greatest number of active constituents. A simple methanolic extraction (10 mL) of dried *M. recutita* L. flower heads (3 g; harvested at bud, early flower, full flower and late flower) was carried out according to Pasquali et al (2003), before isolation using TLC in toluene–acetic acid (4:1). Characterisation was based upon R_f values of plant extract constituents and pure standards before final confirmation using λ_{max} (MeOH) values. HPLC retention times were also recorded using a reverse phase, gradient system of acetic acid–water according to Greenham et al (2003). All measured data were compared with literature values to confirm the presence of active constituents. Constituents identified include apigenin-7-*O*-glucoside, (-)-α-bisabolol, bisabolol oxides A and B, caffeic acid, chamazulene, herniarin, luteolin, cis-en-in-dicycloether and trans-en-in-dicycloether as expected (Willuhn 2004), showing the *M. recutita* L. samples analysed to be of good pharmaceutical quality. Indications are that harvesting could take place earlier in the growth cycle and still contain the major constituents required for biological activity and standardisation. A key finding to support this was the presence of apigenin-7-glucoside only in the budding stage of growth and not in full flower as expected. As commercial products of the plant are often standardised to apigenin-7-glucoside content this warrants further detailed investigation to include samples of *M. recutita* L. from a variety of sources and quantification of the active constituents. The chromatographic profiles generated at each stage of growth could also be used as a quality control tool to detect possible adulteration of German chamomile samples with related plant species (e.g. feverfew), for which profiles have already been developed (Pasquali et al 2003).

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Effects of *Hypoxis hemerocallidea* (Fisch. & C. A. Mey) Corm ('African potato') aqueous extract on renal electrolyte and fluid handling in the rat

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Recent biomedical evidence suggests that the corm of *Hypoxis hemerocallidea* Fisch. & C. A. Mey (family: Hypoxidaceae) has antidiabetic potential and may be useful in the management of adult-onset, non-insulin-dependent, type-2 diabetes mellitus (Mahomed & Ojewole 2003). This 'miracle ' medicinal plant of southern Africa is a tuberous, perennial herb with long, strap-shaped leaves and yellow, star-shaped flowers (Van Wyk et al 2002). The traditional health practitioners of southern Africa have widely employed the tuberous rootstock (i.e., the corm – popularly known as 'African potato') of the herb as a muthi (the South African isiZulu word for medicine) for an array of human ailments. Currently, the humble African potato (AP) has been claimed to be an amazing and wonder plant medicine in the fight against various modern human disorders, including HIV/AIDS-related diseases, arthritis, hypertension, diabetes mellitus, cancer, psoriasis, gastric and duodenal ulcers, tuberculosis, and so on (Van Wyk et al 2002). However, biomedical literature reports have indicated that certain herbal extracts and plant products attenuate the deterioration of kidney function associated with diabetes mellitus. This study was, therefore, undertaken to examine the effect of short- (acute) and long-term (chronic) administration of *Hypoxis hemerocallidea* corm aqueous extract (APE) on renal electrolyte and fluid handling in male Wistar rats. To establish the acute effects of APE, separate groups of anaesthetized control and test rats were challenged with continuous jugular infusions of 0.077 M NaCl at 9 mL h^{-1}. After 3 ½ h equilibration period, consecutive 30-min urine collections were made over the subsequent 4 h of 1 h control, 1 ½ h treatment and 1 ½ h recovery periods for measurements of urine flow and Na^+ and K^+ excretion rates. In the test rats, APE was added to the infusate at doses of 90, 180 or 360 μg h^{-1} for 1 ½ h during the treatment period. For chronic studies, individually-caged rats were treated with APE (30 mg kg^{-1} p.o.) every third consecutive day at 0900 h, followed by the same APE dose (30 mg kg^{-1} p.o.) 8 h later in the day, for 5 weeks. Control rats were given distilled water (3 mL kg^{-1} p.o.). Urine volume and total urinary outputs of creatinine, Na^+ and K^+ were determined from 24-h samples. Acute administration of graded doses of APE provoked a dose-dependent, significant ($P < 0.05$–0.01) decreases in urine flow and urinary Na^+ and K^+ excretion rates. Chronic APE treatment significantly ($P < 0.05$) reduced urinary Na^+ output from the 2nd to the 5th week, without affecting urine flow or K^+ excretion rates. By comparison with control rats, APE significantly ($P < 0.05$) increased plasma creatinine concentration (68 ± 6 μmol L^{-1} vs 55 ± 3 μmol L^{-1}, n = 6 in all groups) with a concomitant reduction in glomerular filtration rate (GFR) by the end of the 5th week (1.52 ± 0.02 mL min^{-1} vs 2.54 ± 0.09 mL min^{-1}). The results of this experimental animal study appear to suggest that APE impairs some functions of the kidney.

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Deregulated glucose transport as a target for anticancer drug development: use of a Glut-1 over-expressing colon carcinoma cell line to screen tyrosine kinase inhibitors for Glut-1-dependent toxicity

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Malignant cells show a significant increase in glucose uptake and metabolism, a phenomenon described as the Warburg Effect, which reflects a switch to anaerobic glycolysis. This change, which is mediated in part by the overexpression of the facilitative glucose transporter Glut-1, occurs in response to tumour hypoxia, oncogene expression and the production of certain growth factors. Glut-1 is an important tumour biomarker, being overexpressed in a wide range of tumours, and correlating with poor prognosis(Airley et al 2001). Therefore, Glut-1 may be an attractive target for novel anticancer agents. Glut-1 is made of two sub units each possessing an active site to which AMP or ATP can bind. Binding of ATP to the site induces a conformational change that reduces glucose uptake. ATP-binding tyrosine kinase inhibitors, such as quercetin, directly bind to and inhibit Glut-1 at its ATP-binding site (Vera et al 2001). Further, certain clinically used tyrosine kinase inhibitors are known to target the ATP-binding sites of tyrosine kinases, such as imatinib (Glivec), which targets the oncogenes C-kit or /bcr-abl. Phase 1 studies of ST1571 in gastrointestinal stromal tumours have shown a rapid decrease in fluorinated deoxyglucose (FDG) uptake by tumours, sometimes as early as 24 h after administration of drug. This may be due to a combination of a decreased tumour volume and a drug-induced, Glut-1-mediated reduction in FDG uptake by the tumour. It may be possible, therefore, that the level of Glut-1 expression in individual tumours may influence the activity of such tyrosine kinase inhibitors. To test this hypothesis, MTT and clonogenic cell survival assays were carried out following 24-h exposures to quercetin (0–1000 μM) and imatinib (0–100 μM) in the human colon carcinoma HT-29 cell line (designated GLAR-HT29), which we have genetically manipulated to constitutively overexpress Glut-1, relative to wild type cells. To evaluate the influence of hypoxia, cells were also exposed to drug in normoxic and anoxic conditions. IC50 data (Table 1) show that although cells were significantly less sensitive to quercetin after exposure to anoxia alone ($P = 0.017$), overexpression of Glut-1 increased sensitivity in both normoxic ($P = 0.03$) and anoxic ($P = 0.06$) conditions. Although imatinib showed greater toxicity, this was not influenced by the level of Glut-1 expression or the level of oxygenation. It has been observed previously that the HT29 cell line constitutively overexpresses c-kit, the major target of imatinib, which may override any Glut-1-dependent toxicity. However, Glut-1 clearly influences toxicity to quercetin. Structurally related ATP-binding TKI's, which may partially exert toxicity through glut-1 inhibition, may therefore be used in the future as possible lead compounds in the design of Glut-1 inhibiting novel anticancer agents.

Table 1 IC50 values under normoxic and anoxic conditions

Cell line	Drug	I	C50 (μM)
HT29 WT	Quercetin	Normoxia:	642.9
		Anoxia:	1200.1
HT29 WT	Imatinib	Normoxia:	181.9
		Anoxia:	274.8
GLAR-HT29	Quercetin	Normoxia:	492.9
		Anoxia:	631.1
GLAR-HT29	Imatinib	Normoxia:	265.1
		Anoxia:	259.9

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Ascorbic acid toxicity in tumours: role of facilitative glucose transporter Glut-1 and hypoxia

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Facilitative glucose transporter Glut-1 is overexpressed and confers poor prognosis in a wide range of tumours (e.g. cervix, breast, lung and bladder). Glut-1 is regulated through many pathways, and follows malignant transformation by viruses, amplification of oncogenes and increased production of growth factors. The control of Glut-1 by hypoxia via the transcription factor HIF-1 (hypoxia-inducible factor 1), however, has led to its use as a marker of hypoxia, which is a microenvironmental feature specific to malignant tissue that leads to increased malignancy, metastasis and treatment resistance (Airley et al 2003). The HIF-1 transcription factor consists of two subunits – HIF-1α and HIF-1β. The HIF-1α subunit is stabilised in hypoxic conditions, being rapidly degraded in normoxia via the ubiquitin proteasomal pathway. Degradation of HIF-1α depends upon a prolyl hydroxylase that is activated by ascorbate (Knowles et al 2003). In normal tissue, ascorbate enters cells via ascorbic acid transporters (SVCT's). However, tumour cells take in ascorbic acid in its dehydroascorbate form, via the HIF-1 regulated Glut-1, where reduction to ascorbate occurs intracellularly (Agus et al 1999). Therefore, it is possible that the induction of Glut-1 expression in hypoxia may serve as a feedback mechanism by which HIF-1 activity is decreased upon reoxygenation. To test this hypothesis, preliminary experiments were carried out to determine the effect of constitutive Glut-1 overexpression upon ascorbate-mediated toxicity in tumour cell lines. To determine the role of HIF-1 activity and hypoxia-inducible Glut-1, experiments were carried out using the prostate carcinoma PC-3 cell line, which we have shown previously, by immunohistocemical analysis, to upregulate Glut-1 in anoxic conditions. However, due to an amplification of the HIF-1α gene, PC-3 cells possess large quantities of functional HIF-1 even in normoxic conditions. To evaluate the role of Glut-1 specifically, we used the colon carcinoma HT-29 cell line, from which we have derived a stable clone that constitutively overexpresses Glut-1 (HT29-GLAR). Neutral Red proliferation assays were carried out to examine the toxicity of ascorbic acid in cloned versus wild type cell lines, in normoxic and anoxic conditions. HT29-GLAR cells were significantly more sensitive to ascorbic acid compared with wild type (IC50 = 28.330 mM (n = 3, s.d. = 1.191) for HT29-GLAR, 75.095 mM (n = 2, s.d. = 5.762) for HT29 WT) in normoxia. Further, although PC-3 cells were slightly more sensitive to ascorbic acid, toxicity did not appear to be affected by the level of oxygenation (IC50 in anoxia, 10.840 (n = 1) vs 12.610 (n = 2, s.d. = 0.127) in normoxia). Although the precise mechanism of ascorbate toxicity is unknown in these experiments, the data suggests that Glut-1 expression leads to increased accumulation of ascorbate by the cell, which may in turn serve a critical role in HIF-1-dependent survival pathways. However, the data obtained using the PC-3 cell line shows that, either the ascorbate toxicity is independent of anoxia-induced cellular changes like Glut-1 upregulation, or that any effect on HIF-1 levels is overridden by the vast amplification of HIF-1 gene expression present in this cell line. This study justifies further work, which will aim to relate Glut-1 over-expression, and the resulting intracellular accumulation of ascorbate to HIF-1 prolyl hydroxylase activity.

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Bone marrow hypoxia as a target in acute lymphoblastic leukaemia: toxicity assays using the bioreductive cytotoxic agent tirapazamine

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Acute Lymphoblastic Leukaemia (ALL), a haematological malignancy of immature lymphocytes, is the most common type of cancer in children. Tumour hypoxia, traditionally a feature of solid tumours, arises when rapidly proliferating cells outgrow their blood supply, tumour vasculature being characteristically poorly organised, torturous and lacking vasomotor function. Hypoxia leads to resistance to radiotherapy and chemotherapy, and as tumour cells adapt to the harsh tumour microenvironment, molecular changes take place, which confer increased malignancy and likelihood of metastasis. One therapeutic strategy, which aims to overcome and exploit tumour hypoxia, is the use of hypoxia-activated bioreductive drugs such as tirapazamine (TPZ), which is currently undergoing phase II clinical trials in lung tumours (Brown & Wang 1998). Recent work has revealed that the bone marrow of leukaemic patients shows hypoxia-associated changes, including increased expression of the transcription factor HIF-1 (hypoxia-inducible factor) (Wellmann et al 2004). Further, HIF-1 induces overexpression of hypoxia-regulated genes, such as the facilitative glucose transporter Glut-1, which we have found previously to be expressed in 3/10 cases in a small series of trephine biopsies taken from ALL patients. The aim of this work was therefore to determine the potential of bone marrow hypoxia as a therapeutic target in ALL, by determining the sensitivity of ALL cell lines to TPZ. In this study, MTT proliferation assays were carried out to determine the viability of two T cell leukaemia cell les (MOLT-3 and MOLT-4) after 3 h exposure to TPZ in normoxic and anoxic conditions. For comparison, the assay was also carried out using the colon carcinoma HT-29 cell line. IC50 values (Table 1) demonstrate that although the MOLT-3 and MOLT-4 cell lines were slightly more sensitive to TPZ in anoxia, the hypoxic cytotoxicity ratio was poor (1.6 for MOLT-3, 3.2 for MOLT-4 cells). In comparison, although the leukaemia cell lines were more sensitive to TPZ in anoxia than the HT29 cell line, the latter showed negligible sensitivity to TPZ in normoxic conditions. The hypoxic cytotoxicity ration is an important determinant of selective toxicity when using hypoxia-selective bioreductive drugs, where toxicity to normoxic cells potentially leads to adverse drug reactions. Although we have demonstrated the sensitivity of ALL cell lines to TPZ, the lack of selectivity to anoxic cells might indicate a consequent adverse effect on normal bone marrow.

Table 1 IC50 of tirapazamine in normoxia and anoxia

Cell line	IC50 (μM)		Hypoxic cytotoxicity ratio
	Normoxia	Hypoxia	
MOLT-3	34.0	21.5	1.6
MOLT-4	30.7	9.7	3.2
HT29	ND	54	N/A

ND, not determined. HT29 cells showed negligible toxicity in normoxic conditions, therefore a hypoxic cytotoxicity ratio was not obtained

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Efficiency of perindopril in sustaining the beneficial effect of endoventricular circular patch plasty and coronary artery bypass graft surgery in patients with left ventricular dysfunction and apical aneurysm

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Endoventricular circular patch plasty (EVCPP) is a widely used surgical procedure developed by Dor et al (2001) for treating left ventricular (LV) systolic dysfunction (LVD) associated with LV apical aneurysm (LVAA). However, pharmacological measures are required to sustain its beneficial effects. Angiotensin converting enzyme inhibitors (ACEIs) are recommended for all patients with LVD whether they are symptomatic or not. ACEIs vary in the degree of improvement they produce. Therefore, the study was carried out to compare the efficiency of two ACEIs in sustaining the beneficial effects of EVCPP along with coronary artery bypass graft (CABG) surgery in patients having ischaemic LVD and LVAA. It was a randomized, parallel group design and open label study. Study protocol was approved by the ethics committee of the hospital and patients' written informed consent was taken. The study comprised of 45 patients undergoing EVCPP, as well as CABG, and having LVD with LVAA from November 2003 to October 2004. They were randomized into group-I (n = 23) receiving captopril and group-II (n = 22) receiving perindopril. Patients were evaluated 1–2 days before surgery and 3- and 6-months following surgery and ACEI administration with respect to 2D echocardiography and colour Doppler assessment and functional status as per New York Heart Association class for heart failure. Age, gender ratio and disease modifying risk factors were comparable in both the groups. EVCPP, along with CABG and ACEI administration, produced favourable effects on LV

function and LV remodeling, as well as functional status, as there was improvement in these parameters in both the groups following 3-months of surgery and ACEI administration. At 6-months following surgery and ACEI administration, the perindopril-treated group showed significant ($P < 0.05$) improvement from baseline status and also from 3-month status in ejection fraction (baseline to 3-months to 6-months: group-I, $22.8 \pm 0.8\%$ to $31.0 \pm 1.1\%$ to $32.0 \pm 0.9\%$; group-II, $23.5 \pm 1.7\%$ to $33.8 \pm 2.4\%$ to $39.3 \pm 1.2\%$), wall motion score index (baseline to 3-months to 6-months: group-I, 2.04 ± 0.27 to 1.54 ± 0.22 to 1.1 ± 0.19; group-II, 2.23 ± 0.26 to 1.52 ± 0.21 to 1.0 ± 0.12), LV end diastolic diameter (baseline to 3-months to 6-months: group-I, 59.8 ± 1.5 mm to 53.1 ± 1.8 mm to 56.3 ± 2.7 mm; group-II, 60.1 ± 2.6 mm to 57.0 ± 1.9 mm to 52.0 ± 1.2 mm), LV end systolic diameter (baseline to 3-months to 6-months: group-I, 47.1 ± 2.1 mm to 40.7 ± 2.5 mm to 42.0 ± 3.7 mm; group-II, 46.3 ± 2.3 mm to 40.8 ± 1.3 mm to 36.1 ± 1.8 mm) and also in functional status (baseline to 3-months to 6-months: group-I, 3.44 ± 0.18 to 2.56 ± 0.18 to 1.9 ± 0.18; group-II, 3.0 ± 0.23 to 2.0 ± 0.19 to 1.2 ± 0.13). There was a more remarkable improvement in mitral valve regurgitation (MR)-grade in the perindopril-treated group after 3-months and 6-months of treatment. Four patients died (two from each group) during postoperative in-hospital stay, while the remainder had a smooth course. Follow-up of about 11 months, on an average, has shown no mortality or major cardiac adverse events in others. In conclusion, our study suggests that captopril and perindopril are both effective in maintaining the beneficial effect of EVCPP performed along with CABG. However, perindopril is more efficient than captopril in sustaining the improvement brought about by the surgical procedure on LV function, LV remodeling and MR-grade, as well as functional status, in patients having LVD and LVAA.

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Effect of progesterone on 17β oestradiol-induced relaxation of rat intestinal smooth muscle

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Many epidemiological studies suggest that oestrogens in hormone replacement therapy (HRT) protect women from coronary heart disease: a component of this cardioprotection appears to involve vasodilatation. However, the Women's Health Initiative (WHI) study, in which oestrogen plus a progestin was taken, has been terminated prematurely, partly due to increased incidence of cardiovascular disease. The properties of synthetic progestins and their metabolites differ greatly and it is not established whether all forms of HRT containing progestins cause adverse cardiovascular effects. It is possible that progestins in HRT could affect the beneficial relaxant effects of oestrogens by direct action on vascular muscle or indirectly via nitric oxide. A previous study showed potentiation, by progesterone, of oestrogen effects on endothelium-dependent production of the vascular relaxant, nitric oxide (Simoncini et al 2004). Whereas Cox et al (2005) recently reported that progesterone decreased nitric oxide synthase in vascular endothelium, with a detrimental effect on vasorelaxation. We previously showed that oestradiol and some metabolites elicit similar relaxant effects in both vascular and intestinal muscle preparations (McCurrie et al 2004). To study the direct effects of progesterone on oestrogen-induced smooth muscle relaxation, in the absence of endothelium-derived factors, we used a simple longitudinal smooth muscle preparation from rat ileum, contracted by carbachol. Segments of terminal ileum from male Hooded-Lister rats (250–350 g) were studied in Krebs'solution (37°C, 95% O_2, 5% CO_2) containing 10 μM indomethacin under 1 g tension. Control concentration–response curves were constructed to carbachol (0.1–30 μM) and repeated in the presence of one of the following: 17β oestradiol (EST, 4 or 8 μM), progesterone ((4-Pregnene-3,20-dione; 4 or 8 μM) or a combination of either EST (4 μM) plus progesterone (4 μM) or EST (8 μM) plus progesterone (8 μM). Steroids were incubated with tissues for 20 min before construction of the second concentration–response curve. No vehicle (EST, 60% alcohol; progesterone, 80% alcohol in water) or time-dependent effects were observed, $N = 5$. Oestradiol (4–8 μM) caused rightward shifts in the carbachol concentration–response curve and concentration-dependent reduction in Emax: Emax was reduced to $71.2 \pm 3.1\%$ ($P < 0.05$). Progesterone (4–8 μM) produced similar concentration–dependent reduction in Emax to $62.5 \pm 5.7\%$ ($P < 0.01$, compared with control). A combination of EST (4 μM) and progesterone (4 μM) also displaced carbachol concentration–response curves to the right and reduced Emax to $74.1 \pm 3.8\%$, which was not significantly different from the reductions observed with EST (8 μM) alone (Student's unpaired t-test). These results show that both oestradiol and progesterone cause similar concentration-related relaxation of intestinal smooth muscle and that addition of progesterone does not significantly change oestrogen-induced relaxation in this preparation. We previously showed that ileum smooth muscle responds to oestrogens in a manner similar to vascular smooth muscle (Mccurrie et al 2004) and conclude that natural progesterone is unlikely to antagonise the beneficial relaxant effects, on blood vessels, of the oestrogens in HRT. However, there are differences between vascular and intestinal muscle. It is also possible that the synthetic progestins present in the wide range of HRT preparations available could affect vascular relaxation produced by oestrogens, since the properties of progestins differ considerably, particularly in androgenic and glucocorticoid activity.

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Effectiveness and tolerability of selective and non-selective antimuscarinic agents in the treatment of clozapine induced hypersalivation

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The atypical antipsychotic clozapine has a complex pharmacological profile (including dopamine D_2, serotonin 5-HT_{2A} and muscarinic M_1/M_3 receptor antagonism, in addition to muscarinic M_4 receptor partial agonism). Despite being associated with a low incidence of extrapyramidal movement disorders, it has a number of other adverse effects, including hypersalivation, which has an estimated incidence of up to 80% (Lieberman 1998). The mechanism underlying this effect is poorly understood, but may involve increased saliva flow, an alteration in the swallowing reflex, or changes in circadian rhythm. Management often involves administration of non-selective or selective muscarinic receptor antagonists, despite the relative lack of evidence to support their use. The aim of this study was to evaluate patient perception of the effectiveness and tolerability of antimuscarinic treatments for clozapine induced hypersalivation. A structured interview was used to assess the degree of hypersalivation experienced by patients from an acute mental health trust who were maintained on clozapine. The effectiveness and tolerability of both non-selective and selective antimuscarinic agents in managing this condition were analysed using a ten point analogue scale (ranging from "no effect" to "very severe"). Fifty-one patients agreed to be interviewed, 48 (94%) of whom described symptoms of hypersalivation. Twenty were treated for hypersalivation with non-selective antimuscarinics, six received the selective muscarinic M_1/M_4 antagonist pirenzepine, while twenty-five patients did not receive drug treatment. Perception of the effectiveness of treatment with non-selective muscarinic antagonists (mean 4.8 ± 3.7) was not significantly different to that with pirenzepine (mean 7.0 ± 1.0), ($P > 0.05$ Mann–Whitney U test). Tolerability of the antagonist treatments employed was generally well-perceived, with only 3 of the 26 treated patients (11.5%), reporting any adverse effects. The only side effect noted was dry mouth, and all subjects complaining of this were treated with non-selective antimuscarinic agents. Both drug treatments showed some effectiveness in managing clozapine induced hypersalivation, and were generally well tolerated, as measured by patient perception. The effectiveness of pirenzepine, which has relatively low affinity for the M_3 receptor (associated with salivary secretion), suggests that clozapine may induce hypersalivation in a mechanism independent of increased saliva flow. This is consistent with the findings of Ben-Aryeh et al (1996), who reported no change in the salivary flow rate of patients treated with clozapine. In view of the relative muscarinic selectivity of pirenzepine, one might predict that it would be associated with fewer adverse effects (such as constipation and blurred vision) than the non-selective cholinergic antagonists used. Despite all of the reported side effects being associated with non-selective antimuscarinic agents, patients described no adverse effects other than dry mouth, suggesting that there was a lack of difference between the drug treatments in inducing other peripheral anticholinergic effects. This may reflect the main aim of the study, leading patients to focus their attention on dry mouth or hypersalivation. Alternatively, the pharmacological nature of clozapine, which is itself an antagonist at M_1 and M_3 muscarinic receptors (responsible for these adverse effects), may mask any autonomic effects resulting from administration of the drugs intended to treat hypersalivation.

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Actions of potassium channel modulators on mouse gastric fundus in vitro

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Potassium channels (K channels) are widely distributed in plasma membranes. One K channel, the ATP-dependent channel (KATP), has been extensively studied in many types of smooth muscle, but there are few reports on the characteristics of these channels in the gastrointestinal tract. KATP channels couple membrane excitation to cell metabolism: channel opening, induced by a fall in intracellular ATP, increases K^+ efflux causing hyperpolarisation, which protects cells from metabolic stress. K channel openers (KCOs) are chemically diverse agents, which target KATP channels and appear to have therapeutic potential as cardioprotective, vasodilator and bronchodilator agents, promoters of hair growth and insulin secretagogues. Previous research has shown that the KCOs, cromakalim and pinacidil, inhibit mouse gastric emptying in vivo (Yeung et al 2001). In these experiments we further explore gastrointestinal actions of KATP channel modulators on mouse stomach in vitro. Strips of longitudinally orientated gastric fundus from Bantin and Kingman mice (25–30 g) were studied under 0.5 g tension in Krebs' solution (37°C, 95% O_2, 5% CO_2). Non-cumulative concentration–response curves were constructed to carbachol (0.3–30 μM): a concentration of carbachol (1 μM) that produced 60% of Emax was selected for further experiments. Preparations were pre-contracted by carbachol (1 μM) and cumulative concentration–response curves constructed to pinacidil (PIN), cromakalim (CROM) or diazoxide (0.1–300 μM) in the absence or presence of glibenclamide (GLIB, 0.1–1 μM), a selective antagonist of KCOs (N = 4–7). Only one concentration of GLIB was used in each tissue. Relaxation is expressed as % reversal of contraction. No vehicle or time effects were observed. Pinacidil and CROM (0.1–300 μM) elicited concentration-dependent relaxation of the fundus, reducing maximal carbachol-induced contraction by $94 \pm 2.5\%$ and $91.3 \pm 3.6\%$, respectively. Diazoxide (0.1–300 μM), a relaxant agent in vascular muscle, was without effect on mouse fundus at these concentrations. Glibenclamide (0.1–1 μM) reduced the relaxant effects of CROM and PIN at all concentrations, causing reduction in Emax to $74 \pm 4.7\%$ and $80 \pm 2.6\%$, respectively, and non-parallel, rightward shifts in concentration–response curves to both agents. The EC50 for CROM and PIN increased from 6.4 μM and 8.7 μM, respectively, in the absence of GLIB to 80 μM and 146 μM, respectively, in the presence of the highest GLIB concentration (1.0 μM). These experiments showed that cromakalim and pinacidil are potent relaxant agents in the mouse gastric fundus, in agreement with results previously obtained in mouse ileum (Yeung et al 2002). Relaxation in the fundus was antagonised by glibenclamide in a concentration-related, but non-competitive, manner. However, in many tissues, including vascular muscle, glibenclamide is reported to be a competitive antagonist of KCOs. Diazoxide, known to be a potent dilator of blood vessels, was without relaxant effect on mouse stomach. These results confirm our previous observations on the inhibitory actions of pinacidil and cromakalim on mouse gastric emptying in vivo (Yeung et al 2001) and mouse ileum in vitro (Yeung et al 2002). However, the mechanism of action of these KATP channel modulators in the mouse gastrointestinal tract appears to differ from that reported for other types of smooth muscle.

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ACE-inhibitory activity of a captopril propyl ester prodrug

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Previously, Moss et al (2003) have described the synthesis and characterisation of a range of carboxyl-ester captopril prodrugs. Delivery of the angiotensin converting enzyme (ACE) inhibitor captopril would benefit from the zero-order kinetics associated with the transdermal route. The transdermal/prodrug route may also avoid the first dose hypotensive effect of ACE inhibitors therapy (Weber et al 2002). These prodrugs were designed rationally via a QSPR (quantitative structure-permeability) approach to have range of log P values (0.84–3.66) and to optimise percutaneous absorption. The metabolic fate of carboxyl-ester captopril prodrugs either in vivo or in vitro is unknown and is under investigation by this group. As part of this investigation we have examined the ACE-inhibitory effect of the propyl ester prodrug (PEP), compared with the parent drug, captopril. It has been reported that the carboxylic acid functionality in captopril is essential for good binding (Attwood et al 1984). Further, synthetic studies have shown that the stereochemical configuration of the captopril moiety has been maintained during esterification to produce PEP (Moss et al 2003). The candidate that has been chosen for further investigation is the propyl ester, as it was the prodrug exhibiting the optimum percutaneous permeability (Moss et al 2003). Captopril and PEP were examined quantitatively for their ability to inhibit angiotensin I-induced contractions of rat aortae in vitro. Thoracic aortae were obtained from male wistar rats (170–230 g) and were cut into helical spirals 50–70 mm in length. Each spiral was mounted under 500 mg passive tension in Krebs ringer solution maintained at 37°C and gassed with 95% O_2–5% CO_2. Contractile responses to angiotensin I and II were recorded isometerically. Angiotensin II produced dose-related contractions, which were unaffected by prior administration of captopril. Angiotensin-I induced contractions are, however, diminished in the presence of captopril, indicating the necessity of the conversion of angiotensin I to angiotensin II by endothelial ACE, and totally inhibited at 10^{-8} M captopril. PEP similarly reduced the responses to angiotensin I with total inhibition at 10^{-7} M. This suggests that the PEP retain some of the ACE-inhibitory properties of captopril despite the masking of the carbonyl functionality, while in vivo metabolism to captopril will result in full activity of the parent molecule.

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Synthesis and in vitro vascular response of a putative captopril methyl ester nitric oxide releasing prodrug, on aortic helical strips

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Delivery of the angiotensin converting enzyme (ACE) inhibitor captopril would benefit from the zero-order kinetics associated with the transdermal route. This route of administration may also avoid the first dose hypotensive effect of ACE inhibitors therapy (Weber et al 2002). The synthesis and characterisation of a range of carboxyl-ester captopril prodrugs has been described previously (Moss et al 2003). These prodrugs have been designed rationally via a quantitative structure-permeability relationship (QSPR) approach to have range of log*P* values from 0.84 to 3.66, allowing substantial enhancements to their percutaneous absorption. Synthetic studies have shown that the stereochemical configuration of the captopril moiety has been maintained (Moss et al 2003). Further to this, addition of a nitric oxide-releasing functionality to the captopril methyl carboxyl-ester prodrug has been proposed, using the method described previously (Ingram et al 2002). This putative prodrug (captopril Me-NO) offers enhanced percutaneous absorption compared with the parent drug. Further, it has the potential to release nitric oxide, providing synergistic therapeutic effects. Therefore, this study aims to investigate the relaxant effect of the captopril Me-NO prodrug on rat aortic helical strips. Isosorbide dinitrate was used to provide a suitable comparison. Thoracic aortae were obtained from male Wistar rats (170–230 g) and were cleaned of all connective tissue and cut into helical strips between 30–50 mm in length without deliberate removal of the endothelium. Each spiral was mounted under 500 mg passive tension in a 25-mL organ bath containing Krebs ringer solution maintained at 37°C and bubbled with 95% O_2–5% CO_2. Isometric tension was recorded with a force-displacement transducer (Swema) coupled to a Lectromed polygraph. The physiological solution used had the following composition (in mM): 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 12.5 $NaHCO_3$ and 11.1 glucose. Tissues were routinely allowed to equilibrate for 1 h before the experiments were started. After the equilibration period, cumulative concentration–response curves to phenylephrine were carried out for all the helical strips. The vasorelaxant effects of nitric oxide releasing prodrug were assessed against submaximally contracted tissue. Isosorbide dinitrate was able to induce up to 80% relaxation of the tissue at a concentration of 1×10^{-5} M. In comparison, the same concentration of captopril Me-NO induced 30% relaxation, demonstrating the vasodilator potential of the captopril Me-NO prodrug.

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Investigation of alkaloids from *Phyllanthus amarus* Schum. & Thonn.

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The *Phyllanthus* species has been found to exert a marked inhibitory effect on hepatitis B virus evident by its exhaustive use in Asian countries in chronic jaundice. *Phyllanthus amarus* Schum. & Thonn. has been reported to inhibit reverse transcriptase and its effect on the in vitro replication of a laboratory strain and clinical isolates of HIV has been evaluated (Notka & Meier 2004). Recently, the alkaloid extract of *P. niruri* L. was observed to have a suppressible activity on strains of HIV-1 in MT-4 cell lines (Naik 2003). The aim of this study was to investigate the alkaloid constituents is in the aerial parts of *P. amarus* Schum. & Thonn. This paper will report the isolation of its major alkaloids. The aerial part of *P. amarus* Schum. & Thonn. were collected in Ho Chi Minh City, Vietnam. A voucher specimen was deposited in the Herbarium of Department of Phamacognosy, University of Medicine and Pharmacy, Ho Chi Minh City, Vietnam. The alkaloidal fraction had a strong hepatoprotective activity. Procedures were carried out to extract these alkaloids. Procedure D, which gave a highest yield of alkaloids, was chosen for extraction on a large scale. According to this method, total alkaloids were extracted with chloroform after alkalizing the powder of *P. amarus* with 25% ammonia solution. The chloroform solution was evaporated under reduced pressure to the concentrated solution. This solution was distributed with 1% HCl solution (200 mL × 5). The combined acid layers were distributed with chloroform (100 mL, many times). The chloroform solution was concentrated to a syrupy extract. The syrupy extract was chromatographed on silicagel (0.040–0.060 μm) using increased ratios of mixtures of chloroform–methanol as mobile phase. Five following fractions, named I, II, III, IV, V, were collected. Fractions III and V were purified by column chromatography on Sephadex LH-20. Two pure alkaloids named Pa1 and Pa2 were obtained. By comparison with published MS and NMR data, Pa1 was identified to be nirurine, a known major alkaloid of *P. niruri* L., but not previously isolated from *P. amarus* Schum. & Thonn. (Houghton et al 1996). Further investigations on the structure elucidation of Pa2 and the pharmacology in vivo on cell lines of nirurine and Pa2 of this plant are in process.

Professor P. J. Houghton, King's College London; Chemical Institute of Hanoi, Vietnam for obtaining the NMR spectra.

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Poster Session 3 – Analytical Chemistry

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Comparison of near-infrared spectroscopic methods for the identification of pharmaceutical excipients and active drug ingredients

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Identification of excipients is commonly accomplished by invasive chemical methods. Near-infrared (NIR) spectroscopy is an ideal, versatile and rapid alternative that can be carried out in the warehouse or holding location of a manufacturing site so reducing the quarantine time. A key paper from Blanco & Romero (2001) proposed the use of cascading libraries for NIR spectroscopy. In this work, an NIR spectral library of excipients and active pharmaceutical ingredients (APIs) was constructed and used to compare five identification methods. Reflectance NIR spectra were collected over the range 1100–2500 nm on a FOSS 6500 Spectrophotometer fitted with a Rapid Content Analyser for 210 pharmaceutical compounds. Five pattern recognition methods were compared, based on second derivative spectra: Wavelength Correlation (WC); WC with wavelength selection (WCWS); Maximum Distance in Wavelength Space (MDWS); Peak Positioning (PP); and Soft Independent Modelling of Class Analogies (SIMCA). A reduced library of 55 compounds (containing at least eight batches of each substance) was used to optimise each method. The number of compounds correctly identified by each individual method is given in Table 1. SIMCA was the best method, but it required extensive data analysis for little advantage compared with the other methods. The next best method was PP; but it was discarded because robustness was an issue over time. MDWS came next, but multiple sample spectra are required for this procedure. While WC was the worst performing method, it was easy to update (i.e. add new compounds) and it also gave a good indication of the 'problem' compounds highlighted by the other methods. Identification was best performed by using a cascading approach, initially using WC to divide the spectral library into groups. Each group was then sub-divided sequentially using WCWS, MDWS and SIMCA. Where the group contained both chemically and physically different compounds, then WCWS was next applied. For groups containing only physically different compounds (e.g. particle size), or mixtures with different ratios of components, then MDWS was optimal. SIMCA was finally used for any groups that still remained unresolved. The procedure was initially applied to the library of 55 compounds; 36 compounds were correctly identified with the remainder grouped into starch and starch derivatives or compounds that differed only by the grade of material (Table 1). Applying the procedure to the 210 compounds, for which there were 1–91 batches for each compound, 167 compounds were correctly identified, leaving 43 that fell into 14 groups. Of these 14 groups, only one involved different chemical compounds (ceratonia, guar galactomannan and tara gum). In this study, there were insufficient batches for these compounds to allow further discrimination. In conclusion, while SIMCA is the best individual method, the cascading approach advocated is much better.

Table 1 Number of compounds identified using different pattern recognition methods

Procedure	Method				
	WC	WCWS	MDWS	SIMCA	PP
Single method (n = 55)	24	—	29	33	30
Cumulative procedure (n = 55)	24	29	33	36	—
Cumulative procedure (n = 210)	124	137	151	167	—

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Determination of anastrozole in rat plasma by liquid chromatography-tandem mass spectrometry with electrospray ionization

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A sensitive and specific liquid chromatographic-tandem mass spectrometry method was developed and validated for quantitation of anastrozole, a potent and selective aromatase inhibitor. Aromatase inhibitors are a class of compounds that act systemically to inhibit oestrogen synthesis in tissues by inhibiting aromatase, an enzyme that catalyses the conversion of androgens to oestrogen. The link between oestrogen and the growth and development of some breast cancers has long been recognised (Baum 2001) and there is substantial evidence that circulating oestrogens promote the proliferation of breast cancer. Many current therapies for breast cancer involve hormonal manipulation, with oestrogen-deprivation of the tumour being an established method of treatment. The objective of this study was to develop and validate an LC-MS/MS method for the determination of low concentrations of anastrozole in rat plasma to support formulation optimisation studies. Anastrozole and its deuterated internal standard were extracted from plasma by solid phase extraction on an automated Gilson 215 SPE workstation using 30 mg StrataX cartridges. The chromatographic separation was performed on reverse phase Aquasil C18 column with a mobile phase of acetonitrile/water (75/25, v/v). The mass spectrometer,