salutaris possess antioxidant properties and are commonly used in Southern Africa for the treatment of numerous diseases including inflammatory diseases. In order to determine their therapeutic use in silica-induced injury, the extracts of S. leptoclada and W. salutaris were investigated on: (i) production of proinflammatory cytokines TNF-\(\alpha\), IL-1\(\beta\), INF-\(\gamma\), (ii) the activation of the transcription factor NF-\(\kappa\)B; and (iii) the induction of DNA damage and lipid peroxidation in the presence of crystalline silica particles. Through its antioxidant property, W. salutaris exhibited a protective effect against silica-induced inflammatory cytokine expression, activation of nuclear transcription factor-\(\kappa\)B and DNA strand breakage. W. salutaris also inhibited lipid peroxidation induced by silica. Similarly, the extracts of S. leptoclada showed protection of cells against silica-induced membrane peroxidation. However, S. leptoclada proved ineffective protecting against silica-induced DNA damage, proinflammatory cytokine expression and NF-\(\kappa\)B activation. Since silica-induced DNA damage, NF-\(\kappa\)B activation, inflammation and lipid peroxidation are involved in the process of silica-induced fibrogenicity and carcinogenicity, W. salutaris may be a potential therapeutic agent against silica-induced cellular injury.
Ethnoveterinary plant extracts: relationships between antibacterial activity and cytotoxicity

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Purpose:
Rural farmers and livestock-owners are familiar with the use of many plants to treat their animals for various ailments, particularly wounds and infectious diseases. Such plants may possess anti-infective properties, but also need to be evaluated for toxic effects, including cytotoxicity.

Methods:
The antibacterial effects and cytotoxicity of the acetone extracts of sixteen plants used widely in South African ethnoveterinary medicine (EVM) were tested against two Gram-positive (Enterococcus faecalis and Staphylococcus aureus) and two Gram-negative (Escherichia coli and Pseudomonas aeruginosa) bacterial strains, and the Vero monkey kidney cell line respectively. A serial microdilution assay was employed for antibacterial activity testing, and a tetrazolium-based colourimetric reaction was used to indicate cell viability, or cytotoxicity, after exposure to the plant extracts.

Results:
Antibacterial screening: Minimum inhibitory concentrations (MIC) reached as low as 0.039 mg/ml in the case of the Rhus lancea extract against S. aureus. Four of the 16 extracts showed no antibacterial effects against any of the test organisms. Regarding the Gram-negative species, 18.75% and 37.5% of extracts were active against E. coli and P. aeruginosa respectively. In contrast, 62.5% of extracts displayed inhibitory effects against the Gram-positive species, although not all the same extracts were active against both E. faecalis and S. aureus.

Cytotoxicity: In comparison to the untreated controls, 4 extracts (Dombeya rotundifolia, Hippobromus pauciflorus, Rhus lancea and Sclerocarya birrea) were not cytotoxic at the highest concentration tested, 1 mg/ml. One extract, Combretum caffrum, exhibited an LC_{50} value below 0.05 mg/ml.

Selective antibacterial activity was calculated taking into account cytotoxicity. By dividing LC_{50} by MIC values, Rhus lancea revealed the highest selectivity index (SI) value against S. aureus (SI = 25.64). Combretum caffrum and Cussonia spicata produced the lowest SI values, indicating concurrent cytotoxicity and antibacterial activity. Hippobromus pauciflorus, Rhus lancea, Sclerocarya birrea and Schotia brachypetala showed the best overall SI values, suggesting good antibacterial activity with relatively low cytotoxicity.

Conclusion:
This study highlights the value of testing EVM and other medicinal plant extracts for cytotoxicity together with antibacterial activity in studies aimed at identifying active extracts possessing specific bioactivity without general cytotoxic effects. We are targeting extracts of plants with low cytotoxicity and high antibacterial activity for isolation of active compounds, and development of active standardized extracts. Fractionation of plant extracts with cytotoxic effects could, however, reveal that the compounds responsible for antibacterial activity may not be the same as those causing the cytotoxic effect.
Mechanisms of the toxic effect of *Harungana madagascariensis* stem bark extracts in-vivo and in-vitro.

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**Purpose:** The leaves and stem bark of *Harungana madagascariensis* (Choisy) Poir. of the family Clusiaceae is a plant used in traditional medicine for the treatment of a variety of diseases. *H. madagascariensis* is a component of Jubi Formula, a herbal preparation which was found to restore the PCV and haemoglobin concentration in anaemic conditions and is a potential substitute for blood transfusion (Erah et al 2003). EMEA (1999) reported that 1: 1000 parts of the plant parts are to be used as tincture in veterinary homeopathy. However, data on toxicity of the plant extract is limited. The mechanisms of the toxic effect of *H. madagascariensis* stem bark extract was investigated on the induction of anti-oxidative enzymes markers like: reduced glutathione (GSH), lipid peroxidation (MDA) and nitric oxide (NO) in mice (in-vivo) and NO in RBC (in-vitro) as a measure to throw more lights into the possible toxicity and mechanisms involved.

**Methods:** Preparation of extracts: The ethanolic extract was obtained by soaking 51g of powdered stem bark in aqueous ethanol (1:1). The filtrate after 24 h was evaporated *in vacuo* on a rotary evaporator to dryness. Additionally extractions in six other solvents: hexane (H), dichloromethane (D), chloroform (C), ethyl acetate (E), acetone (A), and methanol (M) were also prepared according to Eloff (1998).

Acute and Sub-acute treatment of Animals: Extracts were given in-vivo to groups of mice to determine the LD₅₀ and effect for 7 days administration.

Biochemical Assays: Determination of reduced glutathione (GSH) was done according to the method of Ellman (1959). Lipid peroxidation - concentration of malondialdehyde (MDA) was measured by the methods of Ohkawa et al (1979), Nitric oxide (NO) was assayed by to Griess method (Green et al, 1982). Protein concentration was determined by the method of Lowry et al., (1951).

Cytotoxicity assay: The cytotoxicity of the six different extracts of *H. madagascariensis* stem bark were monitored by haemagglutination activity, using formaldehyde fixed equine erythrocytes as described by Sadique et al. (1989)

**Results:** The results obtained showed that the extract gave low LD₅₀ values of 300 and 140mg/ kg when administered i.p. and s.c. respectively This suggests that the plant extract is relatively toxic to mice at these doses and routes. There was a significant elevation of the reduced GSH, MDA and NO both in rats and the released of nitric oxide alone in RBC, an indication that there was a cell injury. These biomarkers enzymes depending on the concentrations produced either antioxidant effect or oxidative stress. The results shows that at high concentrations, both the non-polar and polar fractions of the extract seems to be more delirious on the RBC which could be a pointer to its action in producing oxidative stress in-vivo, however, the reverse is the case for low concentrations.

**References:**
Eloff J.N. 1998 Which extractant should be used for the screening and isolation of antimicrobial components from plants?. Journal of Ethnopharmacology 60, 1–8.
Antimicrobial activity of Venda medicinal plants used in respiratory tract infections

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Respiratory tract infections, such as tuberculosis, pneumonia and bronchitis, have become increasingly difficult to treat. The HIV pandemic has resulted in large population of immune-compromised patients susceptible to tuberculosis, while ineffective and unnecessary use of existing antibiotics has lead to widespread resistance to these agents in bacterial populations. The need for new antimicrobial agents with novel mechanisms of action is needed and the search for these compounds has increasing turned to plants associated with ethnomedical uses.

Aqueous and methanolic extracts of ten Venda plants used in the treatment of respiratory tract infections were assessed for their antimicrobial activity against *Candida albicans* (ATCC 10231), *Haemophilis influenzae* (ATCC 49247), *Klebsiella pneumoniae* (ATCC 13883), *Mycobacterium smegmatis* (ATCC 14468), *Staphylococcus aureus* (ATCC 12600) and *Streptococcus pneumoniae* (ATCC 49619). Inhibition of the growth of the micro-organisms was determined by the disk diffusion and microbroth dilution assays. Extracts with activity were tested against clinical isolates. Phytochemical screening, to detect specific compounds in the plant extract, was performed on thin layer chromatography (TLC), using a variety of mobile phases and stray reagents as well as UV light.

*Syzygium cordatum* Hochst. showed antimicrobial activity against Gram-positive and Gram-negative micro-organisms, as well as *Candida albicans*. The aqueous extract of *Syzygium cordatum* had a minimum inhibitory concentration (MIC) of less than 250 µg/mL for *S. aureus* (ATCC 12600). Phytochemical screening of the *Syzygium cordatum* revealed the presence of anthraquinones, alkaloids, coumarins, lipids, phenols steroids and was also shown to contain anti-oxidant activity. These findings justify the ethnomedical use of *Syzygium cordatum* in the treatment of respiratory tract infections.
Phytochemical analysis and antimicrobial activity of *Piper capense*

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Medicinal plants have become the focus of intense study recently as to whether their traditional uses are supported by actual pharmacological effects, or merely based on folklore. *Piper capense* L.f. is a plant renowned for its use in the treatment of infectious diseases. It therefore holds the promise as a source of a novel antimicrobial compound/s. In this study we determined the antimicrobial activity of *P. capense* against standard microbial strains of *Staphylococcus aureus* (ATCC 12600) and *Candida albicans* (ATCC10231) using the disc diffusion and micro-broth dilution assays. Methanol, hexane and acetone extracts were found to be potent inhibitors of both *S. aureus* and *C. albicans* with MIC values < 1 mg/ml.

Phytochemical analysis using thin layer chromatography (TLC) with various mobile phases and spray reagents, as well as UV visualization revealed the presence of alkaloids, phenols, terpenes and flavonoids. Bioassay-guided fractionation employed solid phase extraction, high performance liquid chromatography (HPLC) and HPLC-MS/MS.

HPLC analyses using a binary mobile phase consisting of water (0.1% formic acid) and methanol (0.1% formic acid) revealed four major peaks, at 11.380, 12.245, 12.894 and 13.582 minutes for the sequentially extracted hexane fraction. The eluents of the HPLC analyses were collected in a drop-wise fashion onto silica TLC plates and exposed to microbes using bioautography which indicated that the peak at 13.582 minutes was the compound with the antimicrobial activity. This study has provided scientific support for the ethnomedical use of the root-bark of *P. capense* in the treatment of infectious diseases. To date the peaks with antimicrobial activity have been identified, the chemical characterization of these peaks has not yet been performed. Isolation of sufficient quantity for chemical characterization is in progress.
Effects of tropane alkaloids of *Datura stramonium* and *D. ferox* (“stinkblaar”) containing respectively both L-hyoscyamine and scopolamine (hyoscine) and only scopolamine in humans and various animal *spp*.

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Equines are hind gut fermenters and these alkaloids are absorbed from the stomach and seriously interfere with the motility of the caecum and colon frequently resulting in fatal impaction colic. It was determined that young Datura baled in hay was just as toxic as the seed often contaminating grain, especially maize (mealies [RSA]or corn [USA] vide poster 2006). Datura spp. grow much faster than maize and, consequently, ripen with the maize and with combine harvesting the seed contaminate the grain as a mixture of the two spp. Seed of *D. ferox* and *D. stramonium* look identical but the former is about double the size of the latter and can thus be differentiated.

A literature search revealed that other spp. of ruminant livestock are, however, also susceptible to intoxication to high doses of the seed or even the vegetative plant despite partial degradation by ruminal micro-organisms.

*D. ferox* contains only scopolamine (L-hyoscine) which is a highly hallucinating substance which results in fatal “head-bashing” in sows and is even used in chemical warfare immobilizing troops in trenches as it is absorbed trans-dermal. A heat stable, highly potent congener is even being used in bombs.

Tertiary amines such as physostigmine, arecoline and pilocarpine rapidly abolishes the hallucinating effects in humans as they, in contrast to the commonly used quaternary ammonium prostigmine, cross the blood-brain barrier. They should be tested against refractive impaction colic of equines.

Sheep and goats are totally refractive to the anti-muscarinic effects of these alkaloids. Strong indications that they, however, result in lysosomal storage disease due to calystegines in the daturas, need to be confirmed.

It is also of importance to note that in nature only the L-isomer is produced and that its potency is thus double that of atropine, the chemically produced racemate of hyoscyamine of which the D-isomer is devoid of activity.
Overview of the 2007 Pet Food Toxicosis Crisis

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Commercially produced animal feeds (particularly dog and cat food) contain added protein to meet the target animal’s daily requirement, obtained from animal sources that are scarce and expensive or plant sources, namely glutens. Glutens vary in protein content ranging from 20-25% (corn gluten feed) to 75-85% (wheat gluten meal). The price is based on the “Crude Protein Content” (CPC), determined by the nitrogen content allowing the fraudulent addition of high nitrogen compounds, falsely elevating the apparent protein content.

Apparently, Chinese vegetable gluten suppliers have been adding melamine for 15 years or more (according to the Chinese melamine manufacturers). Melamine, an industrial chemical made from urea at high temperatures and pressure is normally used as a melamine-formaldehyde polymer resin, in the manufacture of plasticware and melamine table tops, in which state, it is entirely stable and inert. Melamine is cheaper to produce than protein and it has a very high nitrogen content (67%). For each 100 grams of melamine added to the gluten, one obtains an apparent (based on the CPC) yield of 420 grams of protein. Melamine, although a known toxin, when administered in very high doses (in sheep and rats, at 1 to 3% of the food, it causes renal and urine melamine urate crystals and can lead to bladder cancer), is fairly innocuous at low doses.

It is proposed that, in the last year or so, pure melamine, either due to price or availability, was replaced with a mixture of melamine and “melamine waste”. In the process of manufacturing melamine, such “waste” is produced, containing the urea “condensation compounds”, cyanuric acid (cyanurate as the principal impurity) and a few other compounds such as amelide, ameline, melon, melan. Cyanuric acid, by itself, is even less toxic than melamine. However, it appears that melamine combines with cyanuric acid in the renal tubules and the resulting compound precipitates in crystal form causing obstruction and kidney failure. There may be some role played by the other “impurities” listed, above, but work done at the University of Guelph has shown that the crystals were composed exclusively of melamine-cyanurate. Uricase enzyme inhibition studies suggest that this is the more likely mechanism.

There were 5 outbreaks of toxicosis in 2007, involving imported contaminated glutens.
1. Menu Foods (USA) obtained wheat gluten in November 2006, including it in a number of pet foods in December, causing an outbreak in February-March 2007.
2. Aquanutro, (South Africa), used corn gluten in November 2006 leading to an outbreak in December-January 2007 (involving Woolworths pet food). This outbreak was complicated by the additional contamination of the food with ethylene glycol, locally.
3. In early April 2007, Wilbur Ellis (USA) imported rice gluten from China. They sold this to Diamond feeds, who sold to the pork and poultry industry. They also sold this material to Natural Balance, a pet food manufacturer, who supplies a number of other pet food manufacturers with ingredients. The FDA discovered the contamination before an outbreak could occur but it entered the human food chain through the pork and poultry products.
4. On the 8th of March, 2007, Royal-Canin (South Africa), incorporated corn gluten into their Vets-Choice range of dog food, leading to an outbreak in April.
5. It would appear that Aquanutro (South Africa, see above) manufactured another line of pet food in mid-April 2007, incorporating corn gluten that was sold in a dog food line called “Dogsense”, leading to deaths in June and July.
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Session10: Analytical technology in pharmacology and toxicology

Supporting confidence in metabolite identification

J Dahlmann
Quantitative determination of Methotrexate in just 1µl plasma.

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Methotrexate (MTX) is an anti-cancer drug widely used in a variety of tumours. Its effects are due to the inhibition of the enzyme dihydrofolate reductase that is required for thymidylate synthesis. As a consequence DNA and RNA synthesis is blocked, resulting in cell death of rapidly dividing cells. However, adverse side effects, especially nephrotoxicity, in patients receiving high-dose therapy and those with MTX elimination dysfunction continue to restrict its therapeutic use. Therefore, proper monitoring of MTX is of crucial importance in MTX therapy and has generated a need for a sensitive and selective method for MTX analysis in biological samples.

A new LC/MS/MS method has been developed for the quantitative determination of MTX concentrations in limited volumes of plasma. The assay is based on injecting a 2µl sample of diluted plasma, equivalent to 1µl plasma directly onto a LC/MS/MS system equipped with a capture column, switching valve, analytical column and two separate pumps. A column switching technique was employed that allowed direct sample injection onto the HPLC system where the methotrexate and folinic acid (IS) are extracted from the sample. After a wash cycle to displace the protein, the capture column was back-flushed onto the analytical column and the eluent introduced into an electrospray ionisation source operating in the positive ionisation mode. A multiple reaction monitoring technique using the precursor ions and transition m/z of 455.5 → 308 for MTX and 474.4 → 327.2 for folinic acid were used for the quantitation.

The method was validated with respect to linearity, reproducibility, sensitivity and selectivity. The lower limit of quantitation in plasma was 1ng/ml with linearity up to 100ng/ml for MTX, with an intraday variation of ± 5% and an interday variation of < 11%.

The utility of the method was demonstrated by estimation of the pharmacokinetics (PK) of MTX in Sprague Dawley rats after intravenous injection of a single bolus dose of 1mg/kg. This novel analytical method with the advantages of low sample volume and rapid automated sample clean up, allowed a multipoint PK study to be completed in an individual rat.

The ability to use a finger prick for blood sampling, especially in young children on MTX treatment, is now a possibility.
The Effect of Human Vaginal Mucosa Barrier Damage on the Diffusion of Permeant Molecules

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Purpose:
The diffusion of molecules through biological membranes depends on their molecular size, degree of ionization, lipophilicity, charge and the intact state of the mucosal barrier layer. In this study, the diffusion of hydrophobic and hydrophilic permeant molecules across intact and epithelial-stripped human vaginal mucosa was studied.

Methods:
Human vaginal mucosa was either left intact or heat treated (80 degrees Celsius, 30 seconds) and the epithelial layer carefully removed with a scalpel, prior to diffusion studies. A flow-through diffusion apparatus was used for all permeability experiments. Flux values for tritium-labelled tacrolimus (822 Da), cyclosporin A (1203 Da), vasopressin (1083 Da) and r-arecoline (160 Da) were determined over 24 hours and fractions collected 2-hourly (20°C, 1.5 ml/h).

Results:
Steady state flux values for tacrolimus, cyclosporin A and vasopressin were not reached, thus estimated values were calculated (mean at 16, 20 and 24 hrs). Increases in mean estimated steady state flux values obtained for the epithelial stripped tissue were 9.3% (P>0.05), 33.6% (P<0.05), 32.5% (P<0.05) and 31.6% (P<0.05) for cyclosporin A, tacrolimus, vasopressin and r-arecoline, respectively.

Conclusions:
Estimated mean steady state flux values for all the permeants tested across the epithelial-stripped mucosa were statistically significantly higher than those found for the intact tissue, except for cyclosporin A. It would thus seem that large, lipophilic, cyclic oligopeptide molecules e.g. cyclosporin A, do not penetrate mucosa easily, even when damage occur to the mucosal barrier.
Comparative Permeation of Drug Compounds Across Bronchial Tissue

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Purpose:
The molecular diffusion of drugs across bronchial tissue can be viewed as consisting of a continuous epithelium, collagen fibre network and interstitium. Depending on the physiochemical properties of a drug, the diffusional resistance offered by the individual layers can vary greatly. Bronchial epithelium, which is lipoidal in nature and consists of pseudo stratified, ciliated columnar epithelium, offer high resistance to the diffusion of hydrophilic and charged molecules. In an effort to better understand the structure-permeation relationship of drug compounds across bronchial tissue, we investigated a variety of alkylxanthine derivatives substituted at the 1-, 3-, 7- (theophylline, caffeine & theobromine), and 8-positions. Other drugs also studied were salbutamol, ipratropium bromide, isoniazid, sulfamethoxazole and trimethoprim.

Methods:
A flow-through diffusion apparatus was used for all permeability experiments. Prior to the start of each experiment, frozen porcine bronchial tissue specimens were thawed to room temperature in phosphate buffered saline (pH 7.4). Thereafter they were carefully cut, so as not to damage the epithelial surfaces, into approximately 4 mm² sections and placed into the flow-through diffusion cells (exposed areas 0.039 cm²). Permeant samples collected over 2-hourly intervals (20°C, 1.5 ml/h) were analysed by HPLC in conjunction with UV/VIS or MS/MS detection and drug concentration was calculated from the appropriate calibration curves constructed.

Results:
Steady state flux values were calculated (mean at 18 - 24 hrs). The mean for 1, 3-dimethylxanthine was about 42% higher than that of 1,3,7-trimethylxanthine and 3, 7-dimethylxanthine respectively. On the other hand, flux values for salbutamol and ipratropium bromide was more than 50% lower than that of 1,3-dimethylxanthine.

Conclusions:
The higher mean flux rate of the asymmetric substituted xanthine (1- and 3-positions), coincides with the molecular structure necessary for the drug to act as an adenosine antagonist and bronchodilator. Contrary to this, substitution at the 1-, 3- and 7- positions (caffeine) coincides with a lower flux rate and decreased ability of the drug to act as a bronchodilator.
In Vitro transcorneal diffusion and penetration of antimicrobial agents and their potential use in ophthalmological therapy.

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Purpose: To compare the in vitro diffusion of clear solutions as well as ophthalmologic gel formulations of three antimicrobial agents through rabbit and human corneas.

Methods: Diffusion of antimicrobial agents through rabbit and human corneas, the latter not suitable for transplantation, were used for all permeability experiments. Fresh rabbit and human corneas were frozen in liquid nitrogen and stored at -80°C. In vitro flux rates of either 1.0% solutions or 1.0% gel formulations were performed using a flow-trough diffusion apparatus (24 h, 20°C, 1.5 ml/h). The antimicrobial agents metronidazole, azithromycin and clarithromycin were investigated. ANOVA and Duncan’s multiple range test were used to test for steady state and an unpaired t-test with the Welch correction was used to test for significant differences (5% level) of the respective agents. Permeant containing effluent samples, collected in the receiver compartment of the perfusion apparatus over the 2-hour sampling intervals were analyzed by means of binary high-performance liquid chromatography in conjunction with either UV/VIS or MS/MS detection. Since no extraction procedure was required, it was unnecessary to determine recovery rates. Calibration curves were linear over the entire applicable concentration range of the respective antimicrobial agents.

Results: Mean flux rates of metronidazole gel formulation (MW 171.2) across rabbit corneas were found to be 7% higher than those found for human corneas whilst for the metronidazole solution, the mean flux rates were found to be 30% higher in human corneas as compared to rabbit corneas. For the macrolides azithromycin (MW 749.0) and clarithromycin (MW 748.0) the mean flux rates were found to be about six times lower as compared to metronidazole. In comparing the two vehicles (solution / gel) it appears that the gel formulations effects reduced flux rates across both rabbit and human cornea. The physical / chemical properties of the antimicrobial agents might be effected by the gel formulation leading to a decline in flux rate. Although the diffusion of the macrolides, azithromycin and clarithromycin, across the corneal barriers appear to be low, the detected concentrations which do cross are well above the published minimal inhibitory concentrations.

Conclusion: Rabbit cornea can be used in general as an in vitro permeability model for human cornea. Antimicrobial agents, suspended in both solution and gel-formulations, do cross the corneal tissue in concentrations which may have clinical application. The mean flux rate of the solution formulation was for the human cornea in all instances higher. Although the respective gel-formulations showed reduced flux rates across human corneas, it may, however be still be therapeutically advantageous to use a gel formulation for intra-ocular penetration due to a longer contact time. Molecular weight, solubility, lipophylic character and other physical / chemical properties do have an effect in respect of the flux rate over the corneal barrier. The use of rabbit corneas as an in vitro permeability model for human corneas, are thus supported. However, extrapolation of data of animal models towards human tissue has to be approached cautiously.
Acute poisoning is a cause of both morbidity and mortality in many parts of the world. The aim of this study was to characterize acute poisoning cases admitted to the selected hospitals in South Africa.

All cases of patients admitted with a diagnosis of poisoning to the eight hospitals, from January 2005 to June 2005, were evaluated retrospectively. Data were obtained from the hospital medical records and included the following factors: age, gender, race, toxic agents, length of stay, circumstances of poisoning, and whether these poisoned patients had survived or died as a result of poisoning.

Of the total 424 patients admitted for treatment, whose median age was 17.6 years, 57.8% were females, and 89.6% black Africans. Most (59%) poisonings were accidental, and the involved toxic agents were, in descending order, household chemicals (45.7%), modern medicines (17.5%), animal/insect bites (15.8%), and agrochemical chemicals (9.7%), food poisoning (5.4%), drugs of abuse (3.3%), traditional medicines (2.4%), and plants (0.2%). Poisoning by drugs of abuse was more common in males than females. Though most patients spent less than 2 days, 70.1% of female patients stayed more than 2 days being hospitalized. The overall case fatality rate was 2.4%. Of those who died, 80% were 13 to 19 years old, 70% poisoned deliberately, 60% female; and the toxic agents responsible for the death were carbon monoxide (40%), cocaine (40%), and organophosphates (20%).

These findings suggest that an educational intervention is needed and that it should focus on young female black Africans.
Isolation and Characterization of the Major Compounds from *Pentanisia prunelloides*

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*Pentanisia prunelloides* (Rubiaceae) is known as the wild verbena, broad leaved *Pentanisia* (English), *sooibrandbossie* (Afrikaans), *icim amlilo* (Zulu) and *licim amlilo* (Swazi) and is distributed throughout the grasslands of southern Africa. This plant appears to be a cure all plant, as the tuberous root and leaves are extensively used in traditional medicines to treat a wide range of ailments which include fever, rheumatism, chest pain, colds, stomach pain, sore joints, wounds and many other illnesses.1,2,3,4 Anti-bacterial and antiviral tests were positive for the extracts of the leaves and the tuber.3 Nothing much seems to be known about the chemical composition of the plant and the *Pentanisia* genus in general.3

A general screening for possibly active compounds was done, followed by isolation of some of the major compounds incorporating techniques such as TLC, GC-MS and HPLC. Characterisation was done using IR, UV and NMR.

**References:**


Food safety and the Impact of Antimicrobial Resistance on Regulatory Policies in Veterinary Medicine and Stock Remedies

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Purpose:
Increasing resistance to antimicrobial agents is of growing concern to public health. The concern includes infections acquired in hospitals, community infections acquired in outpatient care settings, and resistant foodborne disease associated with drug use in food-producing animals. In South Africa, a significant source of antimicrobial-resistant foodborne infections in humans is the acquisition of resistant bacteria originating from animals. The Department of Health (DoH), Directorate: Food Control’s goal in resolving the public health impact arising from the use of antimicrobial drugs in food-producing animals is to ensure that significant human antimicrobial therapies are not compromised or lost while providing for the safe use of antimicrobials in food animals. The paper gives overview of the regulatory rules and monitoring dealing with antimicrobials registered as veterinary medicines and stock remedies.

Methods:
Veterinary medicine and agricultural stock remedy regulations were studied. Emphasis was placed on guidelines in terms of registrations, how regulators use drug scheduling, who prescribes and dispenses the drugs, who monitor the use of the drugs and the collaboration of regulators, academics and industry.

Conclusion:
The results demonstrate the need for innovative trends in regulatory rules and surveillance of antimicrobial resistance in bacteria. The approach and strategy includes revision of registration procedures of antimicrobials registered as veterinary medicines with Act 101 of 1965 and stock remedies with Act 36 of 1947. Revision of the pre-approval safety assessment for new animal drug applications. Use of toxicological risk assessment to determine the human health effect resulting from the use of antimicrobials in food animals. Use of microbiological risk assessment to facilitate scientific investigations of the risks related to the food chain, including quantification of uncertainty and prioritization of control strategies. Robust monitoring for changes in susceptibilities among foodborne pathogens to drugs that are important both in human and veterinary medicine, research, and risk management.
Cardiovascular effects of a compound isolated from *Leonotis leonurus*

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**Introduction**

*Leonotis leonurus* (L. l.) is used in traditional medicine for treatment of various ailments including high blood pressure\(^1\). In previous studies, an aqueous extract of the leaves and stems was found with a positive inotropic and a negative chronotropic effect in anaesthetized normotensive rats and on the isolated perfused rat heart, while a fraction of the methanol extracts had a positive inotropic and chronotropic effects on the isolated perfused rat heart\(^2\). This study was aimed at the isolation and characterization of a cardioactive compound from *L. l.* on male Wistar rats (250-350g).

**Methods**

83 g powder of washed and dried leaves was refluxed for 24 hours in 3 litres of methanol, cooled, and excess solvent evaporated. One yellowish crystalline compound was isolated from the dried extract through a combination of solvent extraction, column chromatography, thin layer chromatography and re-crystallization in chloroform. NMR and X-ray crystallography was used to determine the structure of the isolated compound. Animals were anaesthetized with sodium pentobarbitone (40mg/kg IP). After tracheotomy, the external jugular vein was cannulated for infusion of test substances and the femoral artery cannulated and connected via a pressure transducer to the PowerLab 4/20T for recording systolic (SP), diastolic (DP), mean arterial pressure (MAP) and heart rate (HR).

The isolated compound was dissolved in normal saline using tween 80 and administered IV in the following doses; 0.5mg/kg, 1.0mg/kg, 2.0mg/kg, 3.0mg/kg, 4.0mg/kg and 5.0mg/kg. Results were expressed as a difference between the base line preceding the administration of each tested substance and at steady state. Using the Student’s t-test, differences between two related means were considered statistically significant for \(p \leq 0.05\).

**Results and conclusion**

NMR and X-ray crystallography revealed the isolated compound to be a novel diterpene which we designated DTP. DTP (0.5 to 5.0mg/kg) significantly \((p < 0.05)\) decreased HR (-36.57 ± 6.43 to -82.86 ± 14.41 mmHg). There was an initial significant decrease in MAP (-3.84 ± 1.30 to -7.68 ± 3.7 mmHg) between the 0.5-2.0mg/kg doses, and then a significant in crease in MAP (35.40 ± 4.65 to 41.63 ± 5.7mmHg) between the 3.0 – 5.0 mg/kg doses.

In conclusion, the novel diterpenoid DTP is one of the compounds responsible for the cardiovascular effects of *L. l.* leaves.

**References**

Isolation and characterization of novel antiplasmodial compounds from *Siphonochilus aethiopicus*

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Purposes:

The rhizomes of *Siphonochilus aethiopicus* are traditionally used to treat colds, coughs, influenza and several other ailments. The aim of this study was to investigate *S. aethiopicus* for antimalarial activity and to isolate and characterize the antiplasmodial compounds.

**Methods:**

*Plant material:* Dried ground roots of *S. aethiopicus* were donated by Nigel Gericke. Plant material was extracted with ethyl acetate and subjected to liquid-liquid extraction.

*Fractionation and structure elucidation:* Bioassay-guided fractionation and high performance liquid chromatography were used to isolate and purify the active compounds. Nuclear magnetic resonance (1-D and 2-D) and mass spectrometry was used to determine the chemical structure of compounds.

*Bioactivity tests:* In vitro antiplasmodial activity was determined against *Plasmodium falciparum* using the parasite lactate dehydrogenase assay. In vivo antiplasmodial activity was evaluated using the Peters 4-day suppressive test. The cytotoxicity screens were performed against the Chinese hamster ovarian (CHO) cell-line using the MTT-assay.

**Results:**

The crude extract of *S. aethiopicus* was active in vitro against both chloroquine sensitive (CQS) and chloroquine resistant (CQR) strains of *P. falciparum* with IC₅₀-values of 2.9 µg/ml and 1.4 µg/ml, respectively. Three structurally-related novel compounds were isolated with IC₅₀-values of 3.4 µg/ml, 18.1 µg/ml and 21.7 µg/ml against the CQS strain, and 1.5 µg/ml, 5.9 µg/ml and 16.7 µg/ml against the CQR strain of *P. falciparum*. No cytotoxicity was observed with the crude extract as well as the compounds. The crude extract was evaluated for in vivo activity against the chloroquine resistant *P. yoelii* NS strain. Preliminary results showed that the plant extract reduces parasitemia during treatment.

**Conclusion:**

The crude extract and compounds isolated were more active against the CQR strain of the parasite compared to the CQS strain.
Antimalarial Study of Compounds Isolated from Xerophyta species

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Secondary metabolites of Xerophyta villosa and Xerophyta retinervis were targeted as a potential source of new antiplasmodial compounds. The compounds were grouped according to their polarity by making use of sequential solvent extraction. These extracts were screened against a chloroquine sensitive (D10) Plasmodium falciparum strain, and the greatest antiplasmodial activity was found with the ethyl acetate extracts of both species.

Six pure compounds were successfully isolated from Xerophyta villosa and two from Xerophyta retinervis by making use of two reverse phase HPLC gradient systems. These isolated compounds were screened against a chloroquine sensitive (D10) Plasmodium falciparum strain. Two of these compounds showed good antiplasmodial activity, three showed mild activity, and one showed no activity.

Cytotoxicity testing of the extracts and isolated compounds was done to determine their toxic properties against living cells, and all tested extracts and compounds showed little toxicity.

The chemical structures of the three most active antiplasmodial compounds were determined by making use of mass spectrometry and nuclear magnetic resonance spectroscopy (1D and 2D). The most active compound was identified as 9-O-acetylhydnocarpin. This is the first study that has shown the presence of 9-O-acetylhydnocarpin in Xerophyta villosa. The second best compound was identified as hydnocarpin. Again this is the first report of the presence of hydnocarpin in Xerophyta villosa and Xerophyta retinervis. The third best compound was identified as the flavonoid luteolin and showed mild antiplasmodial activity.

An ADME study of 9-O-acetylhydnocarpin was conducted in a mouse model, and the bioavailability information was used to design a dosing strategy for in vivo antimalarial testing on Plasmodium berghei infected mice.
Tulbaghia violacea, an indigenous South African plant, is one of 21 species of the genus belonging to the family Alliaceae. It is widely used in the traditional medicinal industry in South Africa. A crude extract from T. violacea aerial parts also proved to be highly fungitoxic against a variety of plant pathogenic fungi. When cultivated as a donor plant, in the event that the development of a natural product is considered, it is essential that the fertilizer program enhances yield, but not at the cost of antifungal bioactivity. To evaluate the latter potential, a trial was conducted in an air-conditioned glasshouse at a 30/20°C day night temperature regime to monitor the effect of N-fertilization on growth and bioactivity under natural light conditions. T. violacea bulbs were divided into three weight classes (5-10, 11-20 and 21+ g) and one bulb from each group planted in a 30 ℓ plastic pot filled with rinsed silica sand of which the particles were 2 mm in diameter. A randomized complete block design was used and each treatment replicated four times. Two nitrogen sources, nitrate and ammonium, were used in order to determine which would give the best results in terms of both growth and antifungal activity. Nitrogen was applied at four different rates, i.e. 30, 60, 120, 180 kg ha$^{-1}$ as a single application at the beginning of the trial period. The main objective was to establish the best nitrogen source and optimum application rate that furnish both a high yield and antifungal activity. The seasonal growth pattern of the plant and bioactivity of a crude extract was monitored monthly over a 12-month trial period. It was concluded that nitrate was the preferred nitrogen source at an optimum application rate of 60 kg ha$^{-1}$ in terms of both yield and antifungal bioactivity. This was particularly true for plants harvested during October. It is suggested that trials be conducted under field conditions in order to verify these findings.
POSTERS
What is the Future of Basic Pharmacology in the University? – A return to the Roots

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This paper is to address the almost neglect of experimental pharmacology (basic) in our universities. In the last 2 decades, simulation seems to have replaced experimental pharmacology. This trend cannot give the proper training of pharmacologist in our universities/industries, in the training of medical and pharmacy students. A call to return to the roots in pharmacology being an experimental based discipline.
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.
Structure-Activity Relationships of Novel Antimalarial Agents

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Purpose:
A possible chemotherapeutic target for antimalarial agents is to interfere with the formation of haemozoin, which is the inert by-product of haemoglobin digestion that occurs in the parasites’ acidic food vacuole. Haemozoin is formed by the spontaneous biomineralisation of toxic Fe(II)PPIX to a chemically inert Fe(III)PPIX dimer of β-haematin. Thus novel compounds, 1-N-substituted cyclised pyrazoline analogues of thiosemicarbazones, porphyrin thiosemicarbazones and 6-, 7- and 8-substituted-4-hydroxyquinolines were synthesised and screened for antimalarial activity and for their ability to inhibit haemozoin formation.

Methods:

³H-Hypoxanthine Incorporation Assay: The sensitivity of the chloroquine-resistant strain (FCR-3) of Plasmodium falciparum to the test compounds was determined using the tritiated hypoxanthine assay over 48 hours of growth.

MTT Cellular Viability Assay: Test compounds were incubated with transformed human kidney epithelial cells for 48 hours and percentage cell viability was determined using the MTT tetrazolium-based assay.

Ferriprotoporphyrin Biomineralisation Inhibition Test: Compounds were incubated with haemin in an acidic acetate buffer (pH 4.4) for 24 hours. Polymerised haematin was re-dissolved in NaOH and the percentage of β-haematin inhibition determined.

Results:

Thiosemicarbazones: The bromoacetophene analogues displayed better antimalarial activity than the acetophene analogues, with 2-chlorobenzylamine (S2, IC₅₀: 17.14 ± 1.88µM) and n-phenyl piperizine (S5, IC₅₀: 18.98 ± 1.59µM) derivatives being the most active. Compound S2 interacted synergistically with quinine, whilst S5 interacted in an additive manner with chloroquine. However these compounds did not inhibit haemozoin formation, with S2 (IC₅₀: 521.61 ± 70.3µM) and S5 (IC₅₀: 3563.02 ± 795µM) as compared to chloroquine (IC₅₀: 23.03 ± 8.33µM)

Porphyrins: P4 (5-(4-phenyl-N⁴-2-chlorobenzylthiosemicarbazone)-10,15,20-(trisphenyl) porphyrin) possessed the best antimalarial inhibitory properties (IC₅₀: 4.36 ± 0.08µM) but did not inhibit haemozoin formation. P1 (5-(4-phenyl-N⁴-m-toluidinethiosemicarbazone)-10,15,20-(trisphenyl) porphyrin) possessed both antimalarial properties (IC₅₀: 5.66 ± 0.50µM) and was 94% as effect as chloroquine in inhibiting haemozoin formation.

4-Hydroxyquinolines: The 6-substituted-4-hydroxyquinolines with various dialkylamino groups exhibited greater antimalarial activity than the 7- and 8-substituted derivatives. The presence of a NO₂ functional group at positions 7- and 8- increased the antimalarial activities in comparison to a NH₂ functional group at the same positions. However, NO₂ significantly increased the toxicity profile. Only the 6-substituted-4-hydroxyquinolines possessed an ability to inhibit haemozoin formation.

Several promising compounds have been identified for further studies.
Novel nucleoside analogues in the induction of colonic cell differentiation and apoptosis

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INTRODUCTION:

Over the past few decades, a major effort has been made to develop anti-cancer agents through both empiric screening and rational design of new compounds. These attempts are made to reduce the severe adverse effects associated with existing cancer chemotherapeutic agents, as well as to reduce the development of drug resistance.

Cytotoxic nucleoside analogues were among the first cancer chemotherapeutic agents introduced. The aim of the study was thus to screen novel nucleoside analogue derivatives for cytotoxic activity, as well as to determine the level of differentiation and the induction of apoptosis in the colonic carcinoma cell lines, viz. HT-29 and Caco-2.

MATERIALS AND METHODS:

All compounds were synthesised using standard organic chemistry techniques. The in vitro cytotoxicity of the synthetic compounds was directed against the HT-29 and Caco-2 cell lines. 5′-Fluorouracil and camptothecan were used as positive controls. In order to determine the cytotoxic effects of these compounds, the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay was used. Results are expressed as IC₅₀ values. The measurement of cell differentiation was determined by the alkaline phosphatase assay, and a colorimetric assay was used for the determination of caspase activity.

RESULTS:

CDK 3 and CDK 8 showed the most significant cytotoxic activity (p<0.05) against both cell lines, with IC₅₀ values ranging from 7.38-14.34 µM. JLP 26.5 induced cell differentiation in the HT-29 cells, whereas JLP 38.2 induced cell differentiation in the Caco-2 cells. Maximal caspase 3 expression was observed between 4-12 hours after addition of the active compounds.
Assessment of Commercial Ginseng and Hypoxis supplement products for trace element and heavy metal contamination

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Purpose:
Natural products extensive use as a source of primary health care requires routine quality control scientific testing methodologies to verify the safety, quality and efficacy of commercially available plant products. This will enhance public confidence in the use of such products in an unregulated environment, as adverse side effects often associated with the use of traditional/herbal products, are generally due to misidentification of plants, lack of standardization and good manufacturing practices, botanical substitution, adulteration, and contamination. The therapeutic effect of plant medicine is alleged to be enhanced by essential trace elements. Some products may however also contain excessive amounts of trace elements and heavy metals. For this study the focus was two products. Ginseng as a popular traditional Chinese medicine, used for its adaptogenic and restorative properties, and African Potato products used as an immune booster for patients infected with HIV/AIDS.

Methods:
Different commercial brands of Ginseng and African Potato products were purchased from pharmacies and health stores in Cape Town for this study. The products were evaluated for content validity and contamination for the following trace element and heavy metals, sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), chromium (Cr), nickel (Ni), lead (Pb) and mercury (Hg), Lithium (Li). The instrument utilized for the trace element and heavy metal analysis was the combination tandem system of Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) that is a powerful, accurate, fast and sensitive, analytical technique.

Results and Conclusion:
Results will be presented for trace element and heavy metal analysis to verify product, and batch-to-batch consistency and safety. It provides a rapid method of identification of trace elements and heavy metal contamination in supplements. It allows for verification that overall raw material content is from the same or similar source, and that the process of production is similar. The trace element and heavy metal “finger print” methodology could be used for similar herbal supplements, other than Ginseng and African Potato products.
INTRODUCTION
Ethanol is mainly metabolized in the liver to intermediate aldehydes and finally to acetic acid and water. There are numerous anecdotal claims for substances that are able to either reduce alcohol intoxication or improve the symptoms of over indulgence. None were ever proven in randomised clinical trials.

Absorbatox® is a finely ground volcanic mineral of which the cation exchange capacity has been artificially enhanced by a patented process. The aim of this pilot study was to evaluate the purported effects of Absorbatox® on blood and breath alcohol levels with and without food and to establish any effect on the so called hangover symptoms.

METHODS
In 2 separate double blind randomized studies, volunteers were administered oral 6g Absorbatox® as follows:

(a) On empty stomach with 75 ml alcohol (blood alcohol levels).
(b) After a meal and an evening of “free” drinking and eating
   Breath alcohol concentration as well as Central Nervous System (CNS) and Gastro-intestinal Tract (GIT) symptoms were recorded.

RESULTS
Absorbatox® had no effect on blood or breath alcohol levels and there was also no change in alcohol absorption with or without food. It did significantly (p = 0.001) improve both GIT and CNS symptoms. Fifty percent of patients experienced a reduction in GIT symptoms whereas 70 percent of patients reported a reduction in the CNS symptoms of hangover. The mechanism of action is however not clear.

CONCLUSION
This pilot study suggests that Absorbatox® reduces the symptoms of hangover by an as yet unidentified mechanism. The reported effects on heart burn also only partially explain the GI benefits.
Endogenous Heparin Levels in the Controlled Asthmatic Patients

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Heparin possesses anti-inflammatory properties and heparin-like substances may show potential as a treatment option for asthmatic patients. The authors thus hypothesized that asthmatic patients have decreased levels of circulating endogenous heparin than the healthy individual.

Endogenous heparin levels in the controlled asthmatic patient were compared to healthy individuals. A total of 53 adults, aged 18-78 yrs, with asthma controlled by combination therapy that includes inhaled corticosteroids, were recruited from the Asthma Clinic at the Johannesburg General Hospital, South Africa. These levels were compared with 26 controls, aged 18-57 yrs, recruited from the general population.

In support of the hypothesis, the blood of the patients contained significantly reduced levels of endogenous heparin in comparison to that of the healthy individual. These results indicate that the anti-inflammatory properties afforded by heparin are lacking in these patients.

Further investigation is now needed to determine whether the heparin levels are inherently low in the asthmatic, and can thus be used as a marker of the disease, or whether the medication being used is a contributing factor in decreasing these levels.
Development of 6-\(^{18}\)F-fluoro-L-DOPA for routine use in South Africa.

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The aim of this study was to develop an optimized method for the remotely-operated synthesis of 6-\(^{18}\)F-fluoro-L-DOPA (6-\(^{18}\)F-FDOPA) based on the fluoroestannylation pathway. This drug is currently unavailable in South Africa, there is a need for a simple reliable and fully automated procedure for its routine production.

For several years 6-\(^{18}\)F-Fluoro-L-DOPA (6-\(^{18}\)F-FDOPA) has been produced for research studies into brain pathology. It is an important biochemical probe for studies of presynaptic dopamine metabolism. With positron emission tomography (PET) 6-\(^{18}\)F-Fluoro-L-DOPA provides a unique \textit{in vivo} assessment of the integrity of the dopaminergic system.

The two step production of \(^{18}\)F \(^2\) that exploits the advantageous yield of the \(^{18}\)O(p,n)\(^{18}\)F reaction will be used for the production of 6-\(^{18}\)F-fluoro-L-DOPA via the electrophilic approach.\(^2\) The various intermediates, the stannyl precursor and the final product (after \(^{18}\)F decay) will be characterized by \(^1\)H, \(^13\)C, \(^19\)F and \(^{19}\)Sn Nuclear Magnetic Resonance (NMR). \textit{In vitro} and \textit{in vivo} studies will be performed on the final product prior to registration with the Medicines Control Council of South Africa.
Inhibition of human neutrophil superoxide production - do all plant extracts have this effect *in vitro*?

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**Purpose:**
Superoxides can be both beneficial and detrimental to the human body. After bacterial or fungal infections and inflammation, reactive oxygen species are produced with the specific aim to destroy the cause of the infection or inflammation. Unfortunately this leads to the suppression of the immune response, tissue damage, skin damage and impaired wound healing. Secondary opportunistic infections can also appear. The aim of this study was to investigate how many of the medicinal plant we studied have additional anti-oxidant effects.

**Methods:**
Extracts were made from the following traditional medicinal plants: *Copaifera officinalis* (Commercially available balsam concentrate), *Boophone disticha* (Bulb: inner scales, outer scales), *Carpobrotus acinaciformis* (leaves, stem), *Schkuhria pinnata* (leaves, stem, root), *Combretum woodii* (leaves), *Lippia javanica* (leaves, seeds), and *Ziziphus mucronata* (leaves, bark).

Neutrophils from healthy volunteers were isolated using Percoll as separation medium. The cells were stimulated with phorbol myristate acetate (PMA) after incubation with the extracts. Luminol was used as chemiluminescent probe.

**Results:**
It is claimed by traditional healers that many of their herbal medicines have anti-septic or anti-inflammatory properties and indeed depending on the dosage most of the plants tested in our lab so far did have the ability to inhibit superoxide production *in vitro* in human neutrophils. Most fruit or vegetable extracts available in health stores are also claiming that their extracts may have antioxidant effects.

Could it be that a healthy portion of fruit and vegetables per day includes all the antioxidants needed by the body and that to take antioxidant supplements can in the long run do more harm than good in allowing opportunistic infections to thrive and a depressed immune system not being able to cope increasing the use of antibiotics.
Pilot study: DermaWave™ Aquaphoresis improves the appearance of cellulite by enhancing transdermal drug delivery

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Cellulite is aesthetically undesirable and difficult to treat. To effectively reduce the cellulite appearance using topical administration of a drug needs to cross the relatively impermeable stratum corneum of the skin.

The efficacy of the topical application of a methylxanthine containing gel in the treatment of cellulite with the aid of the DermaWave™ Aquaphoresis system was evaluated. The DermaWave™ Aquaphoresis system combines electroporation and aquaphoresis to enhance drug delivery through the skin.

The thigh-buttock areas of sixteen healthy women were treated for 5 sessions. Only 1 leg was treated in the trial, the other served as the participant’s own control. Before and after photographs were taken and evaluated. Photographs were evaluated on 3 different aspects; padded appearance, orange peel appearance and skin firmness.

The treatment with electroporation and a methylxanthine gel resulted in a significant decrease in the padded and orange peel appearance as well as increase in skin firmness compared to the untreated control area.
Poisoning by animal bites and insects stings in Botswana

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Objective:
Epidemiological knowledge of poisoning by animals and insects is still limited by a lack of data. The aim of this study was to characterize the profile of victims poisoned through biting by animals and insects.

Methods:
The study included the 99 patients admitted from January 2004 to December 2005 to 4 hospitals in Botswana, whose primary diagnosis was “poisoning by snake bites and insect stings”. Data were collected using a pre-tested data collection.

Results:
Victims of poisoning by snake, scorpions, and other insects were 62.6% males. Some 51.5% of incidents were due to unidentified insects, 25.3% by scorpions, and 23.2% by snakes. With regard to age, the mean age was 24.8 (±18.8) years; 14% were ≤5 years, 20.2% 6-12 years; 12.1% 13 to 19 years, 19.2% 20 to 30 years, and 34.3% over 30 years old. Patients were from rural areas in 50.5%; and the biting took place during daylight in 48.5% of cases; With regard to the length of hospital stay, the mean length of stay was 2.3 (±3.9) days. While 27.3% were hospitalized for more than 2 days, the length of stay was higher among patients from rural than urban areas (p=0.001). Although bites occurred in or around the home in 48% of cases, in patients from rural areas, all bites took place outside the home (p=0.001). The case fatality was 2% and the death was due to snake biting.

Conclusion:
Poisoning by snakes and scorpions affected mainly males aged over 20 years old. The findings of this study suggest that preventive actions must take into account the differences due to the age category, and the danger due to snake biting.
Gender and smoking related differences in baseline DNA damage levels in a healthy African population using the Comet assay

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Background:

The Comet assay is increasingly regarded as one of the more valuable approaches among the various techniques for evaluating genotoxic risks. Applicable to either \textit{in vitro} or \textit{in vivo} methodologies, this assay is a sensitive technique for the detection of various DNA damage, e.g., direct single and double strand breaks, DNA adducts and other classes of damage that result in alkali labile sites, DNA-DNA and DNA-protein crosslinking, single strand breaks associated with incomplete excision repair events. The advantage of the assay is that data is collected at the level of individual eukaryotic cells. In this study we investigated the baseline DNA damage in a healthy African population by using the Comet assay with special attention to the role of smoking and gender as potential confounding variables.

Methods:

\textit{Subjects}: Study subjects consisted of normal healthy volunteers, males and females, employed at the National Institute for Occupational Health.

\textit{Blood Sampling and Cell Preparation}: Blood was collected in BD Vacutainer CPT and mononuclear cells isolated as recommended by the manufacturer. Cell viability was checked by the trypan blue exclusion method and cell concentrations adjusted to $1 \times 10^6$ cells/ml.

\textit{Comet Assay}: The alkaline version of the Comet assay was used in the study.

Results:

Scoring of DNA damage was done using the Comet assay image analysis software, Komet 5, and data analysed using Stata 9. No significant differences were observed in the baseline DNA damage between male and female subjects ($p = 0.7$) and DNA damage in smokers was found to be significantly greater than that of non-smokers in both males ($p = 0.0005$) and females ($p = 0.0038$).
A double blind placebo controlled study on the clinical efficacy and *in vivo* pharmacodynamics of potassium humate in the treatment of allergic rhinitis in patients with grass pollen allergies

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The objective of this study was to establish the safety and the therapeutic efficacy of oral potassium humate (Humiboost™) in reducing the signs and symptoms of hayfever in atopic patients during the grass pollen season.

In this parallel double-blind placebo controlled phase II study potassium humate (1.8g in divided doses/ day) and placebo was randomly assigned to 40 atopic patients presenting with acute symptoms of hayfever.

**Outcome parameters**
Nasal and non-nasal symptom assessment was done on specific symptoms according to a grading scale during the whole duration of the trial.
A global Clinical impression was scored by the doctor at end of study at visit 4.
Measurement of the surface area of a skin prick test was done at baseline (at randomization) and again at end of study (at visit 4). This entailed the introduction of the relevant allergen subcutaneously causing a wheal to develop within minutes.
Nasal smears were performed at the beginning (randomization) and again at end of study (visit 4) with an eosinophil count done at each stage and compared.
Other inflammatory markers, like basophil activity and interleukin concentrations were also evaluated (published elsewhere).
Baseline as well as end of study safety assessments were done, comparing a change in liver and kidney function.

**Results:**
Total symptom score during the first 48 hours of the screening visit was compared to the score of the last 48 hours of the study. No difference was found between the 2 groups in the total symptom score.
Sizes of the wheals were measured at the onset of the study and at the last visit. There was a statistically significant decrease in the size of the wheal in the group using the study drug.
The drug was found to be totally safe with no recording of any adverse events in the active group. A rise in the serum potassium level was found in one subject in the placebo group, which could not be explained.
Onset of action could not be determined due to the fact that there was no significant observable difference in the clinical symptoms between the 2 groups.

**Conclusion:**
A significant reduction was seen in the size of the wheals that developed after the allergen challenge in the group using the study drug, but no conclusion could be made on the efficacy of the drug as an antihistaminic agent, because a very small difference in the symptom scores was found.
Human safety of hormonal growth promotants in food of animal origin

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Purpose:
This report updates South Africa’s position on the human safety of residues of hormone growth promotants used in feedlots. Growth-promoting hormones have been used by the beef industry to improve an animal's ability to more efficiently utilize nutrients and produce leaner, more affordable beef. Since 1989 EC banned the use of the natural hormones 17 β- estradiol, progesterone and testosterone, and the synthetic or xenobiotic hormones zeranol, trenbolone acetate and melengestrol acetate. Third world countries including South Africa, Canada and US have been contesting the EC hormone ban.

Critics have claimed that growth hormonal implants act as carcinogens by causing breast cancer or making girls more susceptible to breast cancer, act as reproductive toxins by causing girls to reach puberty earlier than they used to and also endocrine disrupting agents that enter food chain via direct consumption of food products of treated animals, plants from land fertilized with contaminated manure and/or drinking contaminated water. As endocrine disrupters interfere with participation of steroid hormone balance during foetal development, imbalance can lead to disruptions that manifest as health problems at birth and later in life.

Methods:
The report evaluates a number of scientific papers published in international journals and scientific comments presented international at Codex Alimentarius Advisory meetings, to assess whether there are harmful residues of hormonal implants in beef.

Results:
A review of the data does not indicate any grounds for amending South Africa's regulatory position with respect to growth promotants i.e. there is unlikely to be any appreciable health risk to consumers from eating meat from cattle that have been treated with growth promotants according to Good Veterinary Practice.
Inhibition of complement activation: a possible mechanism of action for potassium humate

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It has been documented that potassium humate inhibits contact hypersensitivity in the rat model. In this study the effects of brown coal derived potassium humate (from the Letrobe valley, Australia) on delayed type hypersensitivity and acute inflammation was investigated.

A delayed type hypersensitivity reaction was induced in rats by sensitisation and challenging with sheep erythrocytes. After sensitisation, potassium humate was administered daily by oral gavage at a dose of 60 mg/kg bodyweight. Seven days later sheep erythrocytes were injected into the right hind footpad of the challenged rats. The degree of inflammation was determined by measuring the increase of foot volume with a plethysmometer. The carrageenan-induced paw oedema model was used to determine the effect of potassium humate on acute inflammation in the hind paw. Potassium humate was administered daily by oral gavage at a dose of 60 mg/kg bodyweight. Carrageenan was injected into the right hind footpad of a rat, which caused an increase in foot volume due to oedema, which was also measured with a plethysmometer.

It was found that potassium humate did not have an anti-inflammatory effect on the delayed type hypersensitivity reaction as opposed to the inhibition caused by dexamethasone at a dose of 30 mg/kg bodyweight. However, potassium humate significantly inhibited foot volume of the carrageenan-induced paw oedema model at a dose of 60 mg/kg bodyweight and compared favourably with indomethacin at 10 mg/kg bodyweight.

Due to the important role complement activation plays in acute inflammation, but not in delayed type hypersensitivity reactions, it was decided to investigate the effect of potassium humate in vitro on both the alternative and classical complement pathways. Results indicated that potassium humate inhibits the activation of both pathways without affecting the red blood cell membrane stability.

The mechanism of action of potassium humate might possibly be due to the inhibition of the complement cascade. This study clearly shows that potassium humate possesses anti-inflammatory properties that can be utilised in the future as a potential treatment for inflammatory disorders associated with the activation of complement. However further investigation in the mechanism by which potassium humate inhibits inflammation needs to be done.
Total Phenolic and Flavonoid content in South African herbal remedies

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Purpose:
To investigate the presence of compounds that might be responsible for the antioxidant properties of three South African medicinal plants, Warburgia salutaris (Bertol. F.) Chiov, Rhoicissus tridentata (L.f.) Wild & Drum and Terminalia sericea (Burch. ex DC.).

Methods:
Organic solvent extraction of phenolics: The polyphenolic extractions of the dried powdered samples were performed using 99.9% methanol containing 1% HCl. The mixture was centrifuged, filtered and concentrated. The resulting residue was redissolved in methanol and the extract was used for analysis.

Hot water extraction of phenolics: Ground plant samples were dissolved in hot double distilled water, heated and the mixture allowed to cool at room temperature. The solution was centrifuged and then filtered. The filtrate was concentrated and resuspended in methanol for UV Spectrophotometer analysis.

Total phenolic content assay: The amount of total phenolics was determined using the Folin-Ciocalteu reagent. The absorbance of the samples was measured after 2 hours at 760 nm using a UV-Vis spectrophotometer and the results expressed in Gallic acid equivalent (GAE) of dry weight of plant.

Estimation of flavonoids content: The flavonoid content in extracts was determined spectrophotometrically based on the formation of a complex flavonoid-aluminum. The absorbance of the reaction mixture of the sample extract and 2% aluminum chloride methanolic solution was measured at 430 nm using UV-Vis spectrophotometer and the flavonoid content was expressed in Rutin hydrate equivalent (RE) of dry plant weight.

Results:
Total Phenolic Content in Organic solvent extract: The highest phenolics concentration was found in the branches of W. salutaris with 20.3 mg/g, with the bark at 15.4 mg/g, the tubers of T. sericea at 14.2 mg/g, roots of R. tridentata at 12.6 mg/g and the leaves of W. salutaris at 12 mg/g.

Total Phenolic Content in water extract: High phenolics content was found in the bark (11.1 mg/g) and the branches of W. salutaris (7.3 mg/g), with the lowest content in the leaves of W. salutaris (4.2 mg/g). Other extracts were 5.2 mg/g for R. tridentata and 4.7 mg/g for T. sericea.

Total Flavonoid Content in organic solvent extract: The highest amount was found in the bark extract of W. salutaris at 8.2 mg/g, R. tridentata roots with 5.7 mg/g, W. salutaris leaves with 4.1 mg/g, branches of W. salutaris with 3.2 mg/g and the tubers of T. sericea were the lowest at 1.6 mg/g.

Total Flavonoid Content in water extract: The highest content was found in the roots of R. tridentata at 2.9 mg/g and the lowest was T. sericea at 0.6 mg/g. The W. salutaris plant had 0.9 mg/g for leaves, 1.1 mg/g for branches and the bark was at 2.6 mg/g.
Effect of gender, age and body mass index on arterial elasticity and endothelin-1 in hypertensive patients on perindopril.

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Purpose:
Increased arterial stiffness and endothelial dysfunction are associated with end-organ damage and are independent predictors of cardiovascular risk. Numerous population studies showed that PWV increase with age in both sexes. Obesity is an independent risk factor for cardiovascular disease and the role for ET-1 in the pathogenesis of obesity-associated hypertension has been suggested. The aim of the study was to assess the effect of gender, age and BMI on the elastic properties of the arteries and on endothelin level in newly diagnosed black hypertensive patients (n=44) at baseline and after nine months of ACE Inhibitor therapy (perindopril 4mg).

Methods:
PWV was measured non-invasively (Powerlab 4 SP system). Aortic and peripheral pulses were recorded (infrared plethysmo-doppler 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:
The decrease in PWV was larger in older than in younger patients. The mean endothelin-1 level decreased significantly in the subgroup of patients with BMI over 30. Patients with the BMI over 30 respond to the treatment with the higher decrease in the endothelin-1 level, SBP and mBP. The mean decrease in the diastolic blood pressure was significantly higher in the female than in male patients. There was a greater decrease in PWV aorta in male than in female patients. Perindopril could significantly reduce arterial stiffness and could provide greater beneficial effects on cardiovascular outcomes, beyond that seen with BP-lowering alone. Perindopril reduced arterial stiffness, as indicated by pulse wave velocity (PVW), elastic vascular resistance (EVR), 2m index and significantly improved systolic BP, diastolic BP, pulse pressure in black hypertensive patients. Interestingly BMI was a predictor for changes in the SBP, mean BP and endothelin-1. Patients with BMI over 30 respond to the treatment with the higher decrease in the endothelin-1. That could be the reason for the significant decrease in SBP and DBP. The higher BMI was associated with the biggest change (decrease) in SBP and DBP. These findings confirm the hypothesis that there is a significantly higher level of endothelin-1 in the obese black patients with BMI of more than 30.
The induction of apoptosis by synthetic compounds in acute and chronic leukaemia

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INTRODUCTION:
Over the years, the treatment of cancer has advanced immensely; however, despite all these efforts, many types of cancers still have a poor prognosis. It is thus vital that further research is aimed at developing new anti-cancer agents through both empiric screening and rational design of new compounds.

This study focuses on the induction of apoptosis and level of differentiation in human leukemic cell lines, HL-60 and K562, by derivatives of synthetic compounds (aromatic esters, biaryls and nucleoside analogues).

MATERIALS AND METHODS:
Synthetic compounds were synthesized using standard organic chemistry techniques. The effect of the compounds on cell viability was determined by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay and results are expressed as IC₅₀ values. The NBT (nitroblue tetrazolium) assay was used to assess differentiation induction. A variety of methods was used to detect apoptosis: fluorescent staining (Hoechst 33258) for percentage of apoptotic cells, colorimetric quantification of caspase -3, -8 and -9 activity and flow cytometric quantification of annexin V binding to phosphatidylserine. The activity of these compounds was compared to both etoposide and camptothecan.

RESULTS:
The aromatic ester, biaryl and nucleoside derivatives proved to be cytotoxic against both the K562 and HL-60 cell lines, with IC₅₀ values ranging from 37-80 M. Several of the derivatives tested induced apoptosis. The cytotoxic effects of these synthetic compounds suggest potential for its application in cancer therapy as promising lead compounds.
The evaluation of low-density lipoprotein cholesterol goals achieved in patients with established cardiovascular disease and/or hyperlipidaemia receiving lipid-lowering therapy
The South African Not At Goal Study (S4-NAG)

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Background:
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 CVD management and thus the scientific community has adopted the European Guidelines on CVD prevention in clinical practice. The South African Not at Goal Study (S4-NAG) is a survey to determine the percentage of patients, on lipid-lowering therapy, who are not at target low-density lipoprotein cholesterol (LDL-C) goal, as defined by the updated South African Guidelines.

Design:
A cross-sectional study

Methods:
In this study dyslipidaemic and/or CVD patients on lipid lowering therapy for > 4 months were enrolled. Patients who agreed to participate in the study had their demographic data and previous medical history documented. Blood samples from these patients were analysed to obtain a fasting lipogram and fasting blood glucose levels. Standardised methods were used for lipid and glucose measurements at a central laboratory.

Results:
In total 1201 patients (age 58±11.4 yrs) were recruited by physicians and General Practitioners from across South Africa. Under the new guidelines, 41% of patients were defined as low risk (LR) and 59% of patients were high risk (HR). In total 63% of LR patients and 77% of HR patients (71% overall) did not achieve their LDL-C target goals of 2.5 and 3.0 mmol/L respectively. LR and HR patients, who did not achieve their LDL-C goal, were on average 19% (0.7 mmol/L) and 31% (1.1 mmol/L) above their LDL-C target levels, respectively.

Conclusions:
In light of the new guidelines, these results suggest that a considerable percentage of patients will fall into the category of “not at goal” LDL-C. Patients that failed to achieve goal were also far above their LDL-C target levels. The adoption of the new guidelines will necessitate enhanced disease management to reduce the disease burden.
Occurrence of Postpartum Depression and Concomitant Vulnerability Factors in the Johannesburg Area.

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Purpose:
Postpartum depression (PPD) is a moderate to severe depression that occurs gradually after the second or third week postpartum. Failure to identify the disorder is a cause for clinical concern since PPD contributes to significant morbidity in both mother and infant. Traditionally the worldwide prevalence of postpartum depression (PPD) is cited as 10-15%, however meta-analysis reveals a wide range; from 0.5% to 60% in selected populations. Cross-cultural differences in both biological and social factors have been cited as possible causes of the disparity, thus research on the identification of vulnerability factors is important to enhance both prevention and treatment strategies.

Methods:
Health-Care Facilities and Participants: Mothers were invited to participate in the study when they brought their children to immunization clinics for routine vaccination. Clinics involved in this pilot project included Crosby, Alexandra (8th Avenue), Baragwanath, Johannesburg General, and Alexandra (Hospital).

Rating Scales: Volunteers completed a demographic questionnaire, the Edinburgh Postnatal Depression scale (EPDS), as well as the World Health Organization – 5 (WBI-5) wellbeing index. “Caseness” for PPD was identified for an EDPS score of ≥12 and a WBI-5 score of <13.

Statistical Analysis: Results were analysed using Instat-GraphPad Prism software version 3.0. A value of \( p < 0.05 \) was considered significant. Variables obtained from the analysis of the questionnaires were analyzed using student’s t-tests, ANOVA, and regressional analysis.

Results:
The sample comprised 141 participants. The occurrence of PPD was determined as 29.3% and 22.7% exhibited poor wellbeing.

Participant Profile: Ninety-two point five percent of the pilot sample was Black; the most common language spoken was Zulu (31.8%) followed by Sotho (12.8%) and Xhosa (12.2%). Forty-nine point seven percent of the women were single and 65.3% of the sample was unemployed. Of the participants with previous premenstrual dysphoric disorder, 34.7% developed PPD and 77.8% women with a previous psychiatric history showed PPD caseness.

Infant Description and Birth Event: There were no statistically significant correlations for PPD caseness with infant characteristics, parity, mode of delivery, analgesia and presence of family or friend during delivery.

Social Circumstances: No significant relationship existed between the number of people living in a household against PPD (\( R^2 0.24 \)) or wellness (\( R^2 0.2557 \)). Thirty percent of those identified with PPD lived in a formal setting with whereas 28.2% with PPD lived in an informal setting.

Conclusion:
The most significant vulnerability factor for development of PPD was a previous or family history of depression.
DOES LEVODOPA ENHANCE NEURONAL DEATH IN PARKINSON’S DISEASE?

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Purpose:
Parkinson’s Disease (PD) is characterized by a profound loss of dopaminergic neurons and a significant reduction in striatal dopamine content. Both the autoxidation and the monoamine oxidase (MAO) mediated metabolism of dopamine involve the production of hydrogen peroxide (H$_2$O$_2$), a compound that can easily be reduced in the presence of ferrous ions (Fe$^{2+}$) to form hydroxyl radicals via the Fenton reaction. Several studies have stressed an accumulation of iron in the substantia nigra as an essential factor in the pathogenesis of PD. It has also been postulated that hydroxyl radicals may convert dopamine (DA) to 6-hydroxydopamine (6-OHDA), a potent neurotoxin. Taking into account that the loss of dopaminergic neurons boosts both DA synthesis and turnover in surviving neurons, it seems plausible that the subsequent autoxidation and metabolism of DA in these cells could contribute to the progressive loss of dopaminergic neurons associated with PD. Levodopa (LD), the immediate precursor of dopamine is used in the treatment of PD. LD elevates striatal dopamine levels and could therefore also accelerate the disease process in the same way. In an attempt to assess the capacity of dopamine to cause oxidative stress, this study includes an investigation into the ability of DA to generate ·OH and the ability of ·OH to convert DA to 6-OHDA. It also involves the study of the capacity of DA to affect lipid peroxidation, protein oxidation and GSH levels in rat brain homogenate. In all these cases, the effects provoked by the presence of Fe$^{2+}$ and H$_2$O$_2$ have also been investigated. The effects of DA on these biological molecules in vitro were then compared to the effects observed after the intraperitoneal administration of LD.

Methods:
Male Wistar rats were used during the study. The formation of 6-OHDA from DA was assessed using a validated high performance liquid chromatography (HPLC) method with electrochemical detection (ECD). The detection of the hydroxylated products of salicylic acid with HPLC-ECD was used to assess hydroxyl radical formation. Analysis of lipid peroxidation was done using the TBA assay. The carbonyl content of proteins was taken as a measure of protein oxidation and the thiol content was used to estimate GSH levels.

Results:
The results of the research confirm the previous reports that DA can be converted to 6-OHDA by ·OH in vitro. The results also show that DA is able to enhance ·OH production by Fe(II)-EDTA/H$_2$O$_2$. Despite the increase in ·OH, DA is a potent inhibitor of lipid peroxidation mediated by Fenton chemistry. DA did however boost the carbonyl content and lower the thiol content of rat brain proteins in the presence of Fe(II)-EDTA/H$_2$O$_2$. Interestingly, no 6-OHDA could be detected in vivo following intraperitoneal LD administration. LD administration also did not seem to have any detrimental effects on lipids, proteins or GSH levels.
ABSORBATOX® 2.4D REDUCES THE SYMPTOMS IN ENGORD: A PLACEBO CONTROLLED STUDY.


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Introduction
The effects of cation exchangers have been described in the treatment of patients with heartburn. Absorbatox® is an alumino-silicate of which the cation binding properties have been significantly enhanced by a patented process.

The aim of this study was to evaluate the effects of Absorbatox® in patients with endoscopically negative gastro-oesophageal reflex disease i.e. severe heartburn but with no endoscopic lesion.

Methods
In this double blind placebo controlled pilot study 25 volunteers were recruited after negative upper GI tract endoscopy and being diagnosed as having ENGORD by a gastroenterologist. All patients (>18 years) were prescribed esomeprazole 20 mg as standard of care to be taken as needed once daily. Patients were then randomized to receive either placebo capsules or 750 mg Absorbatox® 3 times per day for 14 days. Patients had to score severity of symptoms and use of esomeprazole on a daily diary card. The main outcome was severity of symptoms.

Results
Twenty patients completed the study. Absorbatox® treated patients reported a significant reduction in severity of symptoms (p < 0.05) and symptom free days improved by 68% compared to the group who received placebo.

Conclusion
Absorbatox® significantly reduces symptoms of ENGORD and should be evaluated further in a larger trial. The apparent mechanism of action is not clear but may be partially ascribed to hydrogen ion trapping.
Prescribing Patterns of Combination Analgesics to a Medical Aid Patient Population in South Africa

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Background:
The avoidance of pain is one of man’s most basic drives and one of the primary reasons for seeking medical help. Six rationales for using combination (polycomponent or compound) analgesics have been identified. The South African analgesic market contains a large number of combination products, many of which contain meprobamate as a constituent.

Aim:
The primary aim of the study was to determine the extent and pattern of combination analgesic prescribing in a South African patient population and to compare it with the results of previous South African studies.

Method:
A retrospective, cross-sectional drug utilisation study was conducted over a period of one year. Computerised medication records for 2006 were obtained from a large prescription database of a medical aid administrator in South Africa. The database contained 2 070 678 records for medication and procedures.

Results:
A total of 141 659 analgesics were prescribed. Combination analgesics with either paracetamol or aspirin as the main active ingredient accounted for 64.37% of analgesic prescriptions, single component analgesics and antipyretics for 24.70%, and narcotic analgesics for 10.93%. Most of the combination analgesic trade name products were available as tablets and most products contained paracetamol as the main constituent. Just over half of the patients (53.00%) who were prescribed combination analgesics were males, which is in contrast with the results of previous South African studies in which females were prescribed proportionally more combination analgesics. The average age of female and male patients was similar (42.32 (SD = 14.17) years for females and 42.50 (SD = 12.25) years for males). Most patients (85.66%) were 18 years or older. Nearly a third (31.92%) of the combination analgesics prescribed were available without a prescription. Some products had exactly the same dosages of active ingredients but were marketed under different trade names. Three of the trade names prescribed, constituted more than a quarter (29.17%) of the prescriptions, indicating that medical practitioners showed a preference for certain trade names of combination analgesics.

Conclusion:
A wide variety of combination analgesics are available in South Africa. Further studies on combination analgesic prescribing should be undertaken, especially in relation to the diagnoses for which these products are prescribed.
The toxicity profile of a carbohydrate derived fulvic acid (CHD-FA)

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Humic substances are a group of ubiquitous compounds formed during the decay of plant and animal residues in the environment. These substances can be divided into humic acid, fulvic acid and humin on the basis of its solubility in water as a function of pH. Fulvic acid is the fraction that is soluble in water under all pH conditions. Naturally occurring fulvic acid and those derived from bituminous coal are characterized with high levels of heavy metals. These heavy metal levels have become a point of concern. To address this problem an unique method to derive fulvic acid from a carbohydrate source has been developed and patented.

The aim of this study was to examine the toxicity profile of this carbohydrate derived fulvic acid (CHD-FA). The study was conducted in three phases. In phase one, toxicity and sensitivity to topical application was examined by applying the product to the ears of mice over a period of 30 days. Phase two was undertaken to study the long-term effects (6-months) of CHD-FA ingestion (150 mg/kg body mass) whilst phase three tested the effects of CHD-FA ingestion on pregnant Sprague Dawley rats and their off-spring.

From the data obtained from phase one it is evident that CHD-FA, when applied topically, does not produce any hypersensitivity reactions and is non-toxic with regards to liver and kidney function in mice over a one-month period. Results obtained from the second phase indicate that the product is non-toxic with regards to liver and kidney function in rats over a period of six months, when administered orally (gavage). Finally, results gathered from phase three indicated no differences in the weight growth pattern of the pregnant females and morphological studies conducted on the organs of the pups did not uncover any developmental defects or pathological anatomical conditions associated with the CHD-FA. There was no significance differences observed in the litter numbers between the control and treatment groups and the pups did not exhibit any behavioral abnormalities.

On the bases of these results it can be concluded that CHD-FA is safe for use topically or orally and that CHD-FA is safe for use in pregnancy. This needs to be confirmed in a phase 1 clinical trial.
In Vitro Diffusion of a Series of Fluorescent-Labelled Polylysines Across Human Vaginal Mucosa

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Purpose:
The development of several therapeutic peptides (e.g. insulin, oxytocin, vasopressin) has provoked investigation into effective methods of delivery of these peptide drugs. Various compounds have shown that the vagina is an efficient site for drug delivery in women’s health. This study entailed the investigation, and comparison, of the in vitro diffusion of four fluorescent-labelled polylysines (MW 3080, 8570, 25 682 and 54 000) across intact human vaginal mucosa. The purpose was to determine in which molecular weight range peptides diffuse through vaginal mucosa.

Methods:
Fresh human vaginal mucosa was snap-frozen in liquid nitrogen and stored at -85 °C. Prior to an experiment, the tissue was defrosted to 20 °C in PBS buffer, pH 7.4, and placed in the seven flow cells of a flow-through perfusion apparatus. Fluos-polylysine 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. Analysis was done using a spectrofluorometer.

Results:
Flux values progressively increased throughout the 24-hour period and steady state was not reached for all but the 54 000 Da F-P-Lys. The mean estimated flux values (mean at 18, 20, 22 and 24h) increased with a decrease in molecular size. Whole curve comparison indicated statistically significant differences (P < 0.05).

Conclusions:
All four polylysines diffuse across vaginal mucosa. As expected the smaller the molecule, the greater its diffusion was across the vaginal mucosa. The mean estimated steady state flux values for polylysines in the MW range of 3000 to 10 000 Da were approximately 5.5 times greater than those for polylysine molecules in the MW range of 25 000 to 55 000 Da.
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