Hypoglycaemic effects of some medicinal plant extracts in streptozotocin-induced diabetic rats

By

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DECLARATION

I, **Mavuto Gondwe**, hereby declare that the dissertation entitled

'Hypoglycaemic effects of some medicinal plant extracts in streptozotocin-induced diabetic rats'

is the result of my own investigation and research and that it has not been submitted in part or in full for any other degree or to any other university. Where use was made of the work of others, it is duly acknowledged in the text.

Student: **Mavuto M. Gondwe**

Supervisor: **Professor CT Musabayane**

To my late grand mother and grand father; my mother and father;

My wife Lilly and

Francis, Enoch and Chitatata, for their love and support

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ABBREVIATIONS

α	Alpha
AGE	Advanced glycation end-product
AIDS	Acquired Immune deficiency syndrome
AMPK	adenosine monophosphate protein kinase
APE	African potato (*Hypoxis hemerocallidea*) ethanolic extract
ATP	Adenosine triphosphate
β	Beta
BB	Biobreeding
CCM	Complete culture medium
Cu^{2+}	Copper ion
CYP	Cytochrome P
DCCT	Diabetes Control and Complications Trial
dG	deoxyguanosine
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
EDRF	Endothelium - derived relaxing factor
EMEM	Eagle's Minimum Essential Medium
FTE:	*Ficus thonningi* ethanolic extract
GLUT-1	Glucose transporter-1
GLUT-2	Glucose transporter-2
GLUT-3	Glucose transporter-3
GLUT-4	Glucose transporter-4
GLUT-5	Glucose transporter-5
h	Hour

H_2SO_4:	Sulphuric acid
HBSS	Hank's balanced salt solution
HCl	Hydrochloric acid
HDL	High density lipoproteins
h-HSD	Hydroxysteroid dehydrogenase
HIV	Human immunodeficiency virus
HLA	Histocompatibility leucocyte antigen
IL	Interleukin
IR	Insulin resistance
IRS	Insulin receptor substrate
K^+:	Potassium
Kg:	kilogram
LDL	Low density lipoprotein
MAP	Mean arterial pressure
MHC:	Major histocompatibility complex
µg	microgram
µl	microlitre
mg	milligram
mmHg	Millimetres of mercury
mmol	millimole
MMP	Matrix metalloproteinase
MTT	(3,4,5-dimethylthiazol-2-yl)-5-5diphenyltetrazolium bromide
MODY:	Maturity Onset Diabetes of the Young
NaCl:	Sodium chloride
NADPH	Nicotinamide adenine dinucleotide phosphate

NF-κB	Necrosis factor- kappa B
ng	nanogram
NIDDM	Non-insulin dependent diabetes mellitus
NO	Nitric oxide
NOD	Non-obese diabetic
NPY	Neuropeptide Y
O_2^-	Superoxide
OGTT	Oral glucose tolerance test
$ONOO^-$	Peroxynitrite
PAE	*Persea americana* ethanolic extract
PAI-1:	Plasminogen activator inhibitor-1
PGI_2	$Prostaglandin_2$
PKC	Protein kinase C
PPAR-γ	Perixosome proliferator gamma receptors
RAGE	Advanced glycation end-product receptor
SBE	*Sclerocarya birrea* ethanolic extract
sc	Subcutaneous
SEM	Standard error of mean
SHR	Spontaneously hypertensive rats
STZ	Streptozotocin
TGF- β:	Transforming growth factor-β
TNF-α	Tumour necrosis factor- α
TZD	Thiazolidinedione
UKPDS	United Kingdom Prospective Diabetes Study (UKPDS)
UKZN	University of KwaZulu-Natal

USA.	United States of America
VEGF	Vascular endothelial growth factor
WHO	World Health Organisation
WKY	Wistar-Kyoto

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ABSTRACT

Medicinal plant use in Africa and elsewhere is on the increase partly because of inconsistent availability and prohibitive costs of synthetic medicines, and partly because they are thought to possess fewer side effects by comparison with allopathic medicines. Few studies, however, have evaluated their efficacy and toxicity. Accordingly, the present studies were designed to determine hypoglycaemic effects of ethanolic extracts of selected medicinal plants in STZ-induced diabetic rats. African potato (*Hypoxis hemerocallidea,* APE), avocado (*Persea americana,* PAE), marula (*Sclerocarya birrea,* SBE) and *Ficus thonningii* (FTE), are used by various populations in Southern Africa in the traditional management of a multitude of ailments including diabetes mellitus.

Oral glucose tolerance test responses (OGTT) were evaluated in separate groups of non-diabetic and STZ-induced diabetic rats starved for 18 h and loaded with 0.86 g/kg glucose followed by graded doses of APE, SBE, FTE and PAE (60, 120 and 240 mg/kg). Rats treated with deionised water (3 ml/kg) acted as control, while those treated with insulin (100 μg/kg), metformin (500 mg/kg) and glibenclamide (5 mg/kg) acted as positive control groups. Blood glucose concentrations were monitored at 15-min intervals for the first hour, and hourly, thereafter for 3 hours. Long term hypoglycaemic effects of the test extracts were investigated in non-diabetic and STZ-induced diabetic rats, chronically administered with test extracts (120 mg/kg, p.o.) once daily for 6 weeks. Acute effects of test extracts on fluid and electrolyte handling and blood pressure were assessed in anaesthetised non-diabetic and STZ-induced diabetic rats challenged with 0.077M NaCl (9 ml/h) in an 8 h protocol that consisted

of a 3.5 h-equilibration, 1 h control, 1.5 h treatment and 1.5 h recovery period. Solutions of test extracts (0.06 μg/min) were added to the infusate during treatment period. Urine sampling, for volume and electrolytes, and, blood pressure measurements were conducted at 30 min interval over the subsequent 4h of 1h control, 1h 30 min treatment, and 1 h 30 min post treatment. Long term effects of test extracts on fluid and electrolyte handling were conducted in non-diabetic and STZ-induced diabetic rats treated with test extracts at a dose of 120 mg/kg once daily for 6 weeks. In order to assess direct effects of test extracts on renal tubular cells, cytotoxicity studies were conducted by dye-reduction assay (MTT) on MDBK and LLCPK-1 cell lines exposed to graded doses (100 – 1000 μg/ml) for 24, 48 and 72 hours.

Acute and long term studies revealed that test extracts possess hypoglycaemic effects in non-diabetic and STZ-induced diabetic rats which are insulin independent. Long term investigations established that all test extracts increased hepatic glycogen concentrations in diabetic rats. The extracts demonstrated vasodepressive effects in non-diabetic and STZ-induced diabetic rats, probably mediated via reduction of peripheral vascular resistance. Short term studies revealed that all extracts except APE had no effects on fluid and electrolyte handling in the diabetic rat. *In vitro* cytotoxicity studies demonstrated that SBE and PAE were cytotoxic to renal tubular cells, whereas, *in vivo* observations revealed the extracts increased glomerular filtration rate (GFR), as assessed by creatinine clearance, suggesting renoprotective properties.

In conclusion, results have shown that *Hypoxis hemerocallidea, Persea americana,* (*Sclerocarya birrea,* and *Ficus thonningii* possess, hypoglycaemic and antihypertensive effects in experimental animals. These results, in part, explain the traditional use of these plants in the management of many diseases, including diabetes mellitus.

CHAPTER 1

1.0. INTRODUCTION AND LITERATURE REVIEW

1.1. Diabetes mellitus

Diabetes mellitus a metabolic disorder characterised by hyperglycaemia, that results from reduced cellular glucose uptake and metabolism due to impaired insulin secretion and/or action. An estimated 150 million people, within the age range 20-79 years, have diabetes at present and the prevalence is escalating (Ahmed and Goldstein, 2006; Gadsby, 2002), particularly in developing countries (Girach and Vignati, 2006; Kim, Kang, Seo, Choi, Choi and Ku, 2006). It is estimated that by 2025, three quarters of all diabetics will be resident in developing countries (King, Aubert, and Herman, 1998). The steady increase in prevalence is partly attributed to obesity in childhood and adolescence, urbanisation and aging of populations (Brosnan, Upchurch and Schreiner, 2001; King, Aubert, and Herman, 1998; Wild, Roglic, Green, Sicree and King, 2004). Diabetes is a major health concern because it is frequently associated with debilitating health complications such as blindness, kidney function failure, cardiovascular diseases, amputations, and subsequently, death. Quality and duration of life is considerably reduced in diabetics due to persistent hospitalizations and disability. In developing countries, one in ten deaths in economically active age range of 35 to 64 years can be attributed to diabetes (Roglic, Unwin, Bennett, Mathers, Tuomilehto, Nag, Connolly and King, (2005). Furthermore, diabetic complications create financial burden to health care systems (Eidi, Eidi and Zamanizadeh, 2005).

Medical care in diabetes is estimated at US$132 billion annually in the United States of America (Girach and Vignati, 2006).

1.1.1. Diabetes mellitus in Africa

There is paucity of studies that show prevalence and/or incidence of diabetes in Africa. Available data, however, show that generally prevalence is low in rural areas and high in urbanized communities. For example, prevalence of diabetes is 0.9% in rural Tanzania and reaches levels of 8.8 to 12.2 % in urban Tanzania (Abbas, Lutale, Gill and Archibald, 2001). In South Africa, urban Indians have a prevalence rate of nearly 20% (Forouhi and Wareham, 2006).

In the estimations of diabetic prevalence in a number of Sub-Saharan Africa, Tanzania was considered a model whose prevalence was extrapolated to several countries including Malawi Rwanda, Somalia, Uganda, Zambia Djibouti, Kenya, Madagascar, Mozambique, Burundi, Comoros, and the Democratic Republic of the Congo. Prevalence estimates for South Africa were extrapolated to Zimbabwe, Namibia, Lesotho and Swaziland. The criteria used were similarities in conditions including social economic standards, ethnic groups and geographical proximity (Wild, *et al.*, 2004). The seemingly low rates could be a true reflection of diabetes prevalence in sub-Saharan Africa or could merely imply poor prognosis (Beran, Yudkin, de Courten, 2006). Otherwise, prevalence in many developing countries point to an escalation (Abdelgadir, Elbagir, Eltom and Berne, 2006).

Due to generally low socio-economic conditions, the problem of diabetes mellitus in Africa is more acute (Ducorps, Ndong, Jupkwo, Belmejdoub, Poirier, Mayaudon and

Bauduceau, 1997). Risk factors for development of diabetes mellitus in Africa include excessive alcohol intake, smoking, obesity consumption of large food quantities that have toxic compounds to the kidney (Lasky, Becerra, Boto, Otim and Ntambi, 2002).

1.2. Glucose homeostasis

Glucose is the main fuel for most tissues. Oxidative metabolism of glucose allows production of adenosine triphosphate (ATP), which is an energy source for many chemical reactions in the body (Wolfe, 1998). Plasma glucose concentration is a reflection of the balance between glucose uptake in the gut, endogenous production, utilization and storage in the body (Woerle, Meyer, Dostou, Gosmanov, Islam, Popa, Wittlin, Welle and Gerich, 2003). Endogenous production of glucose is through gluconeogenesis and glycogenolysis, while tissue utilization is mainly mediated via glycolysis and, to a lesser extent, the pentose phosphate pathway. Excess glucose is converted in the body into glycogen and fat (Guyton and Hall, 2000).

Following ingestion of a meal, a third of glucose is taken up by the brain tissue where it undergoes complete aerobic breakdown to water and carbon dioxide, 2 to 3 hours later; another third is used by the muscle where it is converted to glycogen or catabolised to lactate or water and carbon dioxide; and the rest is taken up by the liver where it is mainly converted to glycogen and stored (Morifuji, Sakai and Sugiura, 2005). The liver, therefore, plays an essential role in the maintenance of blood glucose. It maintains blood glucose levels by maintaining a balance between glucose uptake and its conversion to glycogen and the release of glucose into circulation via glycogenolysis and gluconeogenesis (Saadat, Pournourmohammadi, Donyavi,

Khorasani, Amin, Salehnia and Abdollahi, 2004). The role of other tissues such as the kidney in endogenous glucose production is minimal. Nevertheless, the contribution of the kidney is significant during prolonged fasting (Tappy, Jéquier and Schneiter, 2000).

1.2.1. Glucose transport into cells

The lipid nature of cell membranes offers a barrier against free glucose entry into cells. There are 2 major types of glucose transporters that overcome this membrane barrier. Sodium–glucose co-transporters are mainly located in the kidney and intestines. These actively transport glucose into cells against concentration gradient by cotransporting sodium. GLUT transporters are a family of glucose transporters that transport glucose into cells passively, down a concentration gradient. They include GLUT-1 transporters which are located in the brain, erythrocytes and endothelial cell; GLUT-2, located in the kidney, small intestines, liver and pancreatic tissues; GLUT-3, expressed in the neurones and placenta; GLUT-4, expressed in the skeletal, cardiac and adipose tissue. Transport of glucose via GLUT-4 into muscle and adipose tissue is the rate-controlling step in insulin-mediated glucose disposal, and this disposal is compromised in conditions of insulin-resistance (Herman and Kahn, 2006). GLUT-5 transporters are located in the small intestine, sperm, brain, kidney and muscle (Shepherd and Kahn, 1999).

1.2.2. Insulin secretion

Insulin, produced by the beta (β)-cells of the pancreas is the principal hormone regulating glucose metabolism. Its presence enhances glucose uptake and metabolism by various tissue cells including skeletal muscle, white adipose tissue and the liver (Barros, Machado and Gustafsson, 2006). During non-pathological states, glucose is the stimulus for insulin secretion in pancreatic beta cells. Glucose entry into the pancreatic β-cells is through GLUT-2 transporters (Herman and Kahn, 2006). Oxidation of glucose and the subsequent increase in ATP/ADP ratio in the β-cells triggers closure of ATP-sensitive potassium channels (Doyle and Egan, 2003). Inhibition of potassium efflux results in cell depolarization leading to influx of voltage dependent calcium ions that stimulate extrusion of insulin. Physiological insulin secretion consists of a basal and bolus component. During fasting, basal insulin secretion maintains glucose levels between 70 and 120 mg/dl, and bolus secretion keeps glycaemic levels below 180 mg/dl after a meal (Fonseca, 2006).

1.2.3. The metabolic effects of insulin

The mechanism by which insulin influences glucose uptake in target tissues is that the binding of insulin to α-subunit part of their receptors, on the cell surface, autophosphorylates the β-subunit on tyrosine residues, intracellulary. This activates tyrosine kinase which further phosphporylates insulin receptor substrates (IRS) leading to a series of pathways that result into insulin signal transduction. Depending on the target tissue cell, the outcome of the signal transduction might be movement of GLUT 4, for instance, from intracellular pools towards the cell surface of skeletal

muscle and white adipose tissues, or reducing expression of gluconeogenesis genes in the liver (LeRoith and Gavrilova, 2006), or indeed, increasing levels and activities of glycogen synthase and glycogen phosphorylase in the liver (Barros *et al.*, 2006).

1.2.4. Insulin regulatory and counteregulatory hormones

β-cells are located in the islets of Langerhans in the pancreas. Other cell types present in islets include α, δ and pancreatic polypeptide (PP) cells. Glucagon, secreted by the pancreatic α-cells, is a major counter-regulatory hormone involved in regulation of glucose homeostasis (Hussain, Daniel and Habener, 2000). Glucagon increases glycaemia by mediating hepatic glycogenolysis (Robertson and Harmon, 2006). Studies have shown that diabetics have basal or increased plasma glucagon concentrations. Furthermore, suppression of this hormone is associated with reduction of plasma glucose concentration (Cherrington, Lacy and Chiasson, 1976). δ-cells also secrete somatostatin which plays an inhibitory role in the secretion of several hormones, including insulin (Robertson and Harmon, 2006).

Counteregulatory effects of epinephrine are due to its direct effects on target tissues and its effects on other participating hormones. For example, adrenaline stimulates increased glucagon secretion by activating β-adrenergic receptors on α-cells of pancreatic islets (Guy, Sandoval, Richardson, Tate and Davis, 2005). Due to its effects on muscle, epinephrine also decreases insulin mediated glucose uptake as a result of glucose-6-phosphate-dependent inhibition of hexokinase (Raz, Katz and Spencer, 1991). In addition adrenaline compromises insulin-induced glycogen synthesis attributed to its inhibition of glycogen synthase (Raz *et al.*, 1991).

Glycogenolysis and inhibition of glycogen synthesis, by both epinephrine and glucagon, are mediated via increased generation of cAMP which activates protein kinase A. Protein kinase A inactivates glycogen synthase and activates phosphorylase (Hodis, Kutinová-Canová, Potměšil, Kameníková, Kmoníčková, Zídek, Farghali, 2007).

Glucocorticoids antagonise effects of insulin. Excessive levels of glucocorticoids may cause insulin resistance and central obesity (Liu, Nakagawa, Wang, Sakurai, Tripathi, Lutfy and Friedman, 2005). In addition, glucocorticoids enhance hepatic gluconeogenesis and impair effects of insulin to reduce glucose production in type II diabetes (Delaunay, Khan, Cintra, Davani, Ling, Andersson, Östenson, Gustafsson, Efendic and Okret, 1997; Liu *et al.*, 2005). In states of intensive glycaemic control resulting in hypoglycaemia, hormones antagonistic to insulin action are released to restore normoglycaemia (Ross, Warren, Kelnar and Frier, 2005). Hence, counteregulatory hormones may offer benefits in specific conditions.

Adiponectin is an adipocyte derived peptide which improves insulin sensitivity via several mechanisms. It modulates fatty acid oxidation, inhibits fatty acid synthesis and uptake in the liver and enhances glucose and fatty acid oxidation in the muscle (Mlinar, Marc, Janež and Pfeifer, 2007). These effects are mediated through stimulation of perixosome proliferator gamma receptors (PPAR-γ) nuclear receptors and activation of AMP kinase (Mlinar *et al.*, 2007).

Like adiponectin, leptin is an adipocyte derived-hormone that circulates in plasma in free and bound form. Acting through its receptors located in the hypothalamus,

hippocampus, cortex, cerebellum, thalamus and choroids plexus, leptin decreases levels of circulating neuropepide Y resulting in reduced appetite (Mantzoros, 1999). Leptin also modulates glucose homeostasis (Levy and Stevens, 2001). In insulin-deficient rats, administration of leptin restores normal glucose levels, enhances glucose metabolism in the post absorptive stages and improves hepatic insulin sensitivity during glucose clamp (Chinookoswong, Wang, and Shi, 1999). Leptin, therefore, acts agonistically to effects of insulin. Moreover, leptin modulates release of thyroid hormones which play a homeostatic role in glucose metabolism (Wang, Chinookoswong, Yin and Shi, 2000).

Thyroid hormones secreted by the thyroid glands also exert glucose homeostatic functions in synergy as well as antagonistic to insulin action. Thyroid hormones may increase glucose production via hepatic gluconeogenesis, to meet increased energy requirements by the body (Lenzen and Bailey, 1984). Thyroid hormones, however, enhance transcription of insulin sensitive glucose transporter-4 (GLUT-4) (Chidakel, Mentuccia and Celi, 2005). This allows increased uptake of glucose by tissue cells thereby offsetting initial increase in blood glucose (Lenzen and Bailey, 1984).

1.2.5. Insulin resistance

Insulin resistance largely caused by central obesity is a key component in the pathogenesis of type II diabetes mellitus. It is defined as the inability of insulin to curtail hepatic glucose production and promote glucose disposal in the peripheral tissues (McGarry, 2002). Insulin resistance constitutes defective insulin receptor function, insulin receptor signal transduction, glucose transport and phosphorylation,

glucose uptake and glycogen synthesis in adipose, skeletal and hepatic tissues (DeFronzo, 1999; Kahn and Flier, 2000; Panunti, Jawa and Fonseca, 2004).

Adipose tissue secretes large quantities of tumour necrosis factor (TNF)-α. According to Mlinar *et al.*, (2007), TNF-α is the main factor that stimulates increased levels of circulating free fatty acids into circulation. Increased intraportal free fatty acids impair insulin clearance. The mechanisms by which this is mediated are still unclear (Kahn and Flier, 2000). It is, however, suggested that accumulation of intramyocellular lipids in striated muscles suppresses glucose uptake, glycogen synthesis and glucose oxidation (Flordellis, Ilias and Papavassiliou, 2005). Moreover, TNF-α inhibits expression of PPAR-γ, tyrosine phosphorylation on the insulin receptor and intermediates of insulin post-receptor signalling (Bailey, 2000).

In addition to TNF-α, adipose tissue also secretes resistin, which has been established as one of the possible links between excessive adiposity and insulin resistance (Liu, Fan, Qiu, Wang, Zhang, Gu, Zhang, Fei, Pan, Guo, Chen and Guo, 2008). This link, however, has been disputed by other investigators (De Courten, Degawa-Yamauchi, Considine and Tataranni, 2004).

Insulin resistance may manifest at the level of glucose entry into cells (Shepherd and Kahn, 1999). Indeed, in obesity insulin resistance is characterised by downregulation of glucose transporters (GLUT)-4 in skeletal muscles (Kahn and Flier, 2000). Insulin resistance is the main target of antihyperglycaemic agents because, if left unchecked, it results in the pathogenesis of a wide allay of disorders including hypertension,

dyslipidaemia, atherosclerosis, or the metabolic syndrome (Chakrabarti, Damarla, Mullangi, Sharma, Vikramadithyan and Rajagopalan, 2006).

1.3. The metabolic syndrome

The metabolic syndrome is the association of metabolic disorders including insulin resistance, hypertension, central obesity and dyslipidaemia (Boney, Verma, Tucker and Vohr, 2005). Dyslipidaemia is characterized by increased levels of plasma triglycerides, low density lipoproteins, very low density lipoproteins and reduced levels of high density lipoproteins. Moreover increased plasma levels of plasminogen activator inhibitor-1 (PAI-1) and fibrinogen levels mediate hypercoagulability which is a major problem in the metabolic syndrome (Flordellis, *et al.*, 2005). Prevalence of metabolic syndrome is on the increase due to increasing levels of obesity and ageing. Metabolic syndrome poses an enormous health challenge as it predisposes victims to cardiovascular diseases and diabetes mellitus. This syndrome can be managed by use of hypolipidaemic agents e.g. fibrates, antihyperglycaemic agents e.g. metformin and thiazolidinediones, and physical exercise to improve insulin resistance (Chew, Gan and Watts, 2006).

1.4. Types of diabetes mellitus

The major types of diabetes mellitus are type 1 and type 2, the former arising from inadequate production of insulin due to pancreatic β-cell dysfunction, and the latter arising from reduced sensitivity of insulin in target tissues and / or inadequate insulin secretion.

1.4.1. Type 1 diabetes

There are two forms of type I diabetes, namely, Immune-mediated and Idiopathic diabetes

1.4.1.1. Immune-mediated diabetes

Immune-mediated type 1 diabetes mellitus, previously known as insulin dependent diabetes mellitus (IDDM), constitutes 5 to 10% of all diabetic cases (American Diabetes association, 2004). The cause of inadequate insulin production is T-cell mediated autoimmune destruction of pancreatic beta cells. The pathogenesis of autoimmune β-cell destruction is initiated months or years before overt diabetes (Kukreja and Maclaren, 1999). The initiating factors(s) for autoimmune destruction is (are) still unclear. It is, however, suggested that, among others, it probably begins with β-cell infiltration of the pancreatic islets by macrophages which present antigens to T-helper cell (Lernmark, Bärmeier, Dube, Hagopian, Karlsen, Markholst, Wilson and Wassmuth, 1990). Major genetic factors responsible for autoimmune destruction in type I diabetes are linked to histocompatibility leucocyte antigen (HLA), located on chromosome 6. Interaction of these and other environmental factors precipitate autoimmune activities resulting in β-cell destruction (Kukreja and Maclaren, 1999).

Risk factors for pathogenesis of type 1 diabetes include genotype, diet and viral infections e.g. rubella, coxsackie, mumps (Wei, Li, Chang, Sung, Li, Lin, Chiang and Chuang, 2006). Coxsackie viral infections are a type of environmental factors that do not initiate, but enhance development of type I diabetes in genetically prone victims (Serreze, Ottendorfer, Ellis, Gauntt and Atkinson, 2000). It is proposed that coxsackie

viral infections probably provide a molecular mimic during replication that triggers a cross-reactive $CD4^+$ T-cell response against the candidate β-cell autoantigen (Serreze *et al.*, 2000). Ultimately β-cell death occurs by apoptosis (Kurrer, Pakala, Hanson and Katz, 1997).

In addition, low birth weight is considered a risk factor for type I diabetes. Low birth weight is indicative of exposure of foetus to maternal viral infection and the interaction of immune cross-reactivity between virus and autoantigens which may lead to destruction of pancreatic β-cells (Wei *et al.*, 2006). Furthermore, low birth weight is commonly associated with a reduced beta cell mass which is a vital factor in the pathogenesis of diabetes. Worldwide, an estimated 4.9 million people have type 1 diabetes (Gadsby, 2002) and, approximately 35100 children have type 1 diabetes in Africa (Beran, *et al.*, 2006).

1.4.1.2. Idiopathic diabetes

Unlike immune-mediated diabetes, idiopathic diabetes does not have established aetiology. It accounts for a minority of diabetic cases, most of whom are Africans and Asians. Idiopathic diabetes mellitus has a strong genetic component (American Diabetes Association, 2004).

1.4.2. Type II diabetes mellitus

Type II diabetes mellitus, formerly known as non insulin dependent diabetes (NIDDM) is the most common type of diabetes mellitus accounting for 90 to 95% of

all diagnosed cases of diabetes (American Diabetes Association, 2004). Type II diabetes is a polygenic disease caused by diminished response of target cells to insulin action, and/or insufficient production of insulin. Inadequate insulin production often characterizes later stages of type II diabetes and is attributed to reduced β-cell mass or beta cell dysfunction (Nattrass and Bailey, 1999). Hepatic gluconeogenesis and renal gluconeogenesis significantly contribute to postprandial hyperglycaemia in type II diabetes (Meyer, Stumvoll, Nadkarni, Dostou, Mitrakou and Gerich, 1998). In recent times, interest has been generated into the possibility of involvement of the hypothalamus - pituitary - adrenal axis in the pathogenesis of type II diabetes. (Koshiyama, Hamamoto, Honjo, Wada, Ikeda, 2006).

Risk factors for type 2 diabetes include genotype, age, previous history of gestational diabetes, behavioral factors including poor diet, and sedentary lifestyle. Genetic predisposition interacts with the environmental factors to influence disease prevalence (Noonan and Banks, 2000). In industrialized nations the increase in prevalence and incidences of diabetes are due in part to the increase in the age of the populations (Sowers and Epstein, 1995). Obesity is present in 50% and 70% of type II diabetic men and women, respectively (Oku, Ueta, Arakawa, Ishihara, Nawano, Kuronuma, Matsumoto, Saito, Tsujihara, Anai, Asano, Kanai and Endou, 1999). It leads to increased lipolysis which results in increased levels of circulating free fatty acids and triglycerides and their subsequent deposition in muscle bed (McGarry, 2001).

1.4.3. Gestational diabetes mellitus

Gestational diabetes, which occurs during pregnancy, is the third commonest type of diabetes. It is derangement in carbohydrate metabolism resulting in hyperglycaemia

with onset or first detection during pregnancy (Xiong, Buekens, Vastardis and Pridjian, 2006). Causative factors for gestational diabetes mellitus are unknown but autoimmune activities in the pancreatic β-cells of pregnant mothers are suggested (McEvoy, Franklin and Ginsberg, 2004).

It has high prevalence in developed countries at 3-19%, and is low in Africa, at 0 to 1% (Seyoum, Kiros, Haileselase and Leole, 1999). The significance of gestational diabetes mellitus is that it poses 16 to 63 % risk factor to the mother for future development of type II diabetes within 5 to 16 years (Hyer and Shehata, 2005). Gestational hyperglycaemia also exposes the foetus to risk of future diabetes, hypertension, and obesity (Boloker, Gertz and Simmons, 2002; Maresh, 2005). In addition to the perinatal morbidity, immediate dangers of gestational diabetes include pre-term birth, macrosomia, pre-eclampsia and caesarean section (Fan, Yang, Gao, Lintu and Sun, 2006).

Excessive foetal exposure to glucose as a result of maternal hyperglycaemia results in permanent reduction in levels of GLUT-1 and GLUT-4 transporters in the newborn and adult offspring of a gestational diabetic mother (Boloker *et al.*, 2002). Downregulation of these transporters may be an adaptive mechanism by which the foetus protects itself against the adverse effects of hyperglycaemia. The foetus adapts to a changed environment in the uterus that may enhance its chance of short-term survival but at the expense of a long-term capacity for normal growth and development, resulting in the development of diabetes (Boloker *et al.*, 2002).

Foetal exposure to excessive levels of glucocorticoids in diabetic pregnancy is one of several factors that subject them to growth retardation, hypertension and diabetes mellitus later in life (Fujisawa *et al.*, 2004). Phillips, Barker, Fall, Seckl, Whorwood, Wood and Walker, (1998) also demonstrated that increased plasma cortisol levels

were strongly associated with hypertension, hyperglycaemia, fasting hypertriglyceridaemia and insulin resistance in adults. The authors asserted that intrauterine exposure to transient stressful conditions such as low protein diet, alcohol and maternal infections, permanently alters the hypothalamic-pituitary-adrenal axis, as a result of excessive foetal exposure to glucocorticoids. Later in adult life, victims express increased cortisol activities accounting for diabetes mellitus, hypertension and other complications.

It has been shown that diabetic pregnancy suppresses expression of 11 beta-hydroxysteroid dehydrogenase (11h-HSD) type 2 in the placenta and foetal kidney (Fujisawa, Nakagawa, Ren-shan and Ohzeki, 2004). 11h-HSD is a high affinity NAD^+-dependent enzyme that inactivates glucocorticoids. The role of glucocorticoids in insulin resistance is well established. Glucocorticoids mediate lipolysis resulting in increased availability of free fatty acids which culminates in reduced glucose oxidation and uptake in peripheral tissues. Moreover, the presence of glucocorticoids also compromises translocation of GLUT-4 transporters to the cell membranes (Mlinar *et al.*, 2007). These substances promote blood vessel vasoconstriction, gluconeogenesis, glucose release and discourage glucose uptake in the peripheral tissues (Mlinar *et al.*, 2007).

There are opposing views about influence of birth weight on future occurrence of chronic diseases including diabetes mellitus. One view asserts that gestational diabetes leads to low birth weight which is a risk factor for type1 diabetes (Boney *et al.*, 2005). An alternative view, however, holds that gestational diabetes mellitus results in overweight children who will subsequently develop diabetes mellitus (Ozumba, Obi and Oli, 2004). Either way, use of weights as predictive risk factor for future diabetic incidence is insignificant as diabetes mellitus occurs in both obese and

lean individuals (Nattrass and Bailey, 1999). Indeed, Curhan, Willett, Rimm, Spiegelman, Ascherio and Stampfer, (1996), reported that low birth weight is associated with future occurrence of hypertension and diabetes, while high birth weight is associated with future incidence of obesity.

1.4.4. Other types of diabetes mellitus

Other specific types of diabetes are: Diabetes due to genetic defect of beta cells, also known as Maturity onset diabetes of the young, (MODY), diabetes due to genetic defects in insulin action, diabetes due to diseases of the exocrine pancreas, diabetes due to endocrinopathies, and, drug or chemical-induced diabetes (American Diabetes Association, 2004).

1.5. Diabetic complications

Complications arising from hyperglycaemia are both microvascular and macrovascular in nature. Microvascular complications, affecting the smaller blood vessels frequently result from type 1 diabetes while macrovascular complications affecting large blood vessels, originate from type II (Bate and Jerums, 2003; González-Correa, Arrebola, Guerrero, Cañada, Muñoz Marin, Sanchez De la Cuesta, De la Cruz, 2006; Girach and Vignati, 2006). The two are the leading cause of morbidity and death in people with diabetes (Brites, Ferna´ndez, Verona, Malusardi, Ischoff, Beresan, Elbert and Wikinski, 2007).

1.5.1. Microvascular complications

Microvascular complications include (a) neuropathy, (b) retinopathy, and, (c) nephropathy.

1.5.1.1. Diabetic neuropathy

Diabetic neuropathy is a term that describes many pathological conditions each characterized by peripheral nerve dysfunction in the presence of diabetes (Apfel, 2006). Aetiology of diabetic neuropathy is multifactorial with hyperglycaemia, and/ or lack of insulin, playing a major role.

Chronic hyperglycaemia induces glycation of myelin. Glycated myelin is prone to phagocytosis by macrophages (Ahmed, 2005). Furthermore, glycated myelin stimulates macrophages to secrete proteases which may cause demyelination. Such nerve degeneration may lead to hyperalgesia or loss of pain or temperature sensation (Berti-Mattera, Gariepy, Burke and Hall, 2006).

Endothelins are a family of peptides that, among others, mediate pain perception. Reduced expression of type-B endothelin receptors in peripheral nerves may be one of the factors responsible for pathogenesis and exacerbation of hyperalgesia and tactile allodynia in diabetes (Berti-Mattera, *et al.,* 2006). Severe cases of peripheral neuropathy result in foot ulcers and amputations (Viberti, 2005).

1.5.1.2. Diabetic nephropathy

Diabetic nephropathy is the most important cause of renal failure in developed countries (Amador-Licona, Juan-Manuel, Guízar-Mendoza, Vargas, Sánchez-Camargo and Zamora-Mata, 2000). Patients with early renal damage manifest with microalbuminuria which progresses into proteinuria (Leiter, 2005). In renal tissue, advanced glycation end products can induce activities of transforming growth factor-β and raise expressions of various extracellular matrix mRNAs. These mediate hypertrophy of the glomeruli, thickening of basal membrane, and expansion of the mesangial extracellular matrix.

Such structural changes are associated with albuminuria and proteinuria (Stitt, 2003). Blocking activities of advanced glycation end products by a phenyl hydrazine compound, aminoguanidine, slows down mesangial expansion and proteinuria (Kedziora-Kornatowska, Luciak, Błaszczyk, and Pawlak, 1998; Amador-licona *et al.*, 2000; Adler, 2003). In addition, hypertension associated with diabetes is one of the most important factors in the pathogenesis and progression of diabetic nephropathy (Bretzel, 1997). In turn, diabetic nephropathy is critical in the development of hypertension in diabetics (Sowers and Epstein, 1995). As pointed out by Lewis, Hunsicker, Bain, and Rohde, (1993), diabetic nephropathy results in progressive deterioration of glomerular function. The decline in renal function, however, can be attenuated by management of hypertension.

Hyperglycaemia causes a redox imbalance. This is as a result of excessive reactive oxygen species generation as well as glycation of enzymes responsible for scavenging reactive oxygen species (Ha and Kim, 1999; Agardh, Stenram, Torffvit and Agardh,

2002). Enzymes involved in cellular defence against excessive reactive oxygen species build up include catalase, glutathione peroxidase and superoxide dismutase. Glutathione and vitamin E are non-enzymatic antioxidants. Studies have shown that superoxides directly mediate vasoconstriction in renal microvasculature (Schnackenberg, 2002). This is either by stimulating synthesis of a vasoconstrictor, adenosine, or by blocking effects or secretion of a vasodilator, prostaglandin$_2$ (PGI_2). These processes, occurring on a subcellular level, ultimately compromise the overall renal function. Renal function failure is itself major risk factor for pathogenesis and/or exacerbation of hypertension (Diepeveen, Verhoeven, Palen, Dikkeschei, Tits, Kolsters, Offerman, Bilo and Stalenhoef, 2005). Increased incidences of cardiovascular complications and renal function deterioration are partly attributed to reduced excretion of electrolytes such as sodium (Roland, O'Hare, Walters and Corrall, 1986; Dahar and Gutkowska, 2000). The UKPDS demonstrated the insufficiency of controlling blood glucose alone in the prevention of macrovascular complications (Bate and Jerums, 2003). This was supported by other trials that showed that reduction of hypertension in addition to managing glycaemia to acceptable levels did reduce incidences of macrovascular complications (Bate and Jerums, 2003).

1.5.1.3. Diabetic retinopathy

Diabetic retinopathy is the commonest cause of blindness in people afflicted with diabetes aged between 20 and 74 years (Girach and Vignati, 2006). Accumulation of advanced glycation end products in the retinal cells and vasculature is critical for pathogenesis and progression of diabetic retinopathy (Stitt, 2003). These cause

reduced cell viability of the retinal cells. Hyperglycaemia-induced secretion of vasoconstrictors such as angiotensin II and endothelin-1 are thought to compromise retinal perfusion (Leiter, 2005). Studies have revealed that diabetic retinopathy is itself a risk factor for nephropathy in addition to all other established risks (El-Asrar, Al-Rubeaan, Al-Amor, Moharram and Kangave, 2002). In these studies, patients with diabetic retinopathy were 13.39, and 3.51 times more likely to have nephropathy than patients with type 1 and type II diabetes, respectively. Furthermore, hyperglycaemia compromises nitric oxide secretion of the retinal microvasculature thereby contributing to disturbed retinal perfusion (El-Asrar *et al.*, 2002).

1.5.2. Macrovascular complications

Macrovascular complications are largely responsible for mortality in diabetes, with cardiovascular diseases accounting for up to 80% morbidity (Akula, Kota, Gopisetty, Chitrapu, Kalagara, Kalagara, Veeravalli and Gomedhikam, 2003). Macrovascular complications include (a) atherosclerosis, (b) reproductive problems, (c) stroke, and (d) coronary artery diseases.

1.5.2.1 Atherosclerosis

Due to hyperglycaemia, formation of advanced glycation end products in arterial collagen results in trapping of low density lipoproteins. This process speeds up the rate of atheroma formation in blood vessels (Ahmed, 2005). In addition advanced glycation end products promote smooth muscle cell proliferation in blood vessels (Basta, Schmidt and, De Caterina, 2004). In coronary artery disease, advanced

glycation end products cause increased deposition of lipids in the cardiac cells, and compromise ventricular compliance (Sobel and Schneider, 2005).

1.5.2.2. Reproductive problems

Hyperglycaemia due to diabetes is a significant underlying cause of a number of sexual disorders associated with diabetes mellitus. About 90 % of diabetics manifest sexual problems characterised by impotence, infertility and low sexual drive (Amaral, Moreno, Santos, Seic and Ramalho-Santos, 2006). These are attributed to hyperglycaemia-induced reactive oxygen species generation. Erectile dysfunction in diabetes is caused by lack of smooth muscle relaxation mediated by nitric oxide due to endothelial dysfunction and autonomic neuropathy (Price, 2006). Moreover, interaction of reactive oxygen species generated as a consequence of diabetes mellitus, with DNA of spermatozoa, results in reduced motility and fertility of human sperm (Amaral, *et al.*, 2006).

1.5.3. Pathogenesis of diabetic complications

Development and progression of diabetic complications are mediated by 4 major pathways including increased sorbitol (polyol) pathway flux, increased formation of advanced glycosylation end products, increased activities of protein kinase C, and increased hexosamine pathway flux (Ceriello, 2003; Lapolla, Fedele and Traldi, 2005).

Generation of reactive oxygen species have been implicated as the main pathogenic factor as well as the result, in these processes. In endothelial cells, glucose enters into cells via plasma membrane transported by insulin-independent GLUT-1 transporters. Its subsequent accumulation in the cells causes generation of superoxide which is considered a key component in the initiation of all other pathways (Ceriello, 2003). Excessive formation of reactive oxygen species results in damage, oxidatively, to DNA, proteins and lipids (Dandona, Thusu, Cook, Snyder, Makowski, Armstrong and Nicotera, 1996). The most affected organs appear to be those whose glucose uptake is independent of insulin influence including the nervous system, kidney, heart and small blood vessels (Ahmed, 2005).

1.5.3.1. The polyol (sorbitol) pathway

In the polyol (sorbitol) pathway, aldose reductase is a key enzyme that has been reported to catalyze the reduction of glucose to sorbitol in non-insulin dependent tissues. Sorbitol does not readily diffuse across cell membranes, and its intracellular accumulation is implicated in the chronic complications of diabetes such as peripheral neuropathy, retinopathy and cataracts (Matsuda, Morikawa and Yoshikawa, 2002). Osmotic changes as a result of increased concentration of sorbitol disrupts normal cellular function, leading to cataract formation (Gugliucci, 2000). Sorbitol is metabolised by sorbitol dehydrogenase to fructose and nicotinamide adenine dinucleotide (NADH). Increased levels of NADH are sensed by many tissues as hypoxia (Gugliucci, 2000).

In this pathway, aldose reductase uses nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor (Gugliucci, 2000). This denies other important pathways access to adequate levels of NADPH. For instance, glutathione, an antioxidant depends on NADPH for its recycling. Inadequate NADPH, therefore, can cause redox imbalance due to insufficient glutathione levels (Gugliucci, 2000). Other reactions that use NADPH as a coenzyme include nitric oxide formation. Compromised nitric acid synthesis as a result of inadequate NADPH can cause vasoconstriction resulting in peripheral vascular resistance and ultimately, hypertension. Thus, use of NADPH in the polyol pathway is one way by which hyperglycaemia causes diabetic complications. Oxidative stress generated via the polyol pathway is considered to be a major contributing factor in the pathogenesis of diabetic neuropathy (Chung, Ho, Lam and, Chung, 2003).

1.5.3.2. Advanced glycation products

Figure 1 illustrates a modified process of protein glycation. Chronic hyperglycaemia promotes nonenzymatic glycation of proteins. This occurs by covalent binding of aldehyde groups of reducing sugars to free amino acid groups of proteins resulting in formation of the relatively unstable Schiff's base (Ahmed, 2005). Rearrangement of Schiff's base results in formation of Amadoli's products and, subsequently, advanced glycation end products (AGEs) (Basta *et al.*, 2004). Advanced glycation end products mediate their adverse effects by interacting with advanced glycation end product receptors (RAGE) found on endothelial cells, monocytes and macrophages (Kedziora-Kornatowska, *et al.*, 1998). Such interaction results in intracellular oxidative stress and activation of NF-κB through activation of mitogen-activated protein kinase

signalling pathway (Peyroux and Sternberg, 2006). NF-κB stimulates transcription of genes for various factors including endothelin-1, vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF- β), inteleukin-6 and tumour necrosis factor-α (TNF-α) (Peyroux and Sternberg, 2006). These mediate vasoconstriction, inflammation and coagulation (Ahmed, 2005). Advanced glycation end products crosslink with collagen resulting in arterial stiffening thereby creating hypertensive complications. Indeed, crosslinking of advanced glycation end products and collagen trap plasma proteins including albumin, low density lipoproteins, and immunoglobulins. Accumulation of these proteins in the subendothelium causes luminal narrowing (Gugliucci, 2000), which may lead to development or exacerbation of hypertension.

Interaction of advanced glycation end products with DNA, proteins and amino acids alters their structure and function. Formation of advanced glycation end products on DNA can cause deleterious expression of the DNA (Gugliuuci, 2000).

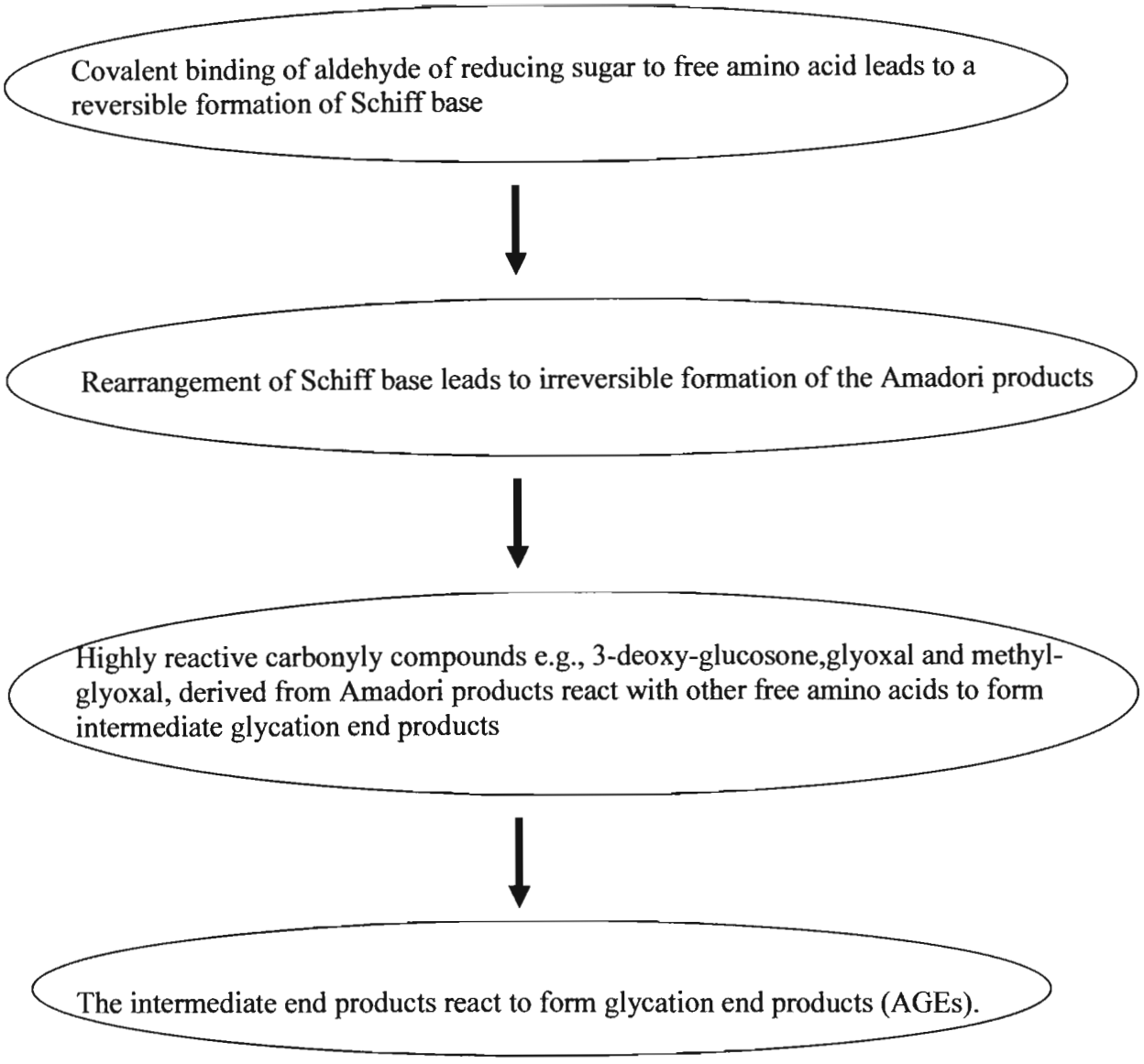

Figure 1. Glycation of protein by glucose and the formation of AGEs.

(Modified from: **Basta G, Schmidt A, De Caterina R**. Advanced glycation end products and vascular inflammation: implications for accelerated atherosclerosis in diabetes. *Cardiovascular Research,* 63:582– 592, 2004.

1.5.3.3. Protein kinase C (PKC)

Hyperglycaemia is also associated with increased levels of circulating diacylglycerols which leads to synthesis of protein kinase C (PKC) (Inoguchi, Xia, Kunisaki, Higashi, Feener and King, 1994). Diacylglycerol generation resulting from hyperglycaemia is possible by several pathways. Diacylglycerols may be a product of phosphodiesteratic cleavage of phosphatidylinositol 4,5-biphosphate or a product of metabolism of phosphatidylcholine, or maybe produced via the glycolytic pathway (Inoguchi, *et al.*, 1994). Induction of PKC production is stimulated by both transient as well as prolonged hyperglycaemia. PKC activates NADPH oxidase, probably the most important enzyme in the generation of reactive oxygen species in blood vessels of the heart, kidney, aorta, and retina (Inoguchi *et al.*, 2003). PKC catalyses phosphorylation of a number of chemical substances including the insulin receptor, thereby reducing activities of its protein-tyrosine kinase (Bollag, Roth, Beaudoin, Mochly-rosen and Koshland, 1986). Moreover, PKC plays a modulatory role in oxidative stress-induced glomerular insult (Ha and Kim, 1999). PKC also elevates levels of vasoconstricting prostaglandins, endothelia and several vascular growth factors that influence vasomotor activities and vascular growth (Sowers and Epstein, 1995). Furthermore, PKC is associated with an increase in the exchange activities of Na^+/K^+ in the renal cells and inhibition of activities of Na^+, K^+-ATPase in proximal tubules (Bertorello, 1992). Thus, the protein kinase C pathway may explain the association between diabetes mellitus and renal function failure.

1.5.3.4. Hexosamine pathway

Under normal physiological conditions, 1-3% of glucose is converted into the hexosamine pathway leading to conversion of fructose 6-phosphate to glucosamine 6-phosphate catalysed by the rate-limiting enzyme, glutamine:fructose-6-phosphate amidotransferase (James, Tang, Ingram, Ly, Thai, Cai and Scholey, 2002). During hyperglycaemic states, excess glucose is shunted into the hexosamine pathway resulting in the formation of UDP-*N*-acetylglucosamine, which is a substrate for glycation of essential intracellular factors including transcription factors. These reactions influence expressions of various genes such as plasminogen activator inhibitor-1 (PAI-1) gene promoter, culminating in the pathogenesis of microvascular complications (Goldberg, Whiteside and Fantus, 2002). Hyperglycaemia activates the hexosamine pathway leading to further deterioration of pancreatic β-cell function in diabetes (Kaneto, Xu, Song, Suzuma, Bonner-Weir, Sharma and Weir, 2001).

Acute exposure to hyperglycaemia has as much adverse effects as long term exposure. Short term hyperglycaemia causes increases in blood pressure that may be caused by suppression of nitric oxide (NO) synthesis (Giugliano, Marfella, Coppola, Verrazzo, Acampora, Giunta, Nappo, Lucarelli and D'Onofrio, 1997; Marfella, Nappo, De Angelis, Paolisso, Tagliamonte and Giugliano, 2000). Similar observations were made by Bohlen and Lash (1993), who reported that acute exposure of intestinal tissues to hyperglycaemia, for periods as brief as 15 minutes, has adverse effects affecting vasodilatory effects of endothelium - derived relaxing factor (EDRF). This period is sufficient to allow onset of reactive oxygen species generation. It is suggested that the low nitric oxide levels are a result of hyperglycaemia-induced

reactive oxygen species generation that preys on nitric oxide (Giugliano *et al.,* 1997; Bohlen and Nase, 2001). Oxygen radicals, e.g. superoxide, O_2^-, react with nitric oxide to produce peroxynitrite, $ONOO^-$, thereby compromising bioavailability and effects of nitric oxide (Beltowski, Wójcicka, Marciniak and Jamroz, 2004). Moreover, peroxynitrite undergoes chemical reactions with nucleic acids, lipids and certain proteins. Reduced nitric oxide levels can lead to a host of adverse effects including platelet adhesion and aggregation, proliferation of smooth muscles, and increased blood viscosity (Giugliano *et al.*, 1997; Brodsky, Morrishow, Dharia, Gross and Goligorsky, 2001).

1.6. Therapy for diabetes mellitus

As reported in the preceding sections, diabetes mellitus is an important risk factor for development of cardiovascular and renal complications. Appropriate therapeutic intervention to prevent hyperglycaemia can prevent the development and progression of diabetic complications (The Diabetes Control and Complications Trial Research Group, 2006). Control of blood glucose achieving less than 7% glycosylated haemoglobin is considered adequate to minimise complications of diabetes (Simó and Hernández, 2002). In addition to achieving glycosylated haemoglobin below 7% via glucose control, control of hypertension to attain blood pressure levels $\leq$ 130/80 mmHg must be pursued to prevent the occurrence of microvascular complications (Bate and Jerums, 2003).

A multi-pronged approach involving lifestyle changes and oral hypoglycaemic drugs and/ or insulin therapy to control glucose metabolism is usually employed. Hence

various drugs are used to achieve these goals. Below is description of some of the major groups of antidiabetic agents used to control blood glucose.

1.6.1. Pharmacological intervention

1.6.1.1. Insulin

Insulin is one of the major hypoglycaemic agents presently used in diabetes mellitus management. It was discovered in 1921 and is the mainstay antidiabetic agent for the management of type 1 diabetes and is also used in late-stage type II diabetes (Emilien, Maloteaux and Ponchon, 1999). There are several types of insulin classified based on their duration of action. These are rapid (ultra short) – acting (e.g. lispro, aspart), short –acting (e.g regular insulin), intermediate – acting (e.g. lente) and long-acting (e.g. glargine, detemir) (Bethel and Feinglos, 2005). Short acting insulin types which control postprandial glycaemia are designed to mimic bolus insulin secretion, while intermediate or long acting insulin are designed to mimic basal glycaemic control (Fonseca, 2006). Insulin is currently administered subcutaneously using multiple daily injections or external pump for continuous delivery (Hirsch, Bode, Garg, Lane, Sussman, Hu, Santiago and Kolaczynski, 2005). Other delivery routes include oral, inhaled, nasal, rectal, ocular, intravaginal and transdermal (Grover, Vats and Rathi, 2000; Takei and Kasatani, 2004). Intensive insulin therapy to maintain optimal glucose levels in type 1 diabetes can reduce incidences and exacerbation of microvascular complications by 50 to 70% (DCCT Research group, 1993).

Shortfalls of insulin therapy include ineffectiveness on oral application due to first pass hepatic metabolism, short shelf life, and severe hypoglycaemia on overdosage,

non-compliance to painful injections and weight gain (DeFronzo, 1999; Takei and Kasatani, 2004). Several observations, however, suggest that non-compliance due to painful daily injections can be overcome by using the oral route (Meyerovitch, Farfel, Sack and Shechter, 1987; Musabayane, Munjeri, Bwititi and Osim, 2000). Since insulin undergoes digestion in the gut, Musabayane *et al.* (2000) demonstrated in the rat that coating insulin in amidated pectin hydrogel beads evades digestion.

1.6.1.2. Oral blood glucose lowering agents

There are 5 classes of antidiabetic agents presently in use, including biguanides, sulphonylureas, thiazolidinediones, benzoic acid derivatives and α-glucosidase inhibitors. Highlighted below are some of their modes of actions, advantages and limitations.

1.6.1.2.1 Biguanides

Biguanides were introduced for use between 1957 and 1960 (Simó and Hernández, 2002). Biguanides (e.g. metformin) mediate antihyperglycaemic effects by sensitizing target tissues cells to insulin action and blocking hepatic gluconeogenesis (Pari and Satheesh, 2006). Metformin (Dianben®) is the principal drug in this class. The drug has been proved effective when used either in monotherapy or in combination with other groups of drugs such as sulphonylureas or insulin (Chadwick, Roux, van de Venter, Louw and Oelofsen, 2007). Its mechanisms of actions are still unclear. Current studies, however, show that on a subcellular level, metformin mediates hypoglycaemic effects by stimulating AMP-activated protein kinase (AMPK).

Stimulation of AMPK results in fatty acid catabolism and enhanced muscle glucose uptake (Zhou, Myers, Li, Chen, Shen, Melody, Wu, Ventre, Doebber, Fujii, Musi, Hirshman, Goodyear and Moller, 2001). Metformin also increases expression or activities of GLUT-4 transporters which also enhances glucose uptake by muscles (Simó and Hernández, 2002). Moreover, biguanides inhibit advanced glycation end product formation. The amino groups on biguanides react with carbonyl groups of dicarbonyl compounds thereby inhibiting advanced glycation end product formation. Therefore, usage of metformin prevents the occurrence of diabetic complications in addition to controlling glucose levels (Kiho, Kato, Usui and Hirano, 2005). Other examples of drugs in this class include fenformin and buformin. Lactic acidosis is the major adverse effect of metformin treatment. This results from accumulation of lactate in blood because metformin inhibits the liver from using lactate to synthesise glucose (Stumvoll, Nurjhan, Perriello, Dailey and Gerich, 1995). Other side effects are diarrhoea, nausea abdominal bloating and flatulence (Zonneveld, 2005).

1.6.1.2.2. Sulphonylureas

Sulphonylureas act by stimulating insulin secretion. They bind to beta cell receptors that are linked to ATP-dependent potassium gated channels. Interaction with these receptors inhibits opening of potassium channels. Potassium build up in the cell causes cellular depolarization evoking opening of calcium channels, and subsequently, influx of calcium ions which mediate exocytosis of secretory granules containing insulin (Simó and Hernández, 2002). Sulphonylureas also minimize hepatic degradation of insulin thereby increasing levels in blood (Harrigan, Nathan and Beattie, 2001). Sulphonylureas usage is restricted to type II diabetes mellitus

because it requires a functioning pancreatic beta cell mass. Examples of agents presently in use include glibenclamide, glipizide, tolbutamide and gliclazide. Hypoglycaemia is the main side effect of sulphonylureas (Porksen, 2006). Factors that enhance episodes of hypoglycaemia include poor nutrition, advanced age, alcohol consumption and renal and hepatic diseases (Harrigan *et al.*, 2001). Other dangers of sulphonylurea class of antidiabetic agents is that they stimulate insulin release by the pancreatic beta cells independent of glucose loading thereby subjecting the beta cells to exhaustion. Furthermore, they cause allergy and weight gain as a result of increased blood levels of insulin.

1.6.1.2.3. Thiazolidinediones

Thiazolidinediones (TZDs) mediate their hypoglycaemic effects by enhancing insulin sensitivity, and reducing gluconeogenesis by the liver (Emilien *et al.*, 1999). This group of drugs targets the perixosome proliferator gamma receptors (PPAR-γ) which are nuclear and expressed mainly in the adipose tissues. Other tissues expressing PPAR-γ include liver, skeletal muscle, kidney, heart, large and small intestine and colon (Bailey, 2000; Song, Knepper, Hu, Verbalis and Ecelbarger, 2004). Interaction TZDs with the receptors leads to modification of expression of major genes responsible for glucose metabolism (Hevener, Reichart, Janez and Olefsky, 2001). Stimulation of PPAR-γ activates adipocytes to secrete adiponectin which induces fatty acid oxidation and inhibits hepatic gluconeogenesis (Mlinar *et al.*, 2006). Activation of these receptors results in improved uptake of glucose and triglycerides by cells, and subsequently, improved insulin sensitivity (Tamsma, Jazet, Beishuizen, Fogteloo, Meinders and Huisman, 2005). When administered at high doses TZDs suppress

hepatic gluconeogenesis (Flordelllis *et al.*, 2005). TZDs are particularly effective in combination therapy with insulin and safeguards against high insulin dose (Bailey, 2000). In addition, TZDs possess anti-inflammatory properties, inhibit reactive oxygen species generation and improve beta cell function (Dandona *et al.*, 2003; Viberti, 2005).

Troglitazone, roziglitazone, and pioglitazone are some of examples of drugs in this class. A major shortfall of thiazolidinediones is that they lead to increased plasma levels of cholesterol (Bailey, 2000). Other side effects of TZDs include fluid retention and oedema, hepatotoxicity, cardiomegaly and haemotoxicity (Song *et al.*, 2004; Chakrabarti *et al.*, 2006). Fluid retention is due to a reduction in glomerular filtration rate and an increase in expressions of renal tubular transporters including α-1 subunit of Na-K-ATPase, the bumetanide-sensitive Na-K-2Cl cotransporter (NKCC2), the sodium hydrogen exchanger and aquaporins 2 and 3, which result in sodium retention (Song *et al.*, 2004).

1.6.1.2.4. Benzoic acid derivatives

Benzoic acid derivatives mediate hypoglycaemic effects by stimulating insulin secretion. Benzoic acid derivatives, bind to ATP sensitive potassium channels on β-cells at receptor different from that bound by sulfonylureas. Interaction of benzoic acid derivatives with their receptors results in reduced levels of insulin release compared with sulphonylureas (Harrigan *et al.*, 2001). Hypoglycaemia may occur on overdose. An example of drugs in this class include repaglinide.

1.6.1.2.5. α-glucosidase inhibitors

Alpha-glucosidase inhibitors mediate their hypoglycaemic effects by lowering glucose absorption into blood by suppressing digestion of polysaccharides and disaccharides into monosaccharides, thus decreasing postprandial blood glucose (Fujisawa, *et al.*, 2005). This is primarily mediated by competitively and reversibly inhibiting the actions of α-glucosidase, a small intestine epithelial membrane bound enzyme which catalyses break down of polysaccharides into monosaccharides. Examples of α-glucosidase are acarbose and voglibose. Side effects include diarrhoea, flatulence abdominal bloating. These adverse effects are primarily due to malabsorption of glucose (Bressler and Johnson, 1992).

1.6.1.3. Adjunct treatment

In specific cases depending on need, non-traditional hypoglycaemic therapy maybe utilized. For example, statins (HMG-CoA [3-hydroxy-3-methylglutaryl coenzyme A] reductase inhibitors) and fibrates are used to control hypercholesterolaemia, hypertriglyceridaemia and increased blood levels of low density lipoproteins (LDL) (Bate and Jerums, 2003). Angiotensin converting enzyme inhibitors and angiotensin II receptor antagonist are 'first choice' drugs used to control hypertension in diabetes because of their effectiveness in treating diabetic nephropathy (Bate and Jerums, 2002). Such renoprotective effects of ACE inhibitors have been confirmed by several investigators including Lewis, Hunsicker, Bain and Rohd, (1994). In addition, angiotensin II has probable adverse mediatory effects on insulin signaling due to its influence in generation of reactive oxygen species (Sowers and Stump, 2004).

Low dose aspirin is also indicated in diabetes to prevent the occurrence of myocardial infarction (Adler, 2003). Furthermore, diabetics on high aspirin doses have reduced prevalence of cataracts. These beneficial effects of aspirin have been confirmed by in *in vitro* and *in vivo* experiments that have shown that aspirin reduces glycation or glycoxidation and formation of advanced glycation end products crosslinks (Ahmed, 2005).

1.6.2. Non-pharmacological intervention

Weight loss as a nonpharmacological intervention for diabetes is significant in that reduced adipose tissue results in lesser production of proinflammatory products cytokine tumour necrosis factor -α (TNF-α) which is thought among others to mediate insulin resistance (Mlinar *et al.*, 2007). Consumption of food with low glycaemic index is beneficial in that it requires less insulin secretion and improves target tissues to insulin effects. Foods with low glycaemic index allow slow absorption of glucose in the gut.

Regular exercise reduces levels of circulating cholesterol. Furthermore, exercise is known to stimulate glucose uptake by the muscles and liver and its conversion to glycogen (Murakami, Shimomura, Fujitsuka, Sokabe, Okamura and Sakamoto, 1997). Studies by Coskun, Ocakci, Bayraktaroglu and Kanter, (2004), have shown that exercise has therapeutic effects by decreasing oxidative stress and maintaining functional capacity of the pancreatic β-cells. Moreover, in diabetic rats exercise improves cardiac output and raises levels of sarcolemmal GLUT-4 protein (De

Angelis, Oliveira, Dall'Ago, Peixoto, Gadonski, Lacchini, Fernandes, and Irigoyen, 2000).

As demonstrated above, pharmacological interventions with synthetic medicines are not without adverse limitations and secondary failures (Park, Ko and Chung, 2005). The failure for monotherapy in most cases to adequately control hyperglycaemia implies that combination therapy be used. This leads to an increase in the cost of maintaining normoglycaemia. Management of diabetes is, therefore, a major challenge. As a result diabetic patients and healthcare professionals are beginning to consider alternative and complementary therapy in the management of diabetes mellitus.

1.7. Novel antidiabetic therapy research

Despite the intensive use of current antidiabetic agents, many type II diabetic patients still exhibit poor glycaemic control and some develop serious complications. This suggests that individual oral agents act on only part of the pathogenic process and only to a partial extent. They do not reinstate normal insulin sensitivity or normal pancreatic β-cell function. In addition, these agents do not prevent gradual loss of pancreatic β-cells, and their efficacy depends upon availability of functional β-cells (Bailey, 2000; Gadsby, 2002). Implications are that higher drug doses will be needed to control the increasingly worsening hyperglycaemia. Moreover, more drug classes will be needed to be used as an increasing number of diabetics will require combination therapy to control their glycaemia (Gadsby, 2002). Research of diverse

nature continues to be undertaken to identify antidiabetic therapies that are effective and affordable, but with fewer side effects.

The incretin hormone glucagon-like peptide-1 (GLP-1) is released by the L-cells of gut after a meal and contributes to maintaining normoglycaemia by regulating insulin and glucagon release, gastric emptying and body weight (Combettes, 2006). Furthermore, GLP-1 is known to mediate β-cell differentiation and proliferation (Bregenholt, Møldrup, Blume, Karlsen, Friedrichsen, Tornhave, Knudsen, Petersen, 2005). Based on the beneficial glucose lowering effects of GLP-1, the antidiabetic drug exenatide has recently been approved. Furthermore, liraglutide, a long-acting GLP-1 analogue, has been shown, in laboratory studies to inhibit STZ-mediated pancreatic β-cell damage (Bregenholt, *et al.*, 2005).

Oestradiol, a reproductive hormone in females is known to play a modulatory role in glucose homeostasis. Oestrogen therapy inhibits hepatic gluconeogenesis and reduces incidences of diabetes in postmenopausal women (Kanaya, Herrington, Vittinghoff, Lin, Grady, Bittner, Cauley and Barrett-Connor, 2003). Other studies have reported that these effects are probably mediated by its influence on expression of GLUT-4 transporters (Barros *et al.*, 2006). These findings have created the need to search for antidiabetic therapy that exploits oestradiol and/ or its receptors.

Long term exposure to a cold environment activates the sympathetic system resulting in increased energy expenditure for warmth, glucose tolerance and insulin sensitivity (Liu *et al.*, 1998; Hori, Ishigaki, Kaya, Tsujita, Terada, Oku and Hori, 2006). β_3-adrenergic receptors which are expressed in most tissues including brown and white

adipose tissues are thought to play a major role in these effects. Liu, *et al.*, (1998) reported that long term stimulation of the β_3- receptors in adipose tissues with a selective agonist evoked positive glycaemic effects similar to chronic cold exposure. These receptors offer a potential therapeutic target worth investigating. The use of genetic therapy to manage diabetes mellitus is being investigated. Type I diabetes can be prevented in victims expressing adverse MHC alleles via genetic engineering of haematopoietic stem cells to restore protective MHC class II expression (Tian, Bagley, Cretin, Seth, Wucherpfennig and Iacomini, 2004).

1.7.1. Experimental animal models of diabetes

Animal models of diabetes have allowed studies and provided understanding of pathogenesis and progression of diabetes mellitus in humans. Furthermore, animal models have allowed the evaluation of various therapeutic interventions which may be potentially used in man. Uses of animal models have made possible studies of disease characteristics in humans by providing genetic and immunological modifications that are not possible in humans (Bach, 1994).

1.7.1.1. Animal models of type I diabetes mellitus

1.7.1.1.1. Streptozotocin (STZ)-induced diabetes in the rat

The streptozotocin-induced diabetic model is an extensively used animal model in studies of human diabetes mellitus. It is a well-described model and the toxicity of STZ is relatively low compared with other diabetogenic agents (Piotrowski, 2003).

Streptozotocin (N-[methylnitrocarbamoyl]-D-glucosamine), STZ, is an antibiotic synthesised by *Streptomycetes achromogenes* and is commonly used in induction of experimental diabetes. STZ selectively destroys β-cells by alkylation of DNA through its nitrosourea moiety (Szkudelski, 2001; Vessal, Hemmati and Vasei, 2003). Other mechanisms of beta cell damage occur via reactive oxygen species and nitric oxide production. Pancreatic beta cells take up STZ via GLUT-2 transporters (Szkudelski, 2001). Intracellular metabolism of STZ leads to liberation of nitric oxide which mediates in DNA damage leading to cell death (Szkudelski, 2001). In addition, STZ mediates mitochondrial production of superoxide ion.

Pancreatic beta cells are vulnerable to reactive oxygen species induced damage because of their particularly low quantities of antioxidant enzymes (Robertson and Harmon, 2005; Valko, Leibfritz, Moncol, Cronin, Mazur and Telser, 2007). The STZ-diabetic model has been used by many investigators as a research tool in studies involving antidiabetic drug discovery or diabetic complications (Musabayane, Ndhlovu and Balment, 1995; Marwaha, Banday and Lokhandwala, 2004; Musabayane, Mahlalela, Shode and Ojewole, 2005). Although considered as a type I model, administration of low doses of STZ may induce type II diabetes resulting in hyperglycaemia due to insulin resistance as opposed to impaired secretion (Piotrowski, 2003).

1.7.1.1.2. Alloxan-induced diabetes

Alloxan, 2,4,5,6[1H,3H]-pyrimidinetetrone, is probably the longest known and most potent diabetogenic agent (Piotrowski, 2003). Uptake of alloxan by the pancreatic β-cells is via GLUT-2 transporters, due to similarity in molecular shape to glucose

(Elsner, Gurgul-Convey and Lenzen, 2006). Alloxan then undergoes reduction intracellulary in the presence of tripeptide thiol, glutathione, to yield dialuric acid whose re-oxidation, regenerates alloxan. Hence a cyclic reaction (redox cycle) is created that ultimately results in reactive oxygen species generation (Szkudelski, 2001; Elsner *et al.*, 2006). The reactive oxygen species so generated interact with pancreatic β-cellular DNA leading to their fragmentation and cell death (Szkudelski, 2001). Thus, like STZ, it is through reactive oxygen species generation that alloxan induces diabetes in experimental animals (Elsner *et al.*, 2006). Alloxan toxicity, however, affects other organs including the liver, kidney, gonads and lungs. Alloxan causes mortality in 37% of animals within a few days (Piotrowski, 2003).

1.7.1.1.3. The Non-Obese diabetic (NOD) mouse

The non-obese mouse model is a genetically homogenous animal mode of insulin dependent diabetes mellitus (Ikegami, Fujisawa and Ogihara, 2004). Development of diabetes in the NOD mouse model is remarkably similar to humans (Kurrer *et al.*, 1997). Like in the BB rat, β-cell cell destruction in the NOD mouse is spontaneous and is initiated by IFN-γ^+ T cells (Kukreja, Cost, Marker, Zhang, Sun, Lin-Su, Ten, Sanz, Exley, Wilson, Porcelli and Maclaren, 2002). The limitations to usage of models that develop diabetes mellitus spontaneously are high costs of the animal models and low availability of genetically impaired animals (Piotrowski, 2003).

1.7.1.1.4. The BioBreeding (BB) rat

This is an animal model type I diabetes that spontaneously develops autoimmune insulin-dependent diabetes whose characteristics are lymphocyte infiltration into the pancreatic islets, and T cell-mediated β-cell destruction (Hessner, Wang, Meyer, Geoffrey, Jia, Fuller, Lernmark and Ghosh, 2004). Development of diabetes is often associated with thyroiditis in the BB rat (Bach, 1994).

1.7.1.2. Animal models of type II diabetes mellitus

The Zucker –ZDF is an animal experimental model that mimics type II diabetes mellitus. It manifests insulin resistance, obesity, hyperglycaemia, hypertriglyceridaemia, hypertension and hypercholesterolaemia (Liu, Pérusse and Bukowiecki, 1998). Leptin is ineffective in suppressing food intake in the Zucker rat. This is because of a mutation of the leptin receptor encoding gene (Carlson, Shelton, White, and Wyss, 2000.

1.7.1.3. Other animal models of experimental diabetes

Other experimental models of diabetes include the transgenic mice and, immunomanipulated models by performing thymectomy (Bach, 1994). Induction of type II diabetes in the rat is also possible by a subjecting the animal to a combination of a high fat diet and low dose STZ injection (Reed, Meszaros, Entes, Claypool, Pinkett, Gadbois and Reaven, 2000; Srinivasan, Viswanad, Asrat, Kaul and Ramarao, 2005). The Goto-Kakizaki is a non-obese type II diabetes rat model that develops

diabetes spontaneously (Tourrel, Bailbe, Lacorne, Meile, Kergoat and Portha, 2002). Experimental animal models are commonly used as tools in order to establish the antidiabetic effects of medicinal plants and other alternative therapy.

1.8. Background to medicinal plant research

Medicinal plant usage by the Neanderthal man, as far back as 60000 years has been recorded (Hart, 2005). In animals, wild chimpanzees have been reported to swallow plant leaves as prophylactics against gastrointestinal nematode infection when commonest, during the rainy season (Hart, 2005). Wood rats, *Neotoma fuscips* use leaf sprigs of California bay, *Umbellularia californica*, in the fumigation of their nests (Hart, 2005). Therefore, this shows that the usage of plants as medicines has not been exclusive to man.

In the developing world, plant medicines are widely available and affordable to the majority of the people, and a large proportion of the populations in such countries rely on medicinal plants (Okine, Nyarko, Kwabena, Oppong, Barnes and Ofosuhene, 2005). Currently, up to 80% of Africans continue to use traditional medicine in the management and treatment of various diseases (Ojewole, 2005). Usage of such forms of therapy has been practiced over generations. Consequently, there is cultural significance attached to such medicines (Light, Sparg, Staffold and Staden, 2005). An increase in the usage of medicinal plants in Africa is partly due to non-availability and high costs of synthetic medicines. For example, insulin availability in government hospitals in Sub-Saharan Africa is often irregular, and the approximate cost for diabetes treatment for an East African type1 diabetic is US$229, annually (Beran *et*

al., 2006). In Sudan, about half of diabetics abandon or reduce insulin treatment because of inconsistent availability or prohibitive costs (Abdelgadir, Elbagir, Eltom and Berne, 2006). Although most of these herbal treatments are effective, they have received little or no scientific scrutiny to establish efficacies and toxicities. Globally, the usage of alternative medicines including herbal medicines is on the rise, because they are thought to possess fewer side effects and have multiple therapeutic effects compared with the synthetic medicines (Jung, Lee, Jeong and Choi, 2004: Hou, Zhang and Wu, 2005). About a quarter of best selling drugs worldwide in 2001 and 2002 were either natural products or traced their origins from natural products (Balunas ang Kinghorn, 2005). Studies have also shown that there is general acceptance of medicinal plant usage if their claimed therapeutic effects are substantiated with scientific evidence (Gilani and Rahman, 2005). This may also have contributed to increased use of medicinal plants.

In general, health research in noncommunicable diseases in Sub-Saharan Africa has not been prioritised and mortality from these diseases is higher in the region than in Western countries (Unwin, Setel, Rashid, Mugusi, Mbanya, Kitange, Hayes, Edwards, Aspray and Albert, 2001). The World Health Organization (WHO) has acknowledged that three quarters of the world's population continue to rely on herbal therapy. According to WHO more than 150 medicinal plants are currently used for management of diabetes (Eddouks, Maghrani, Zeggwagh and Michel, 2005). Furthermore, WHO has encouraged the scientific community to conduct further investigations into the therapeutic potential of natural products with antidiabetic properties.

Research into medicinal potential of plants has already yielded fruits. For example drugs including morphine, aspirin, atropine, artimesinin, colchicine, digoxin, ephedrine, morphine, physostigmine, pilocarpine, and quinine trace their origins from plants (Gilani and Rahman, 2005). Moreover, metformin a major antihyperglycaemic agent originates from a guanidine that was isolated from a plant *Galega officinalis* L. (French lilac) (Vuksan and Sievenpiper, 2005; Martineau, Couture, Spoor, Benhaddou-Andaloussi, Harris, Meddah, Leduc, Burt, Vuong, Le, Prentki, Bennett, Arnason and Haddad, 2006). Furthermore, new antidiabetic drugs including troglitazone and exendin-4 have originated from medicinal plants (Choi, Ko, Park, Jang and Park, 2005).

Medicinal plant research is essential because it provides new drugs, new leads to new drugs and discovery of new chemical compounds (Butler, 2004). A fifth of all new chemical compounds resulted after studies of natural products between 1981 and 2002 (Newman, Cragg and Snader, 2003). Over 200 pure phytochemicals with potent antidiabetic effects have been isolated from plants. For example, pinitol was isolated from soy beans (Bates, Jones and Bailey, 2000). This compound has demonstrated effectiveness at enhancing glucose transport in L6 rat muscle cells and hypoglycaemic effects in STZ-induced diabetic animals (Bates *et al.*, 2000). Other compounds with antidiabetic properties include masoprocol from creosote bush, (Luo, Chuang, Cheung, Quan, Tsai, Sullivan, Hector, Ree, Meszaros, King, Carlson and Reaven, 1998), diphenylamine from onions, arecoline from betel nut (Karawya, Abdel, El-Olemy and Farrag, 1984; Chempakam, 1993), ginsenoside Rb from ginseng (Gong, Jiang, Li, Zhu and Zhang, 1991), and, epigallocatechin gallate from green tea (Waltner-Law, Wang, Law, Hall, Nawano and Granner, 2002). It is, therefore, vital

for the scientific community to investigate the therapeutic efficacy and safety of these natural products in the management of diabetes mellitus.

1.8.1. Some reported medicinal plants with hypoglycaemic effects

The antidiabetic potential of a number of medicinal plants has been investigated and established in various laboratory settings. Presented below are some of the reported medicinal plants with antihyperglycaemic properties in literature. Also presented are other biological effects associated with the reported medicinal plants, besides antidiabetic effects.

Mangifera indica L. (Anacardiceae)

Mango, *Mangifera indica,* of family Anacardiaceae, is widely consumed as fruit in many parts of the world. Aqueous extracts of mango leaves have demonstrated antidiabetic effects in glucose-induced hyperglycaemic rats and mice (Muruganandan, Srinivasan, Gupta, Gupta and Lal, 2005). Mangiferin, catechin and epicatechin are the three main polyphenolic compounds present in the stem bark extracts (Hernandez, Rodriguez, Delgado and Walczak, 2006). Assessment of mangiferin by Muruganandan *et al.*, (2005), indicated hypoglycaemic, antilipidaemic and antioxidant effects. Antihyperglycaemic effects are mediated partly by delayed gastrointestinal absorption of glucose, as a result of inhibiting α-glucosidase actions (Prashanth, Amit, Samiulla Asha and Padmaja, 2001; Muruganandn, *et al.*, 2005). In addition, the hypoglycaemic effects are thought to be mediated by possible extra pancreatic mechanisms.

Cytotoxicity of *M. indica* extracts on T-cells demonstrated protection against T-cell apoptosis, attributed to its antioxidant properties (Hernandez *et al.*, 2007). Other established biological properties of *M. indica* extractives include antiiflammatory (Garrido, González, Lemus, Garc´ıa, Lodeiro, Quintero, Delporte, Núñez-Sellés and Delgado, 2004; Beltrán, Alvarez, Xavier, Hernanz, Rodriguez, Núñez, Alonso and Salaices, 2004), anti-diarrhoeal (Sairam, Hemalatha, Kumar, Srinivasan, Ganesh, Shankar and Venkataraman, 2003), and antioxidant effects (Anila and Vijayalakshmi, 2003).

Maytenus senegalensis (Lam.) Excell (Celastraceae), *Annona senegalensis* Pers. (Annonaceae), *Kigelia Africana* (Lam.) Benth. (Bignoniaceae), and *Lannea welwitschii* (Hiein) Engl. (Anacardiaceae)

ADD-199 is a medicinal preparation used in Ghana, made from several plant species such as *Maytenus senegalensis*, *Annona senegalensis*, *Kigelia africana* and *Lannea welwitschii* (Okine *et al.*, 2005). Phytochemical studies of ADD-199 have shown that it possesses anthocyanins, terpenoids, quinones and alkaloids. These have been shown by other investigators to have blood glucose lowering properties (Okine *et al.*, 2005). Toxicity studies in rats by Nyarko, Okine, Wedzi, Addo and Ofosuhene, (2005), revealed that ADD-199 had no effects on urinary, haematological and plasma biochemical parameters. Subchronic administration of ADD-199 had no effect on blood platelet count and would, therefore, not cause thrombocytosis (Nyarko *et al.*, 2005). Moreover, administration of ADD-199 did not increase levels of urea, creatinine, albumin and bilirubin suggesting that it was neither toxic to the liver or the kidney. In addition the investigators found that ADD-199 did not cause drug

interactions via the hepatic CYP-dependent monooxygenases. The liver CYP monooxygenases are a family of enzymes that are involved in the metabolism of some endogenous substances and xenobiotics (Guengerich, Dannan, Wright, Martin and Kamensky, 1982).

Gongronema latifolium Benth. (Asclepiadaceae)

The aqueous extracts of *Gongronema latifolium* of botanical family Asclepiadaceae is used in the traditional management of diabetes mellitus in the tropics (Ugochukwu and Cobourne, 2003). Ugochukwu and Babady, (2003) demonstrated that leaves of *Gongronema latifolium* of family Asclepiadaceae, possess antihyperglycaemic effects. The major groups of active compounds in *G. latifolium* are saponins. The antihyperglycaemic effects of *G. latifolium* were mediated by increasing activities of glucokinase, hepatic glucose-6-phosphate dehydrogenase and hepatic glucose-6-phosphatase (Ugochukwu and Babady, 2003). Evaluation of ethanolic extracts of *G. latifolium* in normal and STZ-induced diabetic Wistar rats demonstrated up regulation of antioxidant activities (Ugochukwu and Cobourne, 2003). Ugochukwu and Babady also demonstrated potent antioxidant activities of ethanolic and aqueous extracts of *G. latifolium* in type II model of diabetic rats (Ugochukwu and Babady, 2002).

Inula viscosa L. (IV) [Asteraceae]

Inula viscosa L. (IV) belongs to the botanical family Asteraceae. Decoctions of *I. viscosa* have been used in folkloric practice in the Mediterranean region because of its antiiflammatory, antipyretic and antidiabetic properties (Al-Dissi, Salhab and Al-Hajj,

2001). Its hypoglycaemic effects were reported by Zeggwagh, Ouahidi, Lemhadri and Eddouks, (2006). These effects were attributed to inhibition of hepatic gluconeogenesis. Administration of *I. viscosa* in pregnant rats resulted in abortions (Al-Dissi, *et al.*, 2001). Extracts of *I. viscosa* were demonstrated to have inhibitory effects on chitin synthesis in dermatophytes and *Candida albicans.* These findings prove that *Inula viscosa* has antifungal effects (Maoz and Neeman, 2000).

Baccharis trimera (Less) DC (Asteraceae)

Baccharis trimera (Less.) D.C. of botanical family Asteraceae is used in Brazil in folkloric treatment of diabetes mellitus, liver diseases, rheumatism, renal and digestive disorders (Januario, Santos, Marcussi, Mazzi, Pietro, Sato, Ellena, Sampaio, Franca and Soares, 2004; Oliveira, Endringer, Amorim, Brand and Coelho, 2005). Phytochemical evaluation by Oliveira *et al.*, (2005) showed the presence of flavonoids and chlorogenic acids. The authors also reported that laboratory studies in diabetic mice confirmed the antihyperglycaemic effects. Other investigations demonstrated that extractives of *B. trimera* possess anti-proteolytic and anti-hemorrhagic effects against *Bothrops* snake venoms. These effects were attributed to the active compound, a diterpene, 7α-hydroxy-3,13-clerodadiene-16,15:18,19-diolide, isolated from *B. trimera* (Januario *et al.*, 2004). Vasorelaxing properties of *B. trimera,* in smooth muscle of *corpus cavernosum,* isolated from guinea pigs were reported by Hnatyszyn, Moscatelli, Garcia, Rondina, Costa, Arranz, Balaszczuk, Ferraro and Coussio, (2003). In a related study Torres, Gamberini, Roque, Lima-Landman, Souccar and Lapa, (2000), isolated a dilactonic clerodane diterpene from the aerial

parts of *B. trimera* whose vasorelaxant properties on isolated rat portal vein were established.

Helichrysum plicatum DC. subsp. *plicatum* (Asteraceae)

Helichrysum plicatum ssp. *plicatum* capitulums is used in folkloric treatment of diabetes in Turkey (Aslan, Orhan, Orhan, Sezik and Yesilada, 2007). Moreover, laboratory studies supported this traditional usage by demonstrating antidiabetic and antioxidant effects in STZ-induced diabetic rats. Phytochemical evaluation revealed that flavonoids are the main groups of chemical compounds in *H. plicatum* ssp. *plicatum* (Aslan *et al.*, 2007). Further antioxidant effects of *H. plicatum* have been confirmed with reports by Tepe, Sokmen, Akpulat and Sokmen, (2005).

Lepidium sativum L. (Brassicaceae)

Lepidium sativum L. is reported by Eddouks *et al.*, (2005) to possess hypoglycaemic effects in both normal and diabetic animals. These hypoglycaemic effects have been attributed to the presence of imidazole alkaloids. *L. sativaum* also possesses therapeutic potential in management of hypertension. The aqueous extracts of *L. sativum* seeds demonstrated antihypertensive effects in spontaneously hypertensive rats (SHR). In addition the aqueous extracts demonstrated diuretic, natriuretic, kaliuretic and chloride excretion and effects in SHR and normotensive WKY rats (Maghrani, Zeggwagh, Michel and Eddouks, 2005).

Terminalia superba Engl. and Diels (Combretaceae) and *Canarium schweinfurthii* Engl. (Burseraceae)

Antidiabetic effects of *Terminalia superba* and *Canarium schweinfurthii* were reported by Kamtchouing, Kahpui, Dzeufiet, T'edong, Asongalem and Dimo, (2006). The powder of stem bark of *T. superba* of family Combretaceae is used in folkloric management of diabetes mellitus in Senegal. In the Congo and Central African Republic, *C. schweinfurthii* is used for the management of fever, post-partum pain and rheumatism (Koudou, Abena, Ngaissona and Bessie're, 2005). Alkaloids are the principal groups of chemical compounds available between the 2 species. Kamtchouing *et al.*, (2006), suggested that the antihyperglycaemic effect of the plant extracts were mediated by mimicking insulin actions of promoting glucose uptake and subsequent breakdown and blocking liver glucose synthesis. Koudou *et al.*, (2005) isolated essential oil from resins of *C. schweinfurthii*, characterized octylacetate and nerolidol as the main principles in the essential oil, and, reported on analgesic effects of *C. schweinfurthii* essential oil.

Opuntia megacantha Salm-Dyck (Cactaceae)

Bwititi, Musabayane and Nhachi (2000) demonstrated that *Opuntia megacantha*, despite exhibiting favourable glycaemic effects in both normal and diabetic rats is renotoxic. The high phenolic content in *O. megacantha*, responsible for many biological effects has been confirmed (Ndhlala, Kasiyamhuru, Mupure, Chitindingu, Benhura and Muchuweti, 2006).

Caesalpinia bonducella (L.) Flemming (Caesalpiniaceae)

Caesalpinia bonducella Roxb. belongs to family Caesalpiniaceae. Studies have established that *C. bonducella* has anti-inflammatory and antimalarial effects. The plant decoctions are used in ayurveda as antipyretic, antirheumatic, antispasmodic, antiperiodic and in the management of asthma and diabetes (Saeed and Sabir, 2001; Kannur, Hukkeri and Akki, 2006). Phytochemical studies have revealed the presence of phytosterinin, â-sitosterol, flavonoids, bonducellin, aspartic acid, arginine, citrulline, â-carotene and triterpenoids. Kannur *et al.*, (2006) and Chakrabarti, Biswas, Seal, Rokeya, Ali, Khan, Nahar, Mosihuzzaman and Mukherjee, (2005) have validated the ayurvedic antidiabetic use of *C. bonducella* with laboratory studies. These hypoglycaemic effects were attributed to insulin secreting properties of *C. bonducella* and capacity to induce glycogen synthesis (Chakrabarti, Biswas, Rokeya, Ali, Mosihuzzaman, Nahar, Khan and Mukherjee, 2003; Chakrabarti *et al.*, 2005). Antibacterial activities of *C. bonducella* were reported by Saeed and Sabir, (2001).

Euonymus alatus (Thunb.) Siebold., (Celastraceae)

Euonymus alatus is used in folkloric management of pain relief, blood circulation ailments, blood clotting and dysmenorrhea in some Asian countries (Park *et al.*, 2005). *E. alatus* has hypoglycaemic and hypolipidaemic effects mediated by decreasing food intake, and through its influence on expression of PPARγ genes and hepatic lipogenesis related genes (Park *et al.*, 2005). In addition to antihyperglycaemic effects, *E. alatus* has demonstrated potential for the management of cancer. From the methanolic extract of *E. alatus* stem, a phenolic principle with

anticancer potential, 5-caffeoylquinic acid has been isolated (Jin, Lee, Kang, Kim, Park, Kim, Moon and Kim, 2005). These authors showed that 5-caffeoylquinic acid suppressed effects of matrix metalloproteinase (MMP)-9, activities, an enzyme that mediates tumour cell invasion and metastasis. In addition, *E. alatus* induces nitric oxide production in mouse peritoneal macrophages (Chung, Jeong, Kim, Jeong, Kim, Kim, Kang, Ahn, Baek and Kim, 2002). According to Chung *et al.*, (2002), this suggests that *E. alatus,* mediated via nitric oxide production can perform various therapeutic activities including antitumour, antimicrobial and antiviral effects. Management of circulatory problems by *E. alatus* may be mediated through nitric oxide production.

Eucommia ulmoides Oliv. (Eucommiaceae)

The bark of *Eucommia ulmoides* is used in the management of blood pressure, prevent miscarriages and improve the tonicity of the kidney and liver in many places including Japan, China and Korea (Lee, Kim, Cho, Park, Park, Jung, Park and Choi, 2005). The hypoglycaemic effects of leaves of *E. ulmoides* of family Eucommiaceae have been established in scientific investigations (Lee, *et al.*, 2005; Park, Choi, Kim, Jung, Kim, Park, Noh, Park, Park, Lee and Lee, 2006). The major constituents in the plant are flavonoids, triterpenes and polyphenols. Studies by Hung, Fu, Shih, Lee and Yen (2006) have also demonstrated that *E. ulmoides* has dose-related protective effects on carbon tetrachloride (CCl4)-induced chronic hepatotoxity in Wistar rats. These effects were mediated through suppressing oxidative damage and increasing the antioxidant status. Moreover, aqueous and ethyl acetate extracts of *E. ulmoides* demonstrated ability to protect against copper-induced modification of low density

lipoprotein (LDL) oxidation (Yen and Hsieh, 2002). In an earlier study, Hsieh and Yen, (2000) showed that *E. ulmoides* has antioxidant effects against oxidative damage to biomolecules such as deoxyribonucleic acid (DNA) and 2'-deoxyguanosine (2'-dG).

Pterospartum tridentatum L. Willk. (Fabaceae)

The folkloric usage of *Pterospartum tridentatum* flowers in the management of diabetes mellitus has been verified by laboratory studies. Vitor, Mota-Filipe, Teixeira, Borges, Rodrigues, Teixeira and Paulo, (2004) reported that flavonoids are the major groups of active compounds in *P. tridentatum* and their antihyperglycaemic effects may be mediated by their capacity to attenuate oxidative damage.

Sutherlandia frutescens (L.) R. Br (Fabaceae)

Sutherlandia frutescens is endemic to Southern Africa. Decoctions of the plants leaves have been used by certain tribes in the management of a plethora of diseases including diabetes mellitus, influenza, abdominal pain, chicken pox and rheumatism (Chadwick *et al.*, 2007). Laboratory investigations confirmed the antidiabetic effects of the *S. frutescens* extracts in a type II diabetic animal model. Chadwick *et al.*, (2007) suggested that the hypoglycaemic effects were mediated by enhancing insulin sensitivity of the target cells or by stimulating insulin release. *S. frutescens* has antioxidant properties which account for some of its reported anti-inflammatory effects. These effects were reported by Fernandes, Duncan Cromarty and Albrecht, Rensburg (2005) who showed that hot aqueous extract of *S. frutescens* exhibited

superoxide and hydrogen peroxide scavenging activities. *S. frutescens* possesses anti-cancer properties. Tai, Cheung, Chan, Hasman, (2004) showed that ethanolic extract induced cell death on a number of human tumour cell lines. These assertions were supported by reports by other investigators who reported that *S. frutescens* has enormous anticancer potential in (Chinkwo, 2005).

Other investigations have revealed Anti HIV (human immunodeficiency virus) effects of *S. frutescens* were evaluated by Harnett, Oosthuizen, van de Venter, (2005). The authors reported that *S. frutescens* possessed inhibitory properties against HIV target enzymes.

Trigonella foenumgraecum Linn. (cv ghouka) [Leguminosae]

Vats, Yadav and Grover, (2003) have demonstrated that *Trigonella foenumgraecum* has hypoglycaemic effects. These effects are mediated by enhancing hepatic and renal glycogen synthesis. In patients with coronary artery diseases and type II diabetes mellitus, administration of *T. foenumgraecum* reduced blood levels of cholesterol and triglycerides but had no effects on HDL-cholesterol (Bordia, Verma and Srivastava, 1999).

Hibiscus rosa sinensis L. (Malvaceae)

Hibiscus rosa sinensis belongs to botanical family Malvaceae. In ayuverdic practice, petal decoction is used to manage fever and in bronchial catarrh. Studies by Sachdewa and Khemani (2003), have revealed that ethanolic extractives of *Hibiscus rosa sinensis* (Linn.) possess hypoglycaemic and antilipidaemic effects attributed to

mediatory roles that are extra-pancreatic. In these studies, phytochemical investigations revealed the presence of flavonoids.

Eugenia jambolana Lam., (Myrtaceae)

Extracts of *Eugenia jambolana* kernels have potent hypolipidaemic effects demonstrated in laboratory studies (Ravi, Rajasekaran and Subramanian, 2005). These effects were attributed to the presence of flavonoids, saponins, triterpenoids and glycosides. The administration of *E. jambolana* extract mediated more regulated mobilization of plasma triglycerides, and improved insulin secretion levels and actions. The authors asserted that their findings provided scientific basis for the present usage of *E. jambolana* in the management of diabetes mellitus. Compounds isolated from *E. jambolana* include quercetin, myricetin, myricitrin and myricetin 3-*O*-(4-acetyl)-α-L-rhamnopyranoside (Timbola, 2002). In a related study, chronic treatment of *E. jambolana* to diabetic rabbits was shown to induce reduced plasma levels of cholesterol, and triglycerides while increasing high density lipoprotein (HDL) levels (Sharma, Nasir, Prabhu, Murthy and Dev, 2003).

Another study showed that *E. jambolana* extracts have antioxidant effects which were attributed to its hypoglycaemic effects (Ravi, Ramachandran and Subramanian, 2004). Recently, the aqueous and ethanolic extracts of *E. jambolana* fruit were further demonstrated to have hypoglycaemic effects in alloxan induced diabetic rats. In these studies the aqueous extract was more potent than the ethanolic extract (Sharma, Nasir, Prabhu and Murthy, 2006). Phytochemical investigations, however, revealed the presence of both antihyperglycaemic and hyperglycaemic principles. Hyperglycaemic principles were present in the initial fractions after *E. jambolana* fruit extract was

subjected to purification via column chromatography using diethyl amino ethyl cellulose. Other investigations suggested that the antihyperglycaemic effects of *E. jambolana* are mediated by mechanisms similar to both insulin secretagogues and biguanides (Grover, Vats and Rathi, 2000).

Myrtus communis L. (Myrtaceae)

Myrtle oil obtained from leaves of *Myrtus communis* L. of botanical family Myrtaceae is used in Turkey in the traditional management of type II diabetes. *M. communis* is also used in folklore, as an antiseptic, antibacterial, analgesic and antihyperglycaemic remedy (Sepici-Dincel, Açıkgöz, Cevik, Sengelen and Yesilada, 2006). Laboratory studies have confirmed the antihyperglycaemic effects of the oil (Sepici, Gürbüz, Çevik and Yesilada, 2004). Furthermore, α-glucosidase inhibitory activities of the oil have been established as the mechanism of action. Sepici-Dincel *et al.*, (2006), demonstrated that long term administration of *M. communis* extracts to alloxan-induced diabetic rabbits increased their antioxidant status. *M. communis* also demonstrated antioxidant (Montoro, Tuberoso, Piacente, Perrone, De Feo, Cabras and Pizza, 2006) and antigenotoxic effects (Hayder, Abdelwahed, Kilani, Ammar,Mahmoud, Ghedira and Chekir-Ghedira, 2004).

Syzygium cordatum (Hochst.) [Myrtaceae]

On the other hand, acute administration of *S. cordatum* extract to diabetic rats demonstrated hypoglycaemic effects. Long term administration of *S. cordatum* extract

to diabetic animals improved glycogen synthesis compared to untreated diabetic animals (Musabayane, *et al*, 2005).

Rhizoma coptidis (Huang Lian) [Ranunculaceae]

Rhizoma coptidis has been used in the management of diabetes mellitus in China for thousands of years. Recent *in vitro* and *in vivo* laboratory studies suggest that berberine, a major constituent in *R. coptidis,* is responsible for the antihyperglycaemic effects mediated by stimulating insulin release (Leng, Lu and Xu, 2004). The hypocholesterolaemic effects of *R. coptidis* were demonstrated in laboratory studies by Yokozawa, Ishida, Cho and Nakagawa, (2003). The authors showed that oral administration of *R. coptidis* to Wistar rats fed a hypercholesterolaemic diet lowered their serum cholesterol levels.

Iizuka, Miyamoto, Hazama, Yoshino, Yoshimura, Okita, Fukumoto, Yamamoto, Tangoku and Oka, (2000) demonstrated that oral administration of *R. coptidis* extract to tumour bearing mice significantly lowered serum interleukin (IL)-6 levels. Berberine, an active compound isolated from *R. coptidis* demonstrated potent effects against IL-6 both *in vivo* and i*n vitro* studies.

Solanum xanthocarpum Schrad. &Wendl. (Solanaceae)

Fruits of *Solanum xanthocarpum* are used in the traditional management of a number of ailments for its anthelmintic, antiinflammatory, laxative, diuretic and antiasthamatic effects. Leaves of *S. xanthocaprpum* are also used for management of cough, catarrhal fever and chest pains (Rahman, Ahmed, Alimuzzaman and Shilpi,

2003; Kar, Maharana, Pattnaik and Dash, 2006). Phytochemical studies have revealed the presence of steroidal alkaloids. The authors reported that the antidiabetic effects of *S. xanthocaprpum* are mediated by insulin-like activities. Maruo, Bernardi and Spinosa, (2003), investigated the toxic effects of long term consumption of *S. xanthocaprpum* dried fruits to male and female Wistar rats. Results showed that while the extracts had no toxic effects in male rats, it reduced liver and uterus weights in female rats. Evaluation of antinociceptive effects of *S. xanthocaprpum* revealed that it reduced the frequency of acetic acid-induced abdominal constrictions in rats (Rahman *et al.*, 2003).

Solanum lycocarpum St. Hil. (Solanaceae)

In Brazil, *Solanum lycocarpum* St. Hill is used as a hypoglycaemic agent (Perez, Franca, Daldegan and Duarte, 2006). The major constituents of *S. lycocarpum* are steroidal alkaloids. Investigations of the extracts of the plants fruit on haematological parameters in diabetic rats demonstrated that it has potent effects in reducing levels of reactive oxygen species.

Vitis vinifera L. (Vitaceae)

Vitis vinifera is used world wide as food and in traditional management of a number of human maladies including pain, inflammation and blood pressure (Orhan, Aslan, Orhan, Ergun and Yeşilada, 2006). Phytochemical evaluation revealed that the main groups of chemical constituents in *V. vinifera* leaves are flavonoids, tannins and procyanidins. The antihyperglycaemic effects of ethyl acetate extract of *V. vinifera*

were mediated by stimulating insulin release and delaying gastrointestinal absorption of glucose. These effects were attributed to the presence of tannins and flavonoids (Orhan *et al.*, 2006). In addition to antihyperglycaemic effects, *V. vinifera* has antimicrobial properties. These were demonstrated by Karapinar and Sengun, (2007), on *Salmonella typhimurium* present in salad vegetables.

Cissus sicyoides C. Linnaeus (Vitaceae)

Cissus sicyoides is used in the traditional management of a broad spectrum of diseases including gonorhoea, inflammation and respiratory infections in Dominican Republic (Garcia, Saenz, Puerta, Quilez and Fernandez, 1999). Phytochemical studies of *C. sicyoides* have shown that the major compounds in the plant include steroidal-triterpenes, flavonoids, tannins and saponins. The plant was shown to possess hypoglycaemic effects (Viana, Medeiros, Lacerda, Leal, Vale and Matos, 2004). These effects were mediated in a manner similar to biguanides (Viana *et al.*, 2004). Garcia *et al.*, (1999), demonstrated that *C. sicyoides* was effective against both gram-positive and gram negative microbes. Furthermore, *C. cisyoides* demonstrated anti-inflammatory effects that were attributed to its large contents of steroids (Garcia, Quilez, Saenz, Martinez-Dominguez, and de la Puerta, 1999). Other studies have shown that the aqueous extracts of *C. sicyoides* have vasoconstrictor effects on isolated guinea pig aortic rings thought to be mediated by inducing increase in intracellular calcium influx (Garcia, Cartas-Heredia, Lorenzana-Jfmenez and Gijon, 1997). Therefore, while administration of *C. sicyoides* may confer benefits in blood glucose control, it may exacerbate other complications of diabetes mellitus such as

hypertension. Hence studies by Garcia *et al.*, (1997), will help users of *C. sicyoides* to be cautious.

The consensus of a large number of studies that have characterized active principles from medicinal plants show that, generally, major groups of biological compounds with potent antihyperglycaemic effects include flavonoids, vitamins, carotenoids, anthocyanins (Argolo, Sant'Ana, Pletsch and Coelho, 2004; Jung, Lee, Park, Kang and Choi, 2006). These products have multiple beneficial biological effects to humans and experimental animals. For example, flavonoids possess free-radical scavaenging capacity, affect a wide range of body enzymes, have capacity to chelate certain metal cations and provide protection against LDL oxidation (Asgary, Naderi, Sarrafzadegan, Ghassemi, Boshtam, Rafie and Arefian, 1999).

Indeed, as highlighted above, most medicinal plants with hypoglycaemic therapeutic potential also exhibited antihypertensive, renoprotective, antioxidant, antihyperlipidaemic and anticancer properties. This demonstrates that medicinal plants have potential to offer multiple therapeutic benefits.

1.8.2. Plants used in the present study

The plant species used in the present study were selected for study after an extensive ethnobotanical survey that involved interviewing traditional healers and literature survey.

1.8.2.1. *Ficus thonningii* (Blume) [Morarceae]

F. thonningii belongs to botanical family Moraceae. This is shown in Figure 2. The presence of flavonoids in *F. thonningii* were confirmed in studies by Greenham, Grayer, Harborne and Reynolds, (2007). However, there is a paucity of literature on the biological effects of *Ficus thonningii*. The only available literature is on related species of genus Ficus. Members of same genus or family frequently have similar chemical composition and hence biological properties. For example, *Gongronema latifolium* belongs to botanical family Asclepiadaceae. In this family are two other species, *G. sylvestre* and *G. inodorum*. All the three species above possess antihyperglycaemic effects (Ugochukwu and Bababdy, 2003). Hence, reported below are some of the studies done on other species of genus Ficus.

In India, *F. hipida* is used in the folkloric treatment of a number of diseases including diabetes, ulcers, biliousness, psoriasis, anaemia, piles, jaundice, hemorrhage of the nose and mouth, and diseases of the blood (Ghosh, Sharatchandra, Rita and Thokchom, 2004). Laboratory studies by Ghosh *et al.*, 2004, showed that *F. hipida* bark extracts possess hypoglycaemic effects in normal and alloxan induced Wistar

albino rats. These effects were attributed to enhanced glycogen synthesis in muscle and liver and enhanced uptake of glucose by the muscle.

In Taiwan, *F. microcarpa* L. is used as an ornamental plant. Therapeutically, the plant extracts are known to have anti-platelet effects. Phytochemical studies of the bark revealed that *F. microcarpa* posses phenoids, a monoterpenoid, lignans, while the leaves and aerial roots revealed the presence of triterpenoids cycloartanoid, lupanoid, oleanoid, ursanoid (Chiang, Chang, Kuo, Chang and Kuo, 2005). A decoction of *F. carica* L. fruit is used as an antitussive and in the management of respiratory disorders in Italy, while the latex of unripe figs, fruit peduncles or twigs is used to treat warts (Guarrera, 2005).

Leaf decoctions of *F. thonningii* are used in the treatment of rheumatism in Ivory Coast. Also in Ivory Coast, the stem bark of a related species, *F. vallis-choudae* Del. is used in the management of heart problems. Studies by Koné, Atindehou, Terreaux, Hostettmann, Traoré and Dosso, (2004), demonstrated that leaf extracts of *F. thonningii* has antibacterial properties confirming the traditional usage of the plant decoctions against bacterial diseases.

Ficus bengalensis is used in India in the ayuverdic management of a number of diseases. The leaf decoctions are used to treat ulcers, while aerial parts of the roots are used to treat gonorrhea. A number of investigators have demonstrated that aqueous bark extracts possess hypoglycaemic, hypolipidaemic and hypocholesterolaemic effects in laboratory studies (Shukla, Gupta, Gambhir, Prabhu and Murthy, 2004). The authors also isolated 2 compounds from an ethanolic extract which demonstrated antioxidant effects.

A

B

C

Figure 2. *F. thonningii* branches (A), leaves (B) and, roots (C).

1.8.2.2. *Persea americana* Mill (Lauraceae)

Persea Americana Mill, (Figure 3), known in English as avocado, belongs to botanical family Lauraceae. It is native to Mexico but is grown widely throughout the tropics (Abe, Nagafuji, Okawa, Kinjo, Akahane, Ogura, Alfaro and Chilpa, 2005; Adeboye, Fajonyomi, Makinde and Taiwo, 1999). Like *F. thonningii*, there is scarcity of published works on the biological effects of *P. americana.* Hence, the following section contains reported studies on the biological effects of *P. americana* and other related species. Illustrated in Figure 3 are *P. americana* fruit and flowers. Avocado fruit is used as food in most parts of the world. In Nigeria decoction of avocado leaves are used to treat various diseases including hypertension (Adeboye *et al.*, 1999). Elsewhere, in Africa, West Indies, and in South and Central America the bark, fruit and leaves of *P. americana* have been used in traditional medicine in the treatment of hypertension, menorrhagia, stomach ache, bronchitis, diarrhoea and diabetes (Adeyemi, Okpo and Ogunti, 2002). Phytochemical screening of aqueous and methanolic leaf extracts revealed the presence of several bioactive groups of compounds including alkaloids, triterpene glycosides and coumarins saponins, flavonoids and tannins (Adeboye *et al.*, 1999; Adeyemi *et al.*, 2002). As reported by Ramos, Jerz, Villanueva, Dellamary, Waibel and Winterhalter, (2004), and Lu, Arteaga, Zhang, Huerta, Go and Heber, (2005), extractives of avocado possess phytosterols, triterpenes, fatty acids, oligomeric proanthocyanidins and dimmers of flavonols. Ramos, *et al.*, (2004), further reported the presence of 2 glucosylated abscisic derivatives in avocado seeds.

The fruit is also rich in fats, containing between 25% to 33% oil by composition (Ortiz, Dorantes, Gallndez and Cardenas, 2004; Lu *et al.*, 2005), monounsaturated

fatty acids being dominant. Ortiz *et al.*, 2004, pointed out that the nutritional value of avocado fruit oils is equivalent to that of olive oil. Unlu, Bohn, Clinton and Schwartz, (2005), reported that dietary intake of avocado fruit with salad enhances carotenoid absorption from salads and salsa. These effects were attributed to the oil available in fruit. These observations were supported by findings of Lu *et al.*, (2005), who pointed out that carotenoid absorption into the blood stream is enhanced by the high fat content of avocado. Mycotic and parasitic infections have been treated with decoctions of *P. americana* by the Aztec culture (Ramos *et al.*, 2004). Extracts of *P. americana* possess hypotensive properties in anaesthetized normotensive rats (Adeboye *et al.*, 1999). Hypotensive effects of *P. americana* were further supported by findings of Owolabi, Jaja and Coker, (2005), who reported that *P. americana* extracts exhibited vasorelaxing properties attributed to stimulation of release of endothelium derived relaxing factors (EDRF). However, administration of avocado oil rich diet exacerbated angiotensin II induced vasopressor effects in Wistar rats. This suggested that the vasodepressive properties of the avocado extracts might be due to compounds other than avocado fats (Salazar, Hafidi, Pastelin, Ortega and Mendoza, 2005). The authors also reported that avocado oil mediates the composition of fatty acids in cardiac and renal membranes.

P. americana also possesses anti Chagas' disease and anticancerous properties. Methanolic extracts of *P. americana* were effective against epimastigotes of *Trypanosome cruzi*, a causative agent for Chagas' disease (Abe *et al.*, 2005). Antiancerous effects of *P. americana* were reported by Lu *et al.*, (2005). Working on LNCaP and PC3, human prostrate cancer cell lines, the authors demonstrated that the acetone extracts of *P. americana* fruit inhibited proliferation of the cell lines. These

effects were due to lutein, a principal carotenoid and other vitamin compounds present in the extracts.

Assane, Diop, Niang-Sylla, Lopez-Sall, Gueye and Charlevna, (2001) have demonstrated that extracts of a related species of avocado almonds, *Persea gratissima Gaertner* (Lauraceae), possess antiicteric and hepatoprotective properties. The authors reported that lyophilisat of avocado almonds mediated the conjugation and excretion by the liver of bilirubin. Furthermore, the lyophilisat mediated the normalization of aminotransferases enzymes and healed hepatic lesions.

A

B

Figure 3. *P. americana* fruit (A), and flowers (B).

1.8.2.3. *Sclerocarya birrea* (A. Rich) Hochst (Anacardiaceae)

S. birrea (A. Rich) Hochst. subspecies *caffra* (Sond.) Kokwaro known in English as Marula belongs to botanical family Anacardiceae (Figure 4). It is indigenous to Western and Southern Africa. It has been reported to be used medicinally in the treatment of malaria, diarrhoea headache, diabetes mellitus and inflammatory diseases (Ojewole, 2000; Ojewole, 2003). The fruit and its nut are edible. Decoctions of bark are used to manage dysentery and diarrhoea by the Zulu people of South Africa (Eloff, 2001). In Niger, *S. birrea* fruit is used as food (Glew, VanderJagt, Huang, Chuang, Bosse and Glew, 2004). The pulp of the fruit is also used to make an alcoholic drink (Aganga and Mosase, 2001). In Burkina Faso, *S. birrea* decoctions are used to treat a host of human ailments. In addition to food and medicinal usage of *S. birrea* extracts, the wood is used as firewood and for carvings (Emanuel, Shackleton and Baxter, 2005).

Evaluation of the physicochemical composition of *S. birrea* seed by Ogbobe, 1992, suggests that it contains 11.0% crude oil, 17.2% carbohydrate, 36.70% crude protein 3.4% fibre and 0.9% crude saponins. Assessment of the same in seed oil revealed nine different fatty acids with palmitic, stearic and arachidonic acids being the most predominant. Braca, Politi, Sanogo, Sanou, Morelli, Pizza and De Tommasi, (2003) isolated quercetin 3-O-alpha-l-(5' '-galloyl)-arabinofuranoside (1), a flavonol glycoside from the methanolic extract of *S. birrea* leaves and eight other well known phenols from the same extract. From the leaf methanolic extract of cultivated *S. birrea* the group isolated two epicatechin derivatives.

In edible pits of *S. birrea,* Glew *et al.,* 2005, reported the presence of linoleic acid which is a component of membrane phospholipids and a precursor for arachidonic

acid. Also present in edible pits is oleic acid which is an important fatty acid with known cardioprotective effects and other fatty acids which are vital sources of energy when consumed, in communities that face starvation due to lack of food. Aganga and Mosase, (2001) asserted that the presence of tannins in food blocks its digestibility and, therefore, minimizes its absorption in the gut. Tannins are widely prevalent in the bark, leaves and fruits of most species in the Anacardiceae family. In their phytochemical assessment of *S. birrea* bark extracts, Aganga and Mosase reported presence of tannins in the species. Other studies reported that the *S. birrea* bark yields 3.5 to 20.5% tannin (Eloff, 2001).

The folkloric usage of *S. birrea* decoctions in the treatment of dysentery have been confirmed in *in vitro* studies by Eloff, (2001), who demonstrated that leaves and bark extracts had potent antibacterial effects against *Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa* and *P. aeruginosa.* Studies by Belemtougri, Constantin, Cognard, Raymond and Sawadogo, (2001) have shown that *S. birrea* antagonize calcium release from the sarcoplasm, induced by caffeine. Furthermore, Ojewole, 2000 substantiated the medicinal usage of *S. birrea* in the management of arthritis and an inflammatory disorder by showing that it possesses anti-inflammatory effects in Wistar rats. In a separate study, Ojewole, (2003), demonstrated that aqueous extracts of *S. birrea* has potent hypoglycaemic effects.

A

B

Figure 4. *S. birrea* fruits and leaves (A), and, bark (B).

1.8.2.4. **Hypoxis *hemerocallidea*** Fisch. & C.A. Mey. (Hypoxidaceae)

Hypoxis hemerocallidea, formerly known *as H. rooperi,* belongs to botanical family Hypoxidaceae (Figure 5). In English, it is known as African potato but is in fact a corm (Ojewole, 2006). It grows in meanders, valleys, grasslands and mountainous regions of South America, Southern Africa, Australia and coastal regions of Asia (Laporta, Pe´rez-Fons, Mallavia, Caturla and Michol, 2007). It has been used by the Zulus and other tribes of Southern Africa in the management of a variety of maladies including hypertension, diabetes, urinary tract infections, arthritis, and HIV-AIDS infections (Hutchings, 1989). Other studies have established that it possesses anti-inflammatory (Ojewole 2005), antibacterial (Gaidamashvilli and van staden, 2002) and anticancer properties (Albrecht, 1996). The major compound in most of the species of family hypoxidaceae is the relatively inert hypoxoside (*E*)-1,5-bis-(3',4'β-D-glucopyranosyloxy-3'-hydroxyphenyl)-pent-4-en-1-yne which can be converted to the more biologically active aglycone form, called rooperol, (*E*)1,5-bis- 4'-dihydroxyphenyl)-pent-4-en-1-yne), by β-glucosidase (Drewes, Hall, Learmonth and Upfold, 1984; Albrecht, 1996). Laporta *et al.*, 2007 reported that two compounds, hypoxoside and rooperol, isolated from *H. hemerocallidea* extract have more potent antioxidant activities against lipid peroxidation activities than products from other plants such as olive leaf and herbal tea. Other compounds present in *H. hemerocallidea* include β-sitosterol, stigmasterol, β-sitosterol glycoside and sitostanol (Mills, Cooper, Seely and Kanfer 2005).

Despite the numerous positive therapeutic uses of *H. hemerocallidea*, extracts of the plant might be toxic to the myocardium (Ker, 2005). The author reported on a case study where a hypertensive patient with a history of ischaemic heart disease and

diabetes, developed ventricular tachycardia after taking an *H. hemerocallidea* root extract in tea. Although hypoglycaemic effects of *H. hemerocallidea*, *F. thonningii*, *S. birrea* extracts have already been established in laboratory based studies, water was used as the medium of extraction of plant products. Furthermore, little attempt was made to establish the mechanism(s) of actions.

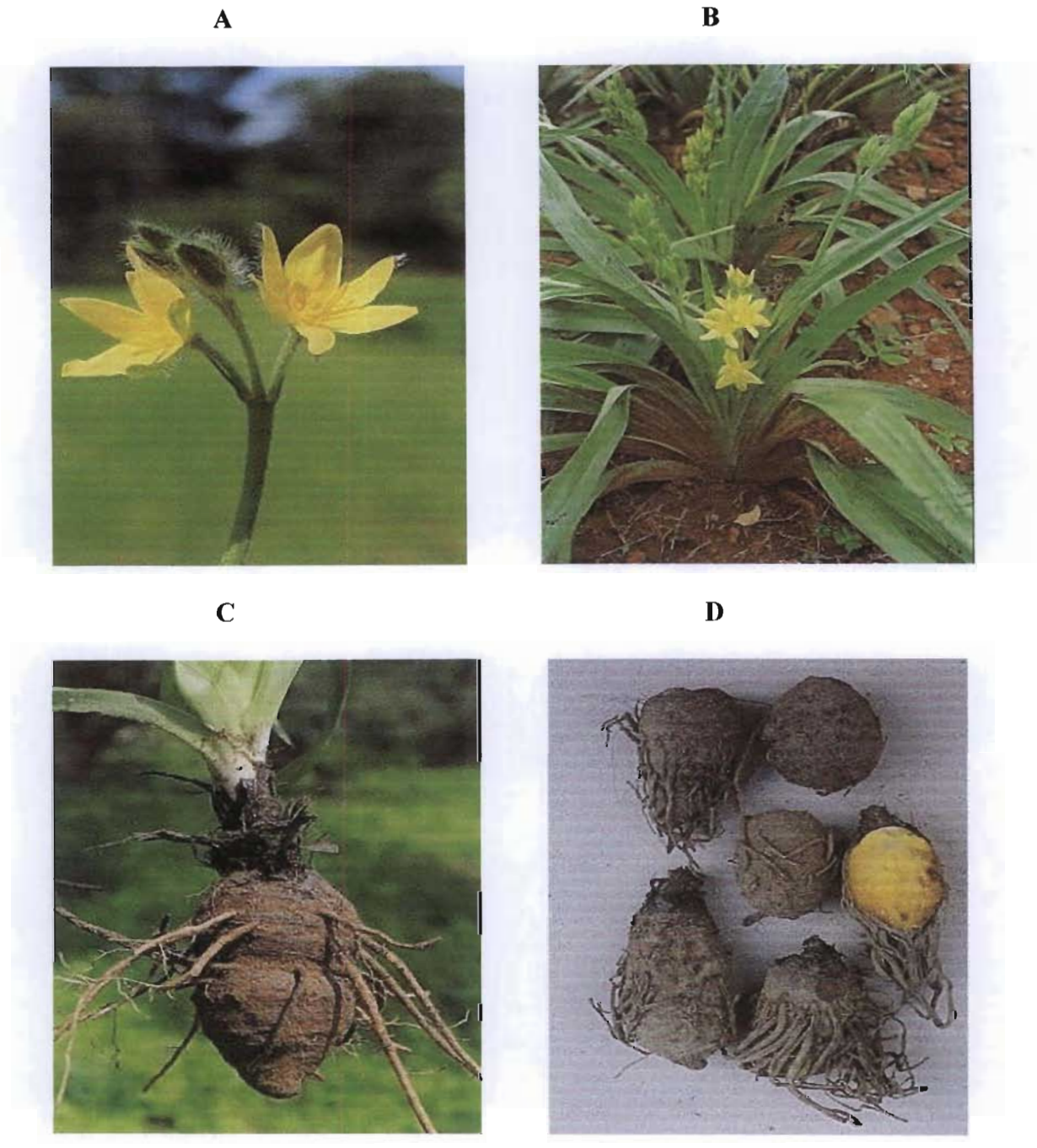

Figure 5 *H. hemerocallidea* flower (A), *H. hemerocallidea* leaves (B), *H. hemerocallidea* corm and roots (C), and, *H. hemerocallidea* corms (D).

1.9. Basis of the present study

As aforementioned diabetes is a chronic ailment with debilitating consequences, reducing quality and life expectancy. The need to look into alternative therapy is supported by emerging evidence that show allopathic medicines do not alleviate diabetes, but only manage hyperglycaemia. Moreover, most of these have one or more serious adverse effects. Herbal medicines have been used as alternative therapy in the amelioration of various diseases, including diabetes in Africa and beyond. Of late, a lot of interest has been generated in medicinal plants as alternative therapy because they are thought to be effective and safe (Jung, *et al.*, 2004; Hou, *et al.*, 20055). Despite wide consumption of medicinal plants, fewer studies have investigated their effectiveness or possible toxic effects. Even the World Health Organisation (WHO), has acknowledged the antidiabetic potential of medicinal plants and has urged increased research into the area (Eddouks, *et al.*, 2005). Since it is relatively less expensive in terms of monetary costs and time, to develop phytomedicines compared to western synthetic medicines, it is hoped that findings of this study will lead to discovery of drugs that are cheaper to the majority of poor patients in the region who cannot access the more expensive synthetic medications.

In the present study the effects of APE, SBE, FTE were investigated in normal and STZ-induced diabetic animals for their antihyperglycaemic effects using short term and long term study designs. Although hypoglycaemic effects of some of the plants have already been established, previous studies did not elucidate their mechanisms of actions. Moreover, these studies did not evaluate whether the plant products had any effects on parameters often considered diabetic complications such as hypertension

and renal function failure. Therapeutic effects of the test extracts were compared with those of standard drugs. Since cardiovascular and renal complications are closely associated with diabetes, the effects of the test extracts on hypertension and renal function were also assessed. Furthermore, effects of the test extracts on the viability of kidney cell lines of proximal and distal origins were also assessed to establish whether the test extracts altered tubular epithelial cells' viability to influences tubular reabsorption and secretion. The present study, therefore, was undertaken with the hope that findings of the study would be used in the development of herbal based drug that is effective, safe and affordable to the majority of poor African diabetics.

1.9.1. General objectives

The main purpose of the present study was to investigate the hypoglycaemic, cardiovascular and renal effects of extracts of *Hypoxis hemerocallidea*, *Sclerocarya birrea*, *Ficus thonningii* and *Persea americana*, in streptozotocin (STZ) - induced diabetic rats.

1.9.2. Specific objectives

The study aimed to investigate effects *Hypoxis hemerocallidea*, *Sclerocarya birrea*, *Ficus thonningii* and *Persea americana* extracts on blood pressure, and renal fluid and electrolyte handling in STZ-induced diabetic animals. The study also investigated *in vitro* the influence of the extracts on cytotoxicity of proximal and distal kidney cell lines.

CHAPTER 2

2.0. MATERIALS AND METHODS

2.1. Chemical reagents and drugs

All chemical reagents were used in the present study were of analytical grade. Reagents and standard drugs were sourced as indicated: potassium hydroxide (Baird and Tatlock S.A. (Pty) Ltd, Johannesburg, RSA), anthrone reagent, metformin, glibenclamide, oyster type II glycogen powder, streptozotocin, dimethly sulphoxide (DMXO), potassium hydroxide, DPX mountant for histology (Sigma St Louis, MO, USA), ethanol, D-glucose, citric acid, sodium chloride, hydrochloric acid (HCl), sulphuric acid (H_2SO_4), isopropanol, paraffin (Merck, Waderville, RSA), porcine insulin (Intervet SA (PTY) Ltd, Isando, South Africa), heparin Novo (Novo Nordisk (Pty) Ltd, Johannesburg, RSA), halothane (Fluothane®, AstraZeneca Pharmaceuticals, Johannesburg, RSA), trapanal (sodium 5-ethyl-59-(1-methyl-butyl)-2-thio-barbiturate; Byk Gulden, Konstanz, Germany), penstrep-fungizone [complete culture medium (CCM)] (Delta Bioproducts, Kempton Park, South Africa), and MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Calbiochem, Darmstadt, Germany).

2.2. Plant materials and extract preparation

The plant species used in the present study were selected for study after an extensive ethnobotanical survey that involved interviewing traditional healers and literature

survey. The plants were identified and authenticated by Professor H. Baignath of the Botany Department, University of KwaZulu-Natal. Voucher specimens of the plant species are deposited at UKZN Botany Department. All the material was harvested in and around Durban, South Africa, between February and May, 2004. This was to eliminate variations in the quantities of chemical constituents that are known to change with seasons. All bark and leafy plant materials were air dried at room temperature (±24°C) before processing, except *H. hemerocallidea* corms.

2.2.1. *Hypoxis hemerocallidea*

Fresh corms of *H. hemerocallidea* were cleaned and pulverized while wet in a Waring commercial blender. The ground material (2 kg) was soaked in 8 l of 95% ethanol at room temperature for 24 hours (with occasional stirring). The material was then filtered and the filtrate was concentrated under reduced pressure (Buchi Lotavapor, BÜCHI Labortechnik, GmbH, Essen, Germany). The material was freeze dried to yield a brown powdery material. The ethanolic crude material so obtained was denoted APE.

2.2.2. *Ficus thonningii* and *Sclerocarya birrea*

The bark materials of *Ficus thonningii* and *Sclerocarya birrea* were air-dried at room temperature and separately extracted as descried for APE. Dried bark material were ground into powder using a Thomas-Wiley Laboratory mill model 4 (Arthur H Thomas Company, Philadelphia, PA., USA). The dried and powdered bark material of *F. thonningii* (5 kg), and *S. birrea* (5 kg) were then soaked in 15 l, respectively, of

95% ethanol at room temperature for 24 hours (with occasional stirring). The decoction was then filtered and concentrated under reduced pressure at 40°C. The concentrate was further air-dried at room temperature to yield a brown powdery material. The crude extract was denoted FTE and PAE, respectively.

2.2.3. *Persea americana*

Leaves of *Persea americana* were air-dried in a room. Dried leaves were ground into powder in a Waring commercial blender. The ground material (5 kg) was soaked in 20l of 95% ethanol and left to stand for 24 hours (with occasional stirring). The material was then filtered and the filtrate concentrated *in vacuo* at 40 °C. Extraction resulted in crude material that was denoted PAE.

The final crude products for all the plants were kept in the freezer until required. Stock solutions of APE, SBE, FTE and PAE crude extract were prepared by dissolving known quantities of extracts in deionised water and administered to animals. Volumes administered varied between 0.25 ml and 0.6 ml, depending on the dosage and size of the animal. Extract solutions were prepared fresh on the day of each experiment.

2.3. Ethical clearance

The experiments were conducted according to the University's guiding principles for the research involving animals. Ethical clearance number HSS/05012A was provided by the University KwaZulu-Natal (UKZN) Ethical committee (See Appendix 1).

2.4. Animals

Male Wistar rats weighing 200-300g were procured from UKZN Biomedical Research Unit. The animals were maintained on a 12-h light/12-h dark regime, with an ambient temperature of 23°C and relative humidity within the range of 30-40%. Animals had *ad libitum* access to standard rat pellets (Epol, Pietermaritzburg, South Africa) and tap water.

Biological effects of test extracts were assessed in healthy non-diabetic and STZ-induced diabetic rats.

2.4.1. Induction of diabetes mellitus

Animals were made diabetic by a single intraperitoneal (*ip*) administration of STZ, at a dose of 60 mg/kg body weight, after fasting overnight. Streptozotocin was dissolved in freshly made citrate buffer solution, pH 6.3. Control animals were injected with citrate buffer solution. Animals that exhibited glucosuria after 24 h, tested by urine test strips (Rapidmed Diagnostics, Sandton, South Africa), were considered diabetic. After 7 days blood was drawn from rat tails to confirm stable diabetes. Animals with blood glucose concentration above 20 mmol/l were considered to have achieved stable diabetic state and were selected for the study.

2.5. Experimental design

The project was divided into 3 phases. These were (i) acute, (ii) chronic and (iii) cytotoxicity studies. Assessment of the hypoglycaemic, renal and cardiovascular

effects of test extracts was conducted in acute and chronic studies. Effects of test extracts on cell viability were investigated on MDBK and LLCPK-1 cell lines. Outlined below were details of the experimental protocols.

2.5.1. Acute studies

2.5.1.1. Oral glucose tolerance test (OGTT)

Effects of test extracts on oral glucose tolerance test (OGTT) were assessed on separate groups of control and treated non-diabetic and STZ-induced diabetic rats, each with 6 animals. OGTT was conducted as previously described by Musabayane, *et al.*, (2005), with slight modifications. Prior to the experiments, animals were starved overnight for 18 hours, according to the methods of Muruganandan *et al.*, (2005; Musabayane *et al.*, 2007). On the day of the experiment, animals in all groups were administered D-glucose monohydrate (0.86 g/kg body weight, p.o.) by means of a bulbed steel tube. This was followed by administration vehicle (deionised water at 3 ml/kg) in control groups, and test extract (SBE, FTE, APE and PAE) or standard drugs (metformin, glibenclamide and insulin) in treated groups. In those animal groups in which the effects of test extracts were to be examined, three doses of each extract (60, 120 and 240 mg/kg body weight) were evaluated in separate groups (6 animals per group). The minimum dose of 60 mg/kg had been selected for the present study after preliminary *in vivo* studies with various plant extracts demonstrated that it was the highest dose to elicit marginal effects in glucose tolerance.

The effects of test extracts were compared with those of standard drugs. In groups where standard drugs were administered, a single dose was used ie metformin (500

mg/kg, p.o.), glibenclamide (5 mg/kg, p.o.) and insulin (100 μg/kg, s.c.). The present dose of insulin was used in previous studies (Musabayane *et al.*, 2000). The following is a summary of experimental groups:

Table 1

OGTT groups of non-diabetic and STZ-induced diabetic rats.

	Group (n = 6)	**Treatment**
(i)	*Untreated/Vehicle treated control group*	Animals in this group were treated with deionised water orally, p.o., by gavage at a dose of 3 ml/kg.
(ii)	*Insulin treated group*	Animals were treated with a subcutaneous injection of, porcine insulin, 100 µg/kg
(iii)	*Metformin treated group*	Animals were treated with metformin 500. mg/kg, p.o.
(iv)	*Glibenclamide treated*	Animals were treated with glibenclamide 5 mg/kg, p.o.
(v)	*SBE treated groups*	Animals were treated with SBE at doses of 60, 120 and 240 mg/kg, p.o.
(vi)	*FTE treated group*	Animals were treated with FTE 60, 120 and 240 mg/kg, p.o.
(vii)	*APE treated group*	Animals were treated with APE 60, 120 and 240 mg/kg, p.o.
(viii)	*PAE treated group*	Animals were treated with PAE 60, 120 and 240 mg/kg, p.o.

Blood glucose was monitored at 15 minute interval during the first hour and then hourly during the subsequent 4 hours. A commercial glucometer (Bayer's Glucometer Elite®, (Elite (Pty) Ltd Health care Division, Newbury, Berkshire, South Africa), was used in the analysis of blood glucose. A drop of blood sample was obtained from a prick at the tips of rat tails, under aseptic conditions. Evaluation of the test extracts for glucose tolerance was conducted in an identical manner in both non-diabetic and STZ-induced diabetic animals.

2.5.1.2. Insulin measurements

In order to determine whether test extracts affect insulin release, insulin levels were monitored. Separate groups of animals were prepared as for OGTT (see section 2.5.1.1.). These were treated with test extracts, at the maximal dose of 240 mg/kg, and standard drugs, after an oral glucose challenge (0.86 g/kg). Blood was collected by cardiac puncture into heparinised pre-chilled tubes 45 minutes after treatment, following light halothane anaesthesia. The blood was then promptly centrifuged (Eppendorf centrifuge 5403, Germany) at 4°C, 4000 rpm for 15 minutes to obtain plasma. Separated plasma was stored at -70°C in a Bio Ultra freezer (Mallkinckrodt, Ohio, USA) until required for insulin assay. Blood glucose, at 60 minutes, of animals challenged with an oral glucose load and treated with test extracts at a dose of 240 mg/kg, and standard drugs were used to monitor effects of insulin.

2.5.1.3. Renal function studies

Effects of test extracts on renal function were assessed in anaesthetized non-diabetic and STZ-induced diabetic animals using a modified procedure that has been previously described (Musabayane, Cooper, Osim, and Balment, 2000). Animals were divided into 2 major groups of non-diabetic and STZ-induced diabetic rats. Under each major group, animals were further divided into separate groups each with 6 animals. The groups were vehicle control and separate groups of animals treated with plant extracts (APE, SBE, FTE and PAE).

The animals were anaesthetized by intraperitoneal injection of trapanal (sodium 5-ethyl-59-(1-methyl-butyl)-2-thio-barbiturate) at 110 mg/kg body weight. The left jugular vein was cannulated with polyethylene tubing (internal diameter, i.d., 0.86 mm; external diameter, o.d., 1.27 mm; Portex, Hythe, Kent, UK) to allow intravenous infusion of 0.077 M NaCl. The bladders were exposed after an abdominal incision and cannulated with polyethylene tubing of the same size to facilitate collection timed urine samples. Tracheostomy was conducted on each animal to allow free breathing. The body temperature was maintained at 37 ± 1°C with a heated table. After surgical procedures, animals were challenged with 0.077M NaCl sustainably administered via the jugular vein, at a rate of 9 ml/h using Harvard Apparatus Syringe Infusion Pump 22 (Instech Solomon, Plymouth Meeting, PA, USA). Following an initial 3.5h equilibration period, eight consecutive urine collections were made into pre-weighed plastic vials at 30-min over the subsequent 4 h for measurements of urine flow, Na^+ and K^+ excretion rates. The control group was designed to check the stability of renal function.

2.5.1.3.1. Treated groups

Renal effects of test extracts were assessed in separate groups of rats following a 3.5h equilibration period. Solutions of test extracts were prepared fresh before use in each case (See Section 2.2.3) Urine samples were collected at 30 minute interval for 1 h (control period), 1 h 30 min treatment, and 1 h 30 min post treatment periods for measurements of urine flow, sodium and potassium excretion rates. The effects of test extracts were assessed in separate groups in which the extract solutions were infused at 0.06 μg/min during the 1h 30 min treatment period. The animals were switched back to the infusate alone for the final 1 h 30 min recovery period.

2.5.1.4. Acute blood pressure studies

Similar groups of animals were prepared as for acute renal studies for assessment of hypotensive effects of test extracts, except that the right carotid artery was also surgically prepared and cannulated with heparinized portex tubing (Hythe, Kent, UK) and then attached to a Stratham MLT 0380 blood pressure transducer compatible with the PowerLab System ML410/W, (ADInstruments Inc., Bella Vista, NSW, Australia) for measurements of MAP. After a 3.5h-equilibration as in acute renal function, blood pressure measurements were conducted at 30 minutes interval over the subsequent 4 h of 1 h control, 1 h 30 min treatment, and 1 h 30 min post treatment.

2.5.2. Chronic studies

Long term hypotensive effects of test extracts were assessed in unanaesthetised non-diabetic and STZ induced-diabetic animals for a period of 6 weeks. Animals were divided into separate groups of control and treated groups (n=8 in each group). Control groups were administered with vehicle (deionised water), 3 ml/kg, p.o), while treated groups were administered with metformin (500 mg/kg, p.o.), glibenclamide (5 mg/kg, p.o.), SBE (120 mg/kg, p.o.), FTE (120 mg/kg, p.o.), (vi) APE (120 mg/kg, p.o.) and PAE (120 mg/kg, p.o). Insulin as a standard drug was not used in long term studies because preliminary findings of acute hypoglycaemic studies revealed that none of test extracts had insulin releasing properties. Animals were placed in clean individual Makrolon polycarbonate metabolic cages (Techniplast, Labotec, RSA), through the duration of the study to allow for the monitoring of parameters under investigation i.e. food intake, water intake, weight changes, urine flow and electrolyte excretion rates. Metabolic cages were cleaned daily.

Animals were treated orally by gavage once daily at 09h00 with the test extracts at the median dose (120 mg/kg) of that used in acute studies using a bulbed steel tubing fitted to a syringe. The following parameters were monitored once weekly at 10h00: mean arterial blood pressure (mmHg), blood glucose (mmol/l), body weight (g), food (g) and water intake (ml), volume of urine voided (ml), and urinary concentrations of Na^+ and K^+ excretion, urine urea and creatinine.

In order to assess the effects of test extracts in preventing cardiovascular complications in diabetes, blood pressure (BP) was measured by the tail cuff method using IITC blood pressure program version 2.31 DPMI (IITC INC / Life Sciences

Instruments, Woodland Hills, CA, USA). The system employs an automatic scanner and pump, sensing cuff and amplifier to measure and count the pulse rate in the animal tail. The results are displayed as data plots and a summary of data of systolic, diastolic and mean arterial blood pressure on the computer screen. Small, medium and large restraining devices and 2 different tail cuffs (10 mm and 15 mm) were used to compensate for the increase in body weight during the 6 week experimental period. Measurements were done a constant temperature of 37 °C maintained by heating lamps. Three blood pressure recordings of each animal were made and the average taken. Animals were restrained individually with tails exposed and placed through the tail cuff device to restrict movements during measurements. Prior to measurements, animals had been warmed for about 15 minutes to allow sufficient vasodilatation of the tail artery. The equipment was calibrated once before each day of mean arterial blood pressure measurements.

2.5.2.1. Terminal studies

After 6 weeks blood was collected from animals via cardiac puncture under halothane anaesthesia. Following blood collection, animals were killed under excess anaesthesia and liver tissue from each animal were quickly excised and blotted of blood. The hepatic tissue was quickly sliced into several portions of known weight and half the quantity transferred to liquid nitrogen. These were preserved at -80 °C until required for hepatic glycogen analysis. The remainder of the hepatic tissue was fixed in 10% neutral buffered formalin for histological examination. Blood was centrifuged (eppendorf centrifuge 5403) and plasma separated and stored at -20° C until required

for insulin, urea and creatinine assays. Liver, kidney, muscle and pancreatic weights were expressed per 100 g body weight of animals at time of sacrifice.

2.5.3. Laboratory analysis

2.5.3.1. Insulin assay

Plasma insulin concentration was determined at Inkosi Albert Luthuli Hospital, chemical Pathology Laboratory (University of KwaZulu-Natal, Durban, South Africa) by electrochemiluminescence immunoassay method on the Hitachi modular EEE Analyzer (Hitachi, Tokyo, Japan) using diagnostic kits from Roche Diagnostics (Roche Diagnostics, Indianapolis, Indiana, USA). The immunoassay is a quantitative method for the determination of plasma insulin utilizing two monoclonal antibodies which together are specific for insulin. The lower limit of detection was 1.39 pmol/l. the intra-assay analytical coefficient of variation ranged from 4.4% to 5.5% and inter-assay coefficient of variation ranged from 4.7% to 8.9%.

2.5.3.2. Measurement of electrolytes, urea and creatinine

Sodium and potassium concentrations were determined by ion activity using the Beckman Coulter (Synchron LX20 Clinical Systems, USA). Urea and creatinine analysis were performed using the Beckman coulter instrument. Creatinine estimation employed the reaction of creatinine and sodium picrate to from creatinine picrate. Urea estimation employed the hydrolytic degradation of urea in the presence of urease. The methods used reagent kits from Beckman Coulter, Ireland, Inc., and

measured using the Berckman Coulter (Synchron LX20 Clinical Systems, Fullerton, USA).

2.5.3.3. Effects of extracts on hepatic glycogen

Hepatic glycogen levels were assessed quantitatively and qualitatively by biochemical and histological techniques, respectively to determine the effects of test extracts on glycogen synthesis after 6 weeks of treatment.

2.5.3.3.1. Biochemical technique

Hepatic glycogen levels were estimated by the methods of Hori *et al.*, (2006), with slight modifications. 0.1g liver tissue samples were homogenized in boiling 1 ml of 30% (w/v) potassium hydroxide (KOH) in centrifuge tubes for 20 minutes. 3ml distilled water and 4 ml absolute alcohol were added to the homogenate. Samples were then left to stand at room temperature for 20 minutes and centrifuged at 1000 g for 10 minutes. Samples were neutralized with 30% hydrochloric acid (HCl) and 2.5 ml was added. 8 ml of 0.2% anthrone reagent made by dissolving 0.2 g anthrone in 100 ml of concentrated H_2SO_4 was added to 1ml of sample and incubated in boiling water for 10 minutes. Glycogen powder from oyster, type II was used as standard. A standard glycogen stock solution was made by dissolving 10 mg glycogen standard in 10 ml distilled water. By serial dilutions of the stock solution, standard glycogen samples were obtained to create a standard curve. Cary dual beam spectrophotometer using Cary Win UV Simple Reads Application, software version 3.00 (182), was used

to read the absorbance, with wavelength set at 620 nm after zeroing with reagent blank.

2.5.3.3.2. Histological techniques

Tissue embedding

Hepatic tissue specimens were removed from formalin, cut into 2mm wide sections. These were placed in plastic cassettes and then quickly placed in ethyl alcohol. Histoprocessing was done in the H2500 microwave processor (Energy beam sciences, England). The sections suspended in alcohol were subjected to a temperature of 67°C for 30 minutes in a microwave oven at a power level of 450W. Specimens were then placed in isopropanol and subjected to temperatures of 74°C for 30 minutes. Specimens were then placed in paraffin at a temperature of 67°C for 30 minutes, after which old paraffin was decanted and fresh one added. Tissues were subjected to the same conditions for 30 minutes. Sections were finally imbedded in fresh paraffin wax and frozen using the Shandon histocentre2. Sample specimens were cut into 2μm wide portions using rotary microtome HM315, placed on surface of lukewarm water and mounted on a slide. A drop of albumin was added to the slide before mounting to allow specimen adherence to slide during staining.

Pas staining

Staining of sections was done according to Bancroft, Stevens and Turner, (1991). Specimen slides were briefly deparaffinized in xylene by dipping in fresh xylene 3

times, each 3 minute long. Slides were then rehydrated in descending grades of alcohol (100, 95, 80, and 70%), each 1 minute long, and then placed in periodic acid for 5 minutes. This was followed by brief rinsing in distilled water. Slides were then placed in Schiff's reagent for 15 minutes. This was followed by rinsing in running water for 10 minutes. Slides were then immersed and kept in haemotoxylin for 5 minutes, followed by rinsing in running water for 5 minutes. Slides were then differentiated in 1% acid alcohol and dehydrated in ascending grades of alcohol (70-100%), and xylene. A drop of DPX was added to the slide and mounted. Fields of interest were digitized with a 3CCD Sony colour video camera interfaced with the Nikon Optiphot photomicroscope (Nikon, Germany) that was linked to the Kontron Systems 300 image analysis system (Elektronik Imaging System, Kontron Elektronik, München Germany).

2.5.4. Cell viability studies

2.5.4.1. MTT assay

To assess direct effects of test extracts on the kidney, the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay was conducted on cell lines of kidney origin. The MTT assay is a quantitative colourimetric method based on the reduced cleavage of the water soluble monotetrazolium salt MTT to a purple formazan in metabolically active cells. The reduction of the MTT salt to formazan is done by the respiratory chain and other electron transport systems (Freimoser, Jakob, Aebi and Tuor, 1999). The porcine renal proximal tubular cell line, LLC-PK-1 and Madin-Darby bovine kidney (MDBK) cells (Highveld biologicals, Lyndhurst, South

Africa) were grown and maintained at 37°C in Eagle's Minimum Essential Medium (EMEM) (containing 0.1 mM Hepes buffer) supplemented with 5% heat inactivated foetal calf serum, 1% L-glutamine and 1% penstrep-fungizone [complete culture medium (CCM)] (Delta Bioproducts, Kempton Park, South Africa). Once the cells reached confluency, they were detached from the culture flask (75 cm^2) with 0.025% (w/v) trypsin and resuspended in CCM. Cell viability was determined in the presence of 0.2% (w/v) trypan blue in a haemocytometer. A 200-µl aliquot of the cell suspension (1.5 x 10^6 cells) was transferred into separate 96-well microtitre plates (Greiner Bio-one GmbH, Germany). Thereafter, the viability of cells incubated at 37°C for 24, 36 and 72 hours, containing various concentrations of SBE, FTE, APE and PAE in separate wells was assessed (0, 100, 200, 400, 600, 800 and 1000µg/ml, n = 6 for each dilution). The wells were aspirated after each incubation and washed with Hank's balanced salt solution (HBSS). All supernatants were discarded. The cells were resuspended in 100µl CCM containing 10µl of MTT (5 $mg.ml^{-1}$ MTT salt in HBSS) (Calbiochem, Darmstadt, Germany) and incubated for 4 hours at 37°C. After 4h, the plates were centrifuged (20 min, 2000 rpm at room temperature). The supernatant was removed and any resulting formazan crystals were then solubilized with 100µl Dimethyl sulphoxide (DMSO). After one hour, the optical density was determined specrophotometrically using an ELISA plate reader (Bio-Tek Instruments) at 595 nm and a reference wavelength of 655nm. Absorbance was expressed as percentage cleavage activity. Percentage cell viability was calculated as mean absorbance of control cells/mean absorbance of treated cells.

2.6. Data presentation

Data are presented as means ± SEM. Statistical comparisons were made using One-way ANOVA, $P < 0.05$, with Dunnett's post test. Effects of test extracts were compared against effects in respective control groups at the corresponding time. Statistical tests were performed using GraphPad Prism version 4.03 for Windows (GraphPad Software, San Diego California USA).

Based on their OGTT responses in non-diabetic rats, extracts were classified into two groups, namely, group A and group B. Group A extracts exhibited a dose-dependent hypoglycaemic effect while hypoglycaemic effects of group B extracts were devoid of dose-dependence.

CHAPTER 3

3.0. RESULTS

3.1. Plant extraction yields

Table 2 gives a summary of percent yields of crude extracts used in the present study. The percentage yield varied from 3.2 to 12.6%.

Table 2

Yield percent of crude extracts after ethanolic extraction

Plant material	Yield percent (%, w/w)
H. hemerocallidea corm*	10
F. thonningii bark	3.2
P. americana leaf	7.6
S. birrea bark	12.6

Yield percent (%, w/w): Percentage weight of dry crude material obtained after extraction, over the weight of plant material before extraction. **H. hemerocallidea* percent yield was calculated as weight of dry crude extract over wet weight of starting material

3.2. Acute studies

3.2.1. OGTT responses

3.2.1.1. Non-diabetic rats

OGTT responses of standard drugs in non-diabetic rats are shown in Figures 6. Results show that blood glucose levels rapidly increased following glucose load (0.86 g/kg). In vehicle treated non-diabetic control group, blood glucose concentration reached peak levels of 6.9 ± 0.2 mmol/l, from fasting levels of 4.2 ± 0.1 mmol/l, within the first 15 minutes. All standard drugs exhibited hypoglycaemic effects compared with untreated control, which were statistically significant, $P < 0.05$. The hypoglycaemic effects of exogenous insulin (100 µg/kg, s.c.), were more potent than metformin (500 mg/kg) and glibenclamide (5 mg/kg).

APE and SBE demonstrated hypoglycaemic effects that were dose-related during the first 30 minutes of treatment. Based on this observation in OGTT responses in non-diabetic rats, APE and SBE were classified as group A extracts. FTE and PAE were classified as group B extracts because they were devoid of dose dependent pattern in their hypoglycaemic effects.

Group A extracts (APE and SBE)

Effects of group A test extracts (APE and SBE) on glucose tolerance test in non-diabetic animals are shown in Figure 7. By comparison to vehicle treated control

group, all groups treated with APE and SBE demonstrated significant hypoglycaemic effects. The extracts markedly reduced blood glucose in non-diabetic groups 30 minutes after administration. Effects of APE lasted up to 45 minutes while those of SBE were sustained up to 120 minutes after treatment. In both extracts, the lowest doses (60 mg/kg) did not demonstrate significant blood glucose lowering effects compared with vehicle treated controls. Although less potent compared with standard drugs, these effects were statistically significant, $P < 0.05$, compared with vehicle treated controls.

Group B extracts (FTE and PAE)

OGTT responses for group B extracts (FTE and PAE) in non-diabetic rats are shown in Figure 8. FTE demonstrated significant hypoglycaemic effects, $P < 0.05$, during the first 15 and 30 minutes but showed no effects during the rest of the experiments. PAE exhibited hypoglycaemic effects beginning 15 minutes after treatment. However, the effects of the dose of 240 mg/kg were more prolonged to 120 minutes after treatment. Unlike group A extracts the lowest dose of group B extracts (60 mg/kg) decreased blood glucose in non-diabetic rats.

Both groups of test extracts demonstrated antihyperglycaemic effects beginning at 15 minutes of treatment, exhibiting peak effects during 30 to 45 minutes with glycaemic levels returning to baseline at 120 minutes. The maximal dose of 240 mg/kg demonstrated most potent antihyperglycaemic effects.

3.2.1.2. STZ-induced diabetic rats

Figure 9 compares OGTT responses of standard drugs with vehicle treatement in STZ-induced diabetic animals. After glucose challenge (0.86 g/kg), blood glucose concentrations rose steadily within 30 minutes to peaks (32 ± 1 mmol/l, n = 6) in STZ-induced control animals. These hyperglycaemic levels declined slightly but remained elevated after 4 hours. Glibenclamide (5 mg/kg) did not exhibit any hypoglycaemic effects in STZ-induced diabetic animals, in contrast to effects observed in non-diabetic animals shown in Figure 6. Exogenous insulin administration (100 µg/kg) demonstrated the most potent effects, reducing blood glucose to 13.2 ± 0.8 mmol/l (n = 6), at the end of experiment a value that was significantly (P <0.05) high by comparison with control animals at the corresponding period (27.8 ± 0.7 mmol/l, n = 6). Metformin reduced blood glucose to 14.2 ± 0.5 mmol/l (n = 6) at the end of the experimental period. Unlike in Figure 6, effects of hypoglycaemic effects of metformin in STZ diabetic animals were delayed starting later, at 45 minutes, but were prolonged to end of experiment.

Group A extracts (APE and SBE)

Figure 10A shows the effects of APE on glucose tolerance in STZ-induced diabetic rats. APE exhibited significant blood glucose lowering effects beginning 45 minutes after treatment, $P < 0.05$. These effects were dose-dependant and were more prolonged in comparison to effects observed in non-diabetic rats, shown in Figure 7A. The lowest APE dose of 60 mg/kg had no statistically significant effects on blood glucose by comparison with control animals. As shown in Figure 10B, SBE also

demonstrated significant reduction in blood glucose levels, $P < 0.05$, which were dose-related. Like APE, in Figure 10A, these effects were prolonged to the end of the experiment. Unlike APE (Figure 10A), the lowest dose of 60 mg/kg demonstrated significant hypoglycaemic effects, $P < 0.05$, 3 h after treatment.

Group B extracts (FTE and PAE)

Illustrated in Figure 11 are the effects of group B extracts on glucose tolerance in STZ-induced diabetic rats. FTE exhibited significant blood glucose reductions, $P < 0.05$, beginning 120 minutes after treatment, that were dose related. The administration of the lowest FTE dose (60 mg/kg) demonstrated significant hypoglycaemic effects beginning 4 hours after treatment. PAE significantly reduced blood glucose, dose-dependently, beginning 60 minutes after treatment, $P < 0.05$. The lowest PAE dose (60 mg/kg) showed significant effects 180 minutes after treatment. Hypoglycaemic effects of all group B extracts were sustained to the end of experiment.

3.2.1.3. Summary of OGTT responses to test extracts

Non-diabetic rats

- APE and SBE demonstrated dose-dependent blood glucose lowering effects.
- FTE and PAE demonstrated blood glucose lowering effects that were not dose-dependent.

STZ-induced diabetic rats

- APE, SBE, FTE and PAE demonstrated dose-dependent blood glucose lowering effects.

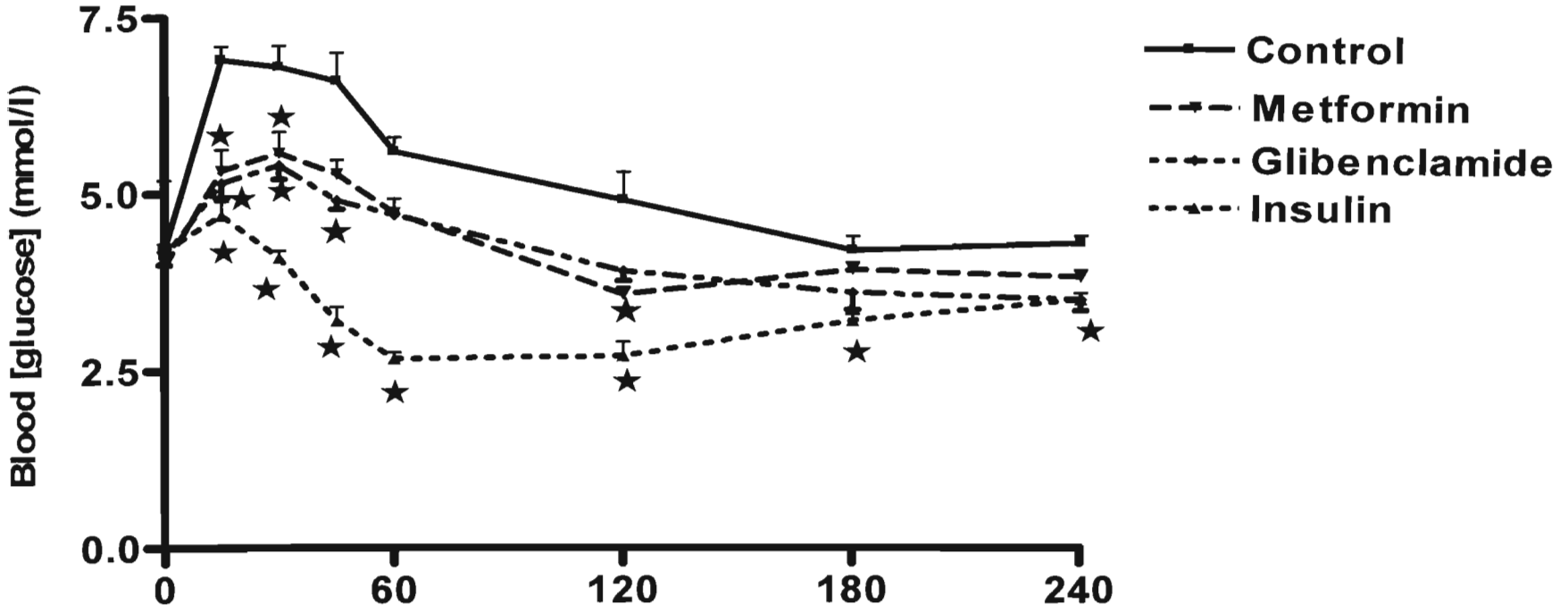

Figure 6. Effects of a single acute oral administration of vehicle and standard drugs on blood glucose in glucose-challenged non-diabetic rats. Values are means ± S.E.M. n = 6, *significant values at $P<0.05$ vs control group at corresponding time.

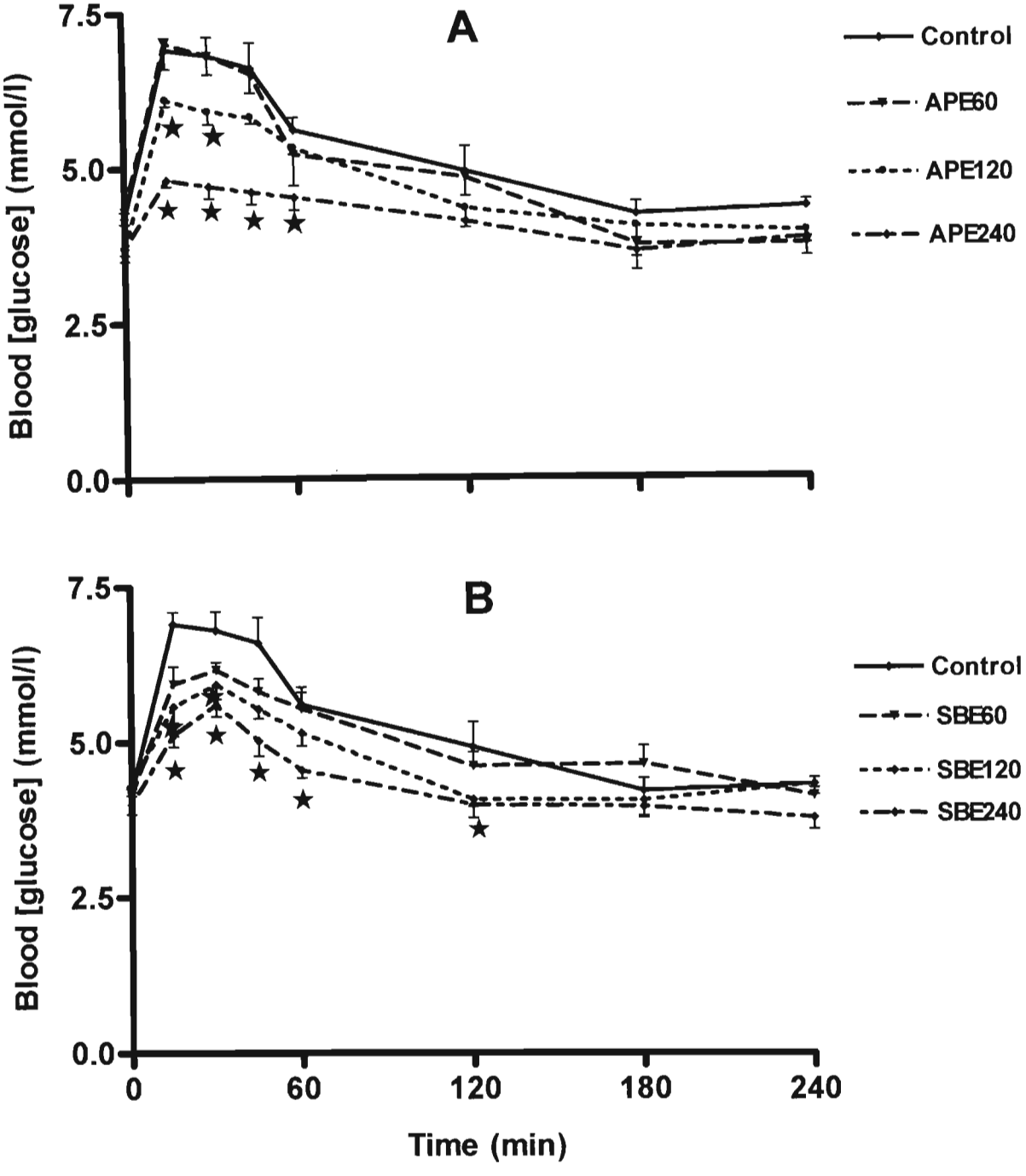

Figure 7. Effects of a single acute oral administration of APE (A), and SBE (B) on blood glucose in glucose-challenged non-diabetic rats. Values are means ± S.E.M., n = 6, *significant values at $P<0.05$ vs control group at corresponding time.

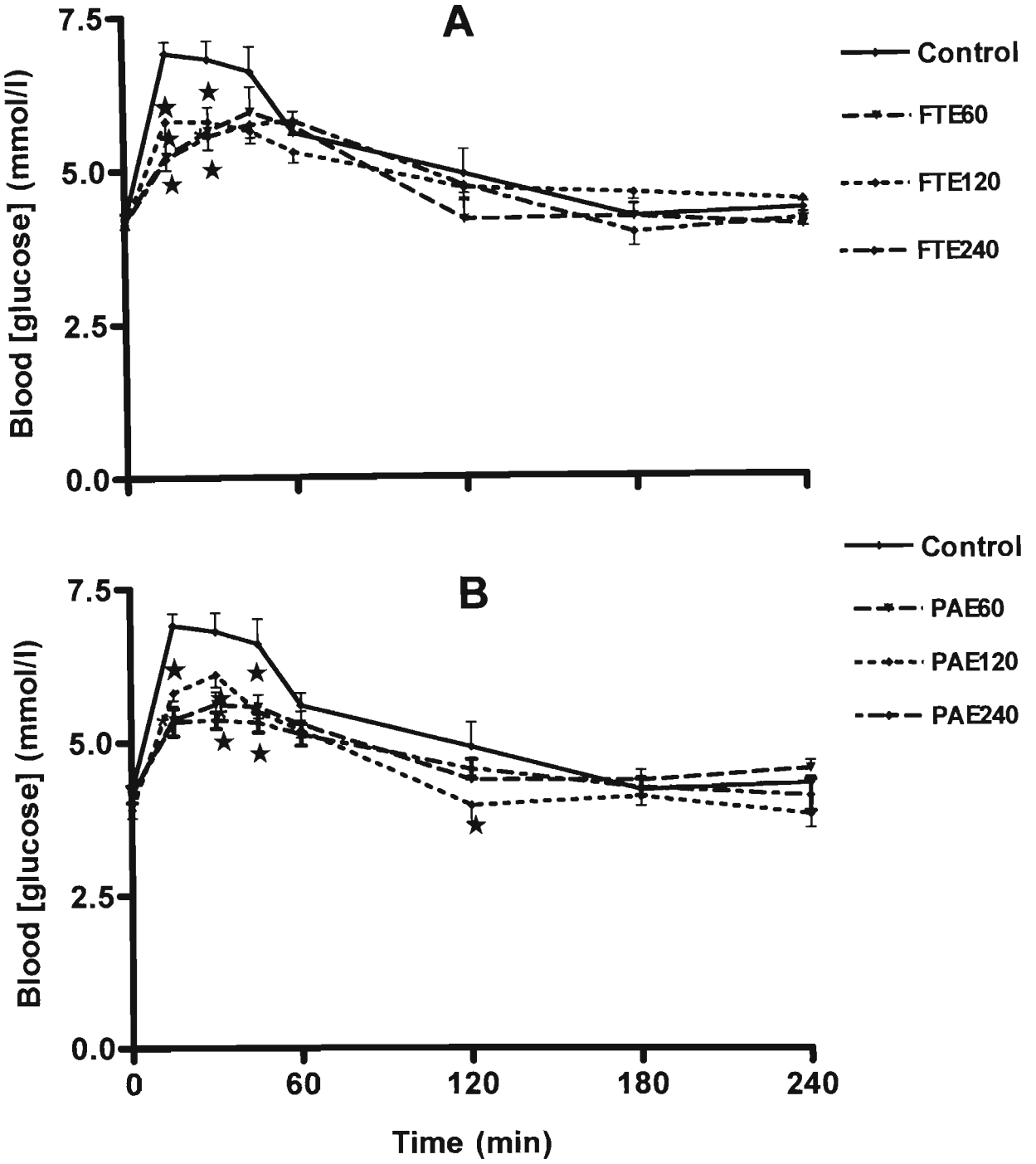

Figure 8. Effects of a single acute oral administration of FTE (A) and PAE (B) on blood glucose in glucose-challenged non-diabetic rats. Values are means ± S.E.M., n = 6, *significant values at $P < 0.05$ vs control group at corresponding time.

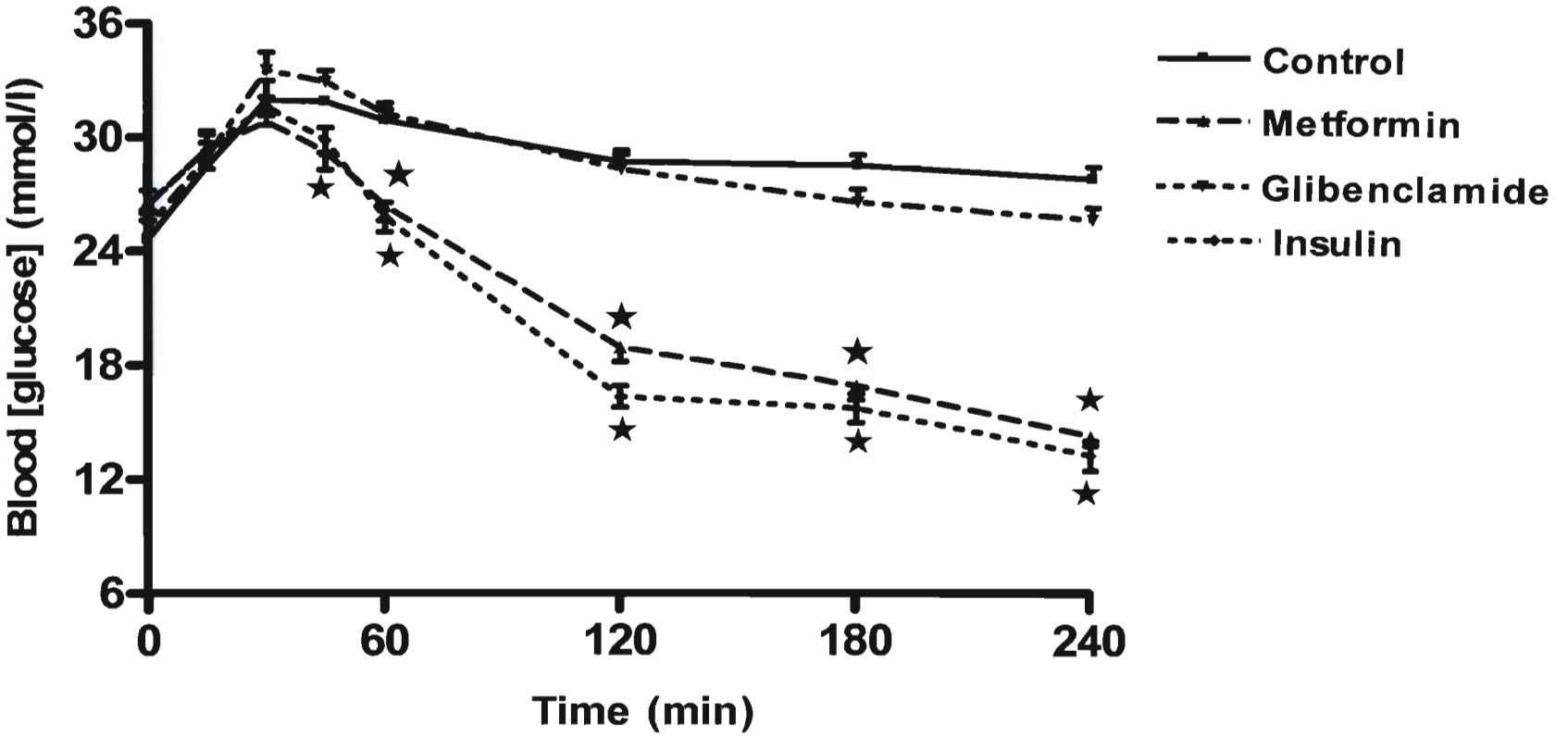

Figure 9. Effects of a single acute oral administration of vehicle and standard drugs on blood glucose in glucose-challenged STZ-induced diabetic rats. Values are means ± S.E.M., n = 6, *significant values at $P<0.05$ vs control group at corresponding time.

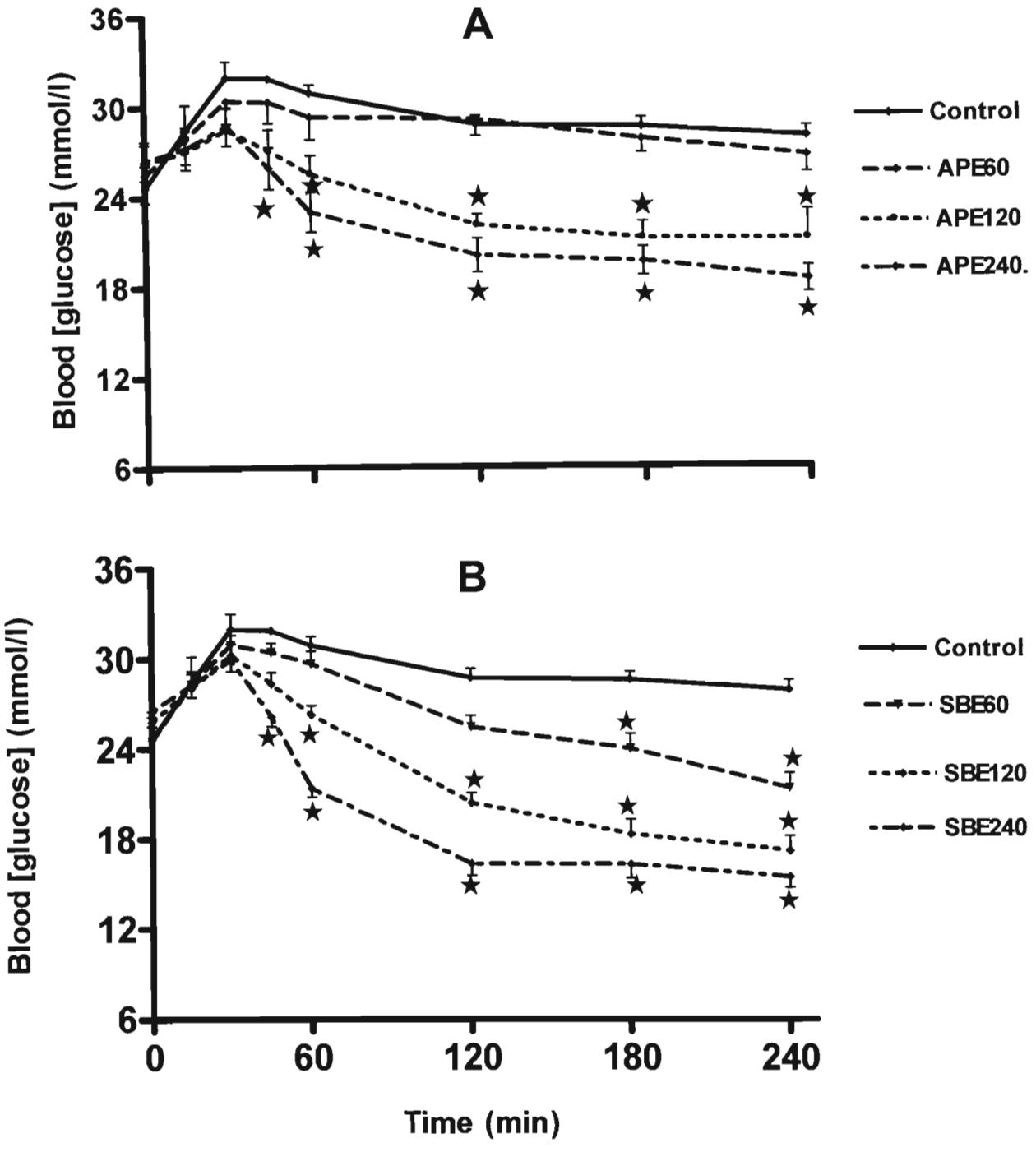

Figure 10. Effects of a single acute oral administration of APE (A), and SBE (B) on blood glucose in glucose-challenged STZ-induced diabetic rats. Values are means ± S.E.M., n = 6, *significant values at $P<0.05$ vs control group at corresponding time.

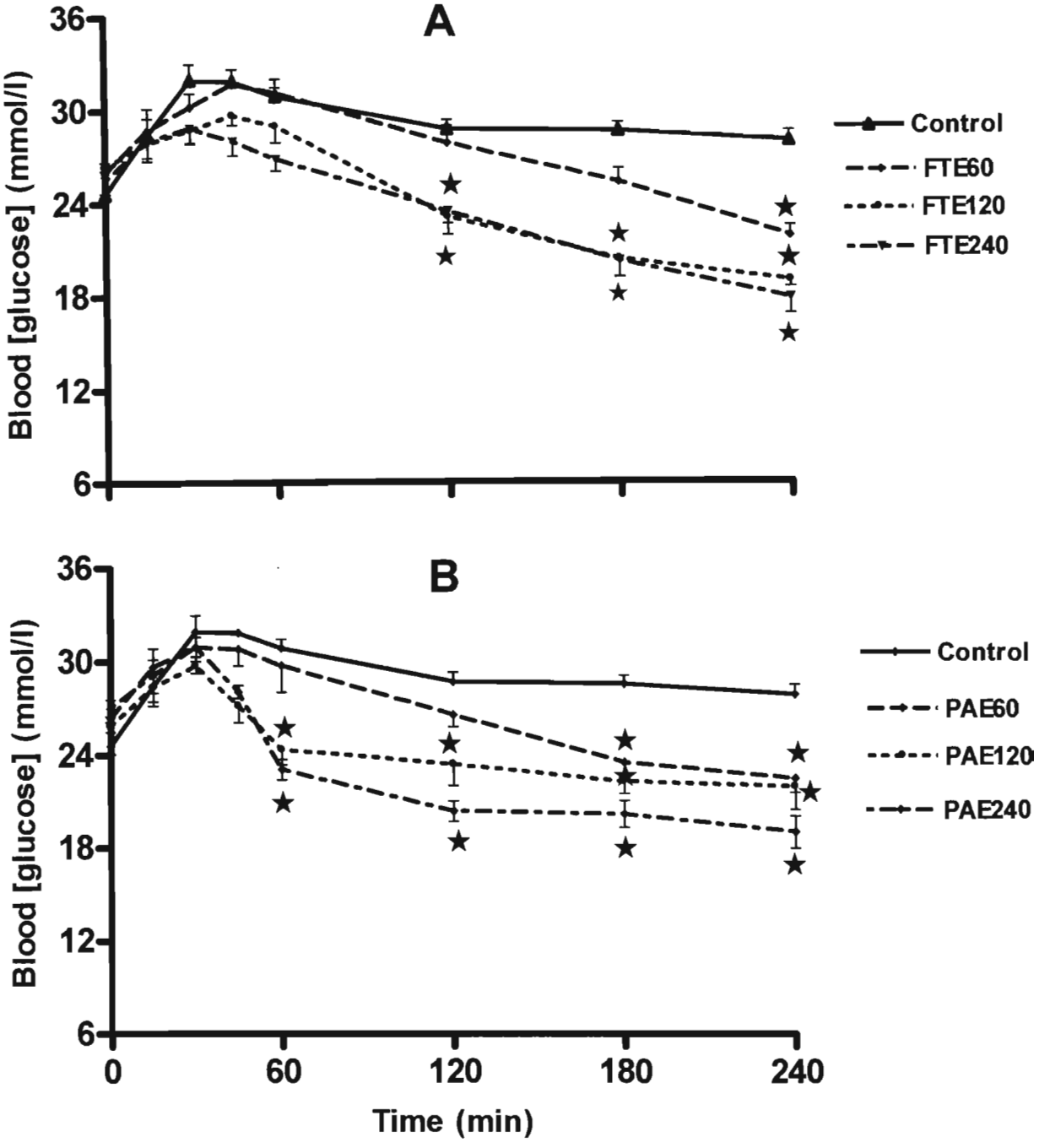

Figure 11. Effects of a single acute oral administration of FTE (A), and PAE (B) on blood glucose in glucose-challenged STZ-induced diabetic male rats. Values are means ± S.E.M., n = 6, *significant values at $P<0.05$ vs control group at corresponding time.

3.2.1.4. Acute effects of test extracts on insulin release

Table 3 shows the effects of acute administration of standard drugs and test extracts on insulin release (45 min) and blood glucose concentrations (60 min) in separate groups of non-diabetic rats, following a glucose load. As shown in the table glibenclamide and exogenous insulin significantly ($P < 0.05$) increased insulin release in comparison to vehicle treated non-diabetic rats. Metformin demonstrated no significant effects on insulin release in non-diabetic rats. All standard drugs demonstrated blood glucose lowering effects that were significantly ($P < 0.05$) pronounced after 60 min.

3.2.1.4.1 *Non-diabetic rats*

Extracts of groups A (APE and SBE) and B (FTE and PAE) did not affect insulin release in non-diabetic rats (Table 3). Glucose concentrations were, however; significantly lower ($P < 0.05$) in rats treated with APE (240 mg/kg) after 60 min, but not in rats treated with SBE. Group B extracts (240 mg/kg) did not affect blood glucose concentration after 60 minutes.

3.2.1.4.2. *STZ-induced diabetic rats*

Table 3 shows the effects of acute administration of standard drugs and test extracts on insulin and blood glucose concentrations in STZ-induced diabetic rats. In contrast to non-diabetic rats, exogenous insulin treatment significantly ($P < 0.05$) raised plasma levels of insulin. Neither metformin nor glibenclamide had any effects in

comparison to vehicle treated STZ-induced diabetic rats. Blood glucose concentrations in rats treated with insulin and metformin were significantly ($P < 0.05$) reduced after 60 minutes. These effects were absent in STZ-induced diabetic rats treated with glibenclamide, in contrast to corresponding non-diabetic group.

Group A (APE and SBE) and B (FTE and PAE) extracts

Extracts of groups A (APE and SBE) and B (FTE and PAE) did not affect insulin levels in STZ-induced diabetic rats (Table 3). However, all extracts with the exception of FTE demonstrated significant ($P < 0.05$) blood glucose lowering effects after 60 minutes.

Table 3.

Effects of acute administration of test extracts at maximal dose of 240 mg/kg on plasma insulin release. Data are expressed as mean ± S.E.M., $n = 6$ rats in each group. *Significant values at $P< 0.05$ compared to corresponding vehicle treated control values.

Group/Treatment	Plasma insulin (ng/ml) [45 min]	Blood glucose (mmol/l) [60 min]
Non-diabetic		
Control	14.4±0.5	5.6±0.2
Glibenclamide	17.7±0.1*	4.7±0.1*
Metformin	13.8±0.1	4.7±0.2*
Insulin	18.9±0.2*	2.7±0.1*
APE	14.5±0.3	4.5±0.2*
SBE	14.1±0.1	5.2±0.4
FTE	14.5±0.2	5.8±0.2
PAE	14.0±0.1	5.1±0.2
STZ-induced diabetic		
Control	2.4±0.1	30.8±0.6
Glibenclamide	2.3±0.3	31.2±0.6
Metformin	2.4±0.2	26.3±0.8*
Insulin	13.7±0.3*	25.7±0.8*
APE	2.3±0.1	22.9±1.3*
SBE	2.3±0.2	21.3±0.5*
FTE	2.5±0.1	26.8±0.8*
PAE	2.4±0.1	23.0±0.7*

3.2.2. Acute renal effects of test extracts

Effects of short term intravenous administration of test extracts on Na^+, K^+ and urine excretion rates in anaesthetised rats are depicted in Figure 12. Control animals were challenged with 0.077M NaCl at a rate of 9 ml/h which allowed delivery of 693 μmol/h Na^+. During the 4-h post equilibration period of sustained infusion of 0.077M NaCl, Na^+ excretion and urine flow rates stabilised and approximated rates of infusion in the control animals. In groups treated with test extracts, infusate of extracts were added during the 1.5 h treatment period.

3.2.2.1. *Non-diabetic rats*

Group A extracts (APE and SBE)

By comparison to corresponding control values, APE markedly depressed ($P < 0.05$) excretion rate of Na^+ from the pre-treatment value of 619 ± 38 μmol/h to 107 ± 25 μmol/h, 30 minutes during the treatment period. K^+ excretion rate was reduced from pre-treatment values of 248 ± 28 μmol/h to 42 ± 11 μmol/h during the same period (Figure 12B). Urine flow rate declined from 10.0 ± 0.7 ml/h to 1.0 ± 0.2 ml/h 30 minutes during post treatment (Figure 12C). APE-treated animals failed to revert to pre-treatment values of electrolyte and fluid excretion rates during recovery. SBE exhibited no effects on Na^+, K^+ excretion and urine flow rates.

Group B extracts (FTE and PAE)

Shown in Figure 13 are the effects of intravenous administration of group B extracts on Na^+, potassium excretion and urine flow rates in anaesthetised rats. FTE modestly elevated urinary Na^+ excretion rate during the first 30 minutes of treatment (Figure 13A). However, the difference was not statistically significant by comparison with the control values. Intravenous administration of FTE showed no effects on K^+ excretion and urine flow rates in non-diabetic rats.

PAE reduced Na^+ excretion rate by 30 minutes during post treatment from a baseline value of 644 ± 44 μmol/h to 437 ± 67 μmol/h, by comparison with corresponding control value, $P < 0.05$, (Figure 13A). Animals returned to baseline values at the end of experiment. PAE significantly depressed K^+ excretion rates after 60 minutes during treatment and recovery period. PAE demonstrated no effects on urine flow rate (Figure 13C).

3.2.2.2. *STZ-induced diabetic animals*

Results of effects of acute intravenous administration of test extracts on Na^+ and K^+ excretion, and urine flow rates in anaesthetised STZ-induced diabetic rats are illustrated in Figure 14. During the 4-h post equilibration period control animals demonstrated significant reduced rates of Na^+ excretion rate of approximately 255 μmol/h, by comparison to non-diabetic control rate of about 693 μmol/h. K^+ excretion rates were higher in STZ-induced diabetic rats by comparison to non-diabetic control

values. Urine flow rates were reduced to 6 ml/h in comparison to 9 ml/h in control non-diabetic rats.

Group A extracts (APE and SBE)

By comparison with corresponding control values, APE depressed Na^+ excretion rate from a pre-treatment value of 242 ± 7 μmol/h, to 211 ± 9 μmol/h, 30 minutes during the treatment period, $P < 0.05$ (Figure 14A). These effects were sustained to the end of recovery period where further reductions occurred to 61 ± 7 μmol/h. APE infusion also depressed urinary K^+ excretion rate from pre-treatment value of 445 ± 5 μmol/h to 377 ± 3 μmol/h, after 30 minutes during treatment (Figure 14B). Suppressive effects of APE on K^+ excretion rate was sustained into recovery period. APE significantly ($P < 0.05$), depressed urine flow rate from a pre-treatment value of 6.0 ± 0.3 ml/h to a post treatment value of 3.7 ± 0.0 ml/h, after 60 minutes during the treatment period. These effects were sustained into the recovery period.

During the first 30 minute treatment phase, SBE reduced Na^+ excretion rate from a pre-treatment value of 249 ± 5 μmol/h to 211 ± 7 μmol/h, 30 minutes during the treatment period, which was significant by comparison with the corresponding control value, $P < 0.05$ (Figure 14A). Na^+ excretion rates remained depressed, to 188 ± 9 μmol/h, $P < 0.05$, at the end of the experiments. Infusion of SBE reduced K^+ excretion rate from a pre-treatment value of 466 ± 5 μmol/h, to 420 ± 7 μmol/h 30 minutes after treatment (Figure 14B). However, effects were only significant ($P < 0.05$), after 60 minutes during treatment and these were sustained into the recovery

period where the final value was 372 ± 6 μmol/h. Acute intravenous administration of SBE had no effects on urine flow rates in STZ-induced diabetic rats (Figure 14C).

Group B extracts (FTE and PAE)

Effects of administration of group B extracts on electrolyte excretion and urine flow rates in anaesthetised STZ-induced diabetic animals are shown in Figure 15. FTE reduced K^+ excretion rate from a pre-treatment value of 257 ± 9 μmol/h to 237 ± 8 μmol/h, after 30 minutes of treatment (Figure 15A). However, this effect was not statistically significant compared with the control value, until 30 minutes during recovery phase. K^+ excretion rates were sustainably depressed by intravenous infusion of FTE, $P < 0.05$, from 30 minutes during treatment phase to recovery period (Figure 15B). Urine flow rate was unaffected by administration of FTE (Figure 15C).

By comparison with corresponding control values, PAE reduced Na^+ excretion rate from a pre-treatment value of 281 ± 6 μmol/h to 176 ± 9 μmol/h, $P < 0.05$, 30 minutes after treatment (Figure 15A). During the same period, intravenous administration of PAE also reduced K^+ excretion rates from baseline values of 508 ± 6 μmol/hr to 344 ± 7 μmol/h, and urine flow rate, from an initial value of 6.0 ± 0.4 μmol/h to 5.0 ± 0.6 μmol/h.

3.2.2.3. Summary of acute renal effects of test extracts

Non-diabetic rats

- APE and PAE reduced Na^+ and K^+ excretion, and urinary flow rates.
- SBE and FTE demonstrated no effects on Na^+ and K^+ excretion, and urinary flow rates

STZ-induced diabetic rats

- APE, SBE, FTE and PAE reduced Na^+ and K^+ excretion, and urine flow rates.

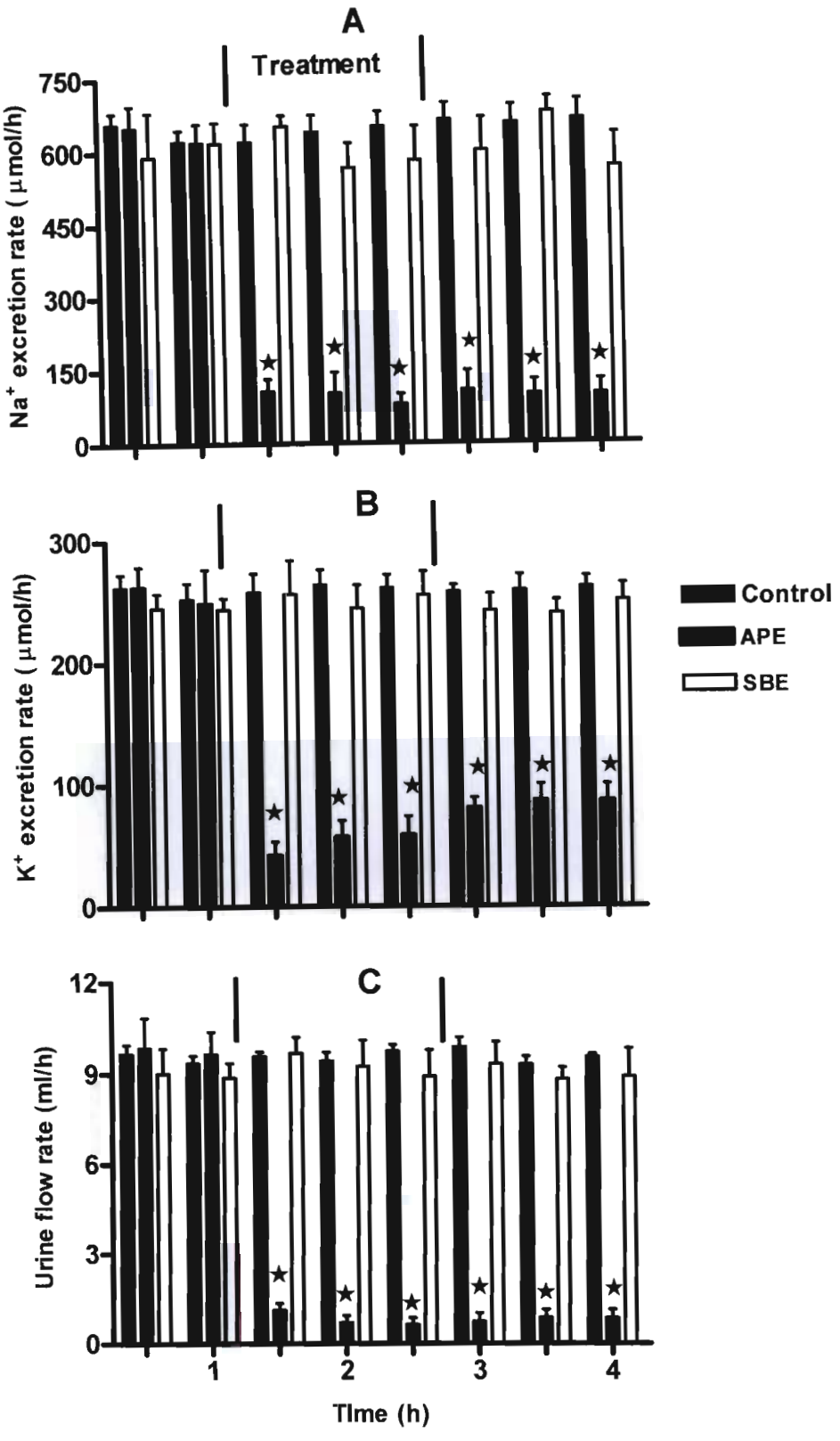

Figure 12. Na^+ (A) and K^+ (B) excretion, and, urine flow (C) rates in 0.077 M NaCl infused control non-diabetic rats, and rats administered APE and SBE at 0.06 µg/min for 1 h 30 min. Values are mean ± S.E.M., n = 6. ★significant values at $P<0.05$ vs. control group at corresponding time.

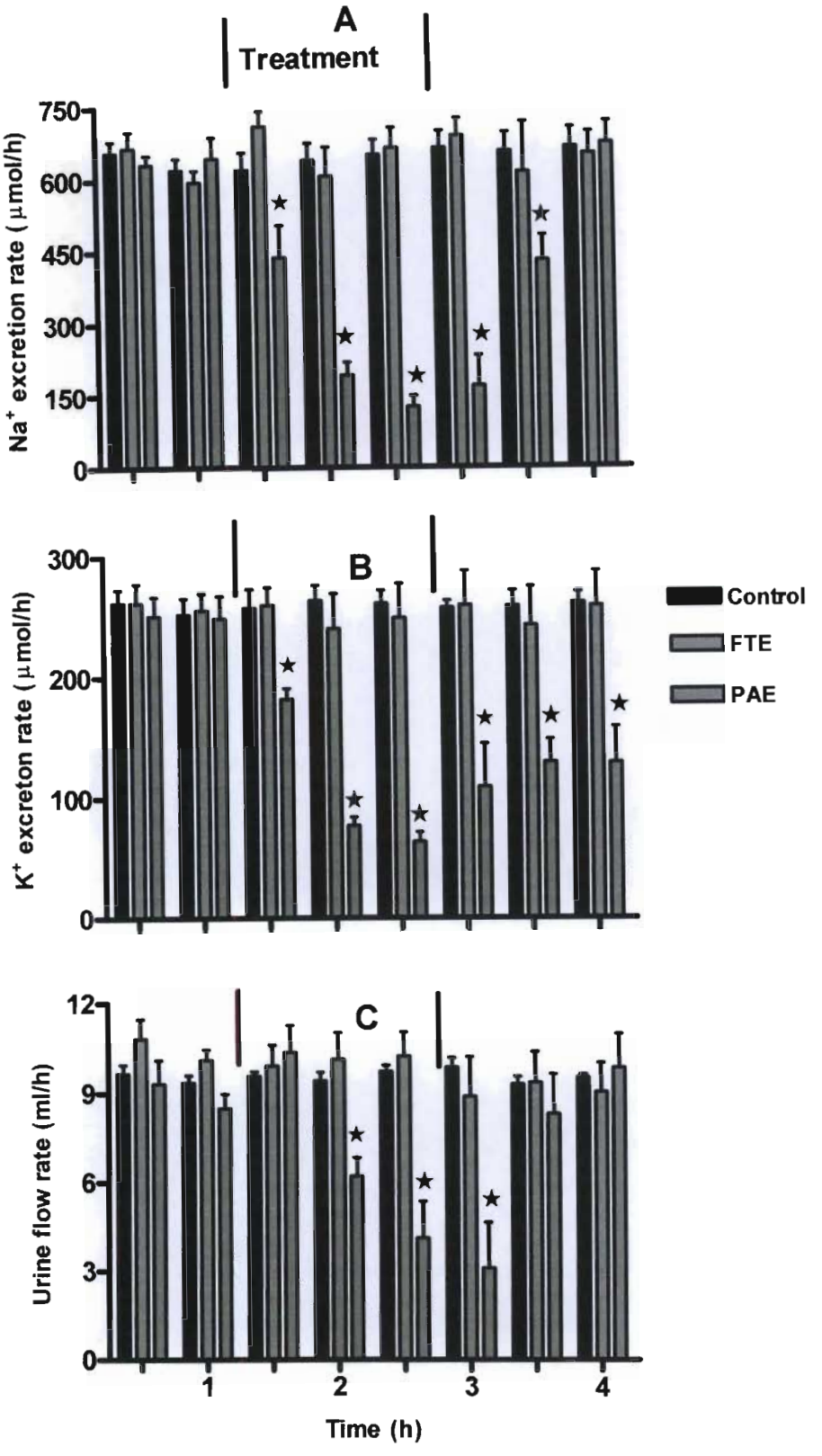

Figure 13. Na^+ (A) and K^+ (B) excretion, and, urine flow (C) rates in 0.077 M NaCl infused control non-diabetic rats, and rats administered FTE and PAE at 0.06 µg/min for 1 h 30 min. Values are mean ± S.E.M., n = 6. ★significant values at $P<0.05$ vs. control group at corresponding time.

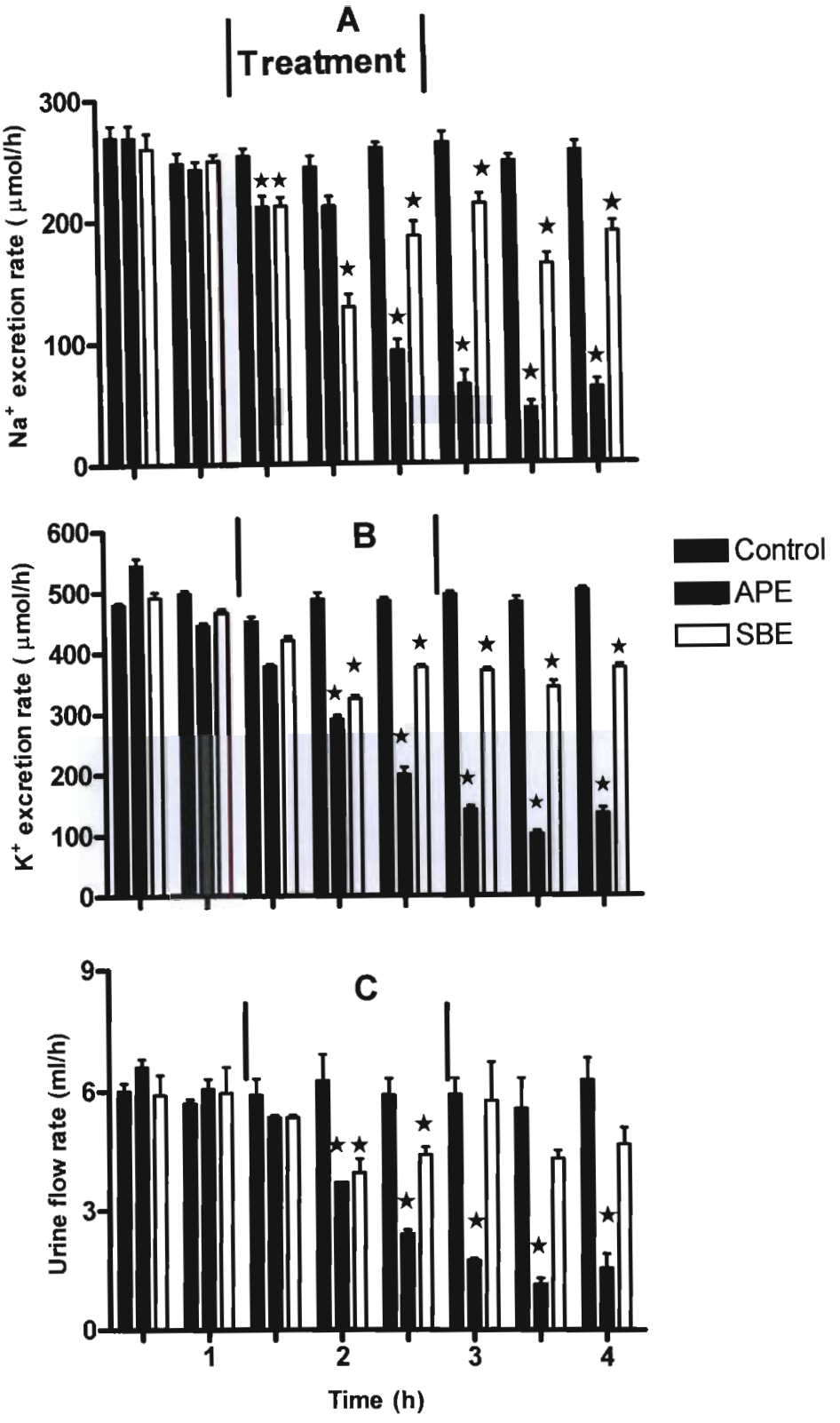

Figure 14. Na^+ (A) and K^+ (B) excretion, and, urine flow (C) rates in 0.077 M NaCl infused control STZ-induced diabetic rats, and rats administered APE and SBE at 0.06 µg/min for 1 h 30 min. Values are mean ± S.E.M., n = 6. ★significant values at $P<0.05$ vs. control group at corresponding time.

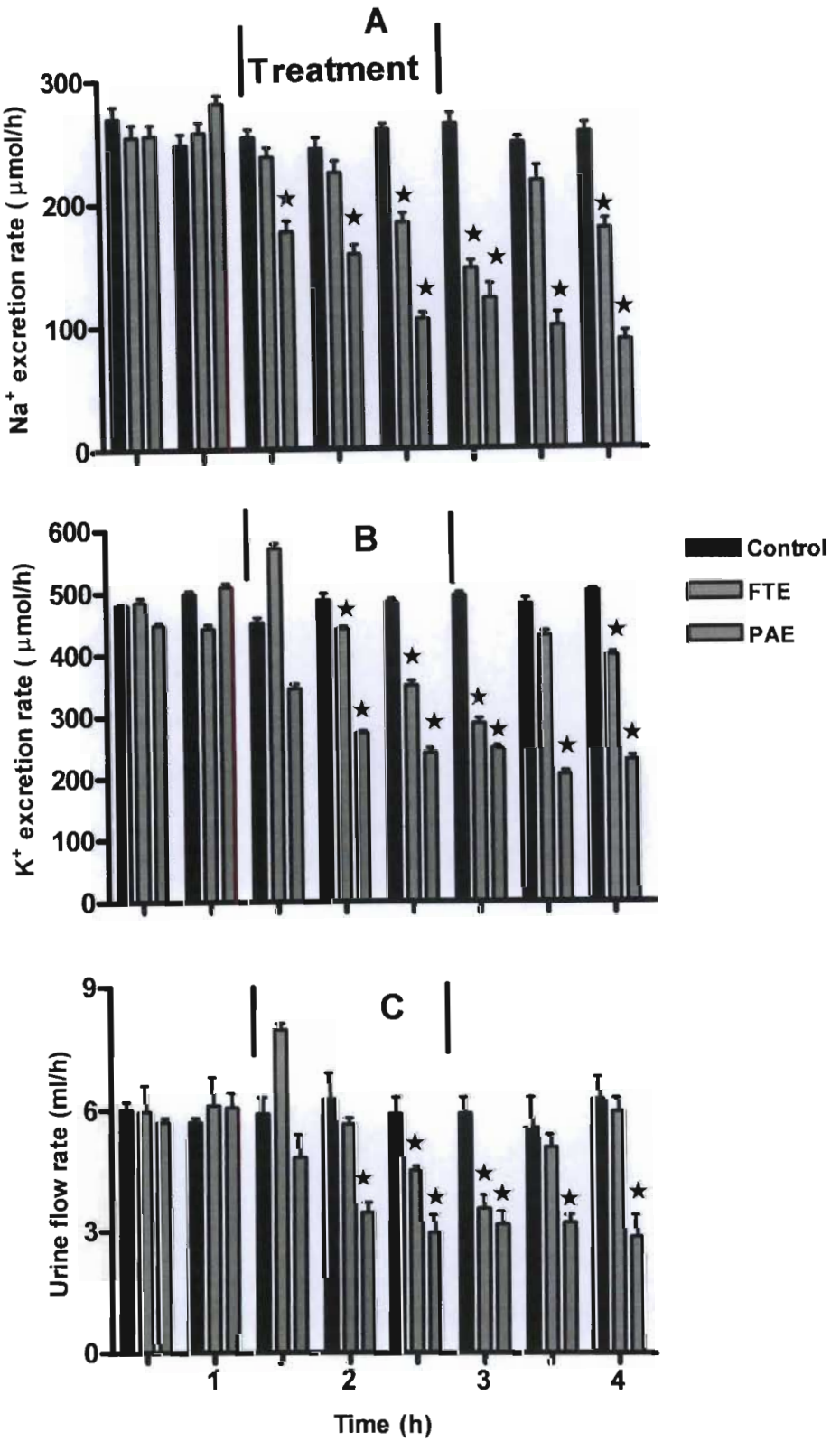

Figure 15. Na^+ (A) and K^+ (B) excretion, and, urine flow (C) rates in 0.077 M NaCl infused control STZ-induced diabetic rats, and rats administered FTE and PAE at 0.06 µg/min for 1 h 30 min. Values are mean ± S.E.M., n = 6. ★significant values at $P<0.05$ vs. control group at corresponding time.

3.2.3. Acute effects of test extracts on mean arterial blood pressure

Figure 16 shows the effects of acute intravenous administration of test extracts on mean arterial blood pressure in non-diabetic rats. Control animals were challenged with 0.077M NaCl intravenously infused at a rate of 9 ml/h. The mean arterial blood pressure values of control animals stabilised at approximately 120 mmHg during the 4h post-equilibration period.

3.2.3.1. *Non-diabetic rats*

Group A extracts (APE and SBE)

Figure 16A shows the effects of acute intravenous administration of group A extracts on mean arterial blood pressure in anaesthetised non-diabetic rats. APE mediated the most potent vasodepressive effects, in comparison to SBE in non-diabetic rats. APE reduced MAP values from pre-treatment values of 120 ± 2 mmHg to post-treatment levels of 112 ± 1 mmHg during the first 30 minutes of treatment. APE mediated vasodepressive effects, $P < 0.05$, which were sustained during the recovery period and did not revert to pre-treatment levels.

Intravenous infusion of SBE resulted in transient vasodepressive effects, $P < 0.05$, reducing pressure from 121 ± 2 mmHg to 115 ± 1 mmHg after 30 minutes of treatment. MAP rose gradually during the last 30 minutes of treatment period to reach values at the end of experiment that compared to the pre-treatment data.

Group B extracts (FTE and PAE)

Shown in Figure 16B are the effects of intravenous administration of group B test extracts on blood pressure in anaesthetised non-diabetic rats. FTE demonstrated transient effects reducing blood pressure from a pre-treatment value of 119 ± 1 mmHg to 98 ± 1 mmHg during the first 30 minutes of treatment. Animals failed to attain pre-treatment values during recovery period, with final values being 109 ± 0.8 mmHg. A similar vasodepressive pattern was observed in rats treated with PAE. PAE treatment reduced the blood pressure from a pre-treatment value of 123 ± 1 mmHg to f 101 ± 1 mmHg. Unlike FTE, animals attained pre-treatment values during recovery.

3.2.3.2. *STZ-induced diabetic animals*

Figure 17 shows the effects of acute intravenous administration of test extracts on blood pressure in anaesthetised STZ-induced diabetic rats. Control animals were challenged with 0.077M NaCl intravenously infused at a rate of 9 ml/h. Control diabetic rats demonstrated steady mean arterial pressure during the 4h post-equilibration period. However, these values were lower by comparison to control non-diabetic rats. In treated groups infusates of extract solutions were added during 1.5h treatment period.

Group A extracts (APE and SBE)

The effects of group A test extracts on blood pressure are shown in Figure 17A. APE demonstrated sustained vasodepressive effects significantly reducing blood pressure

($P < 0.05$) from an initial value of 113 ± 7 mmHg to 109 ± 8 mmHg during the first 30 minute of treatment period by comparison with corresponding control value. Vasodepressive effects of APE were prolonged into the recovery period. Animals treated with APE exhibited severe hypotension and failed to recover. The mean arterial blood pressure of the group treated with APE was 67 ± 4 mmHg at the end of experiment.

Intravenous administration of SBE demonstrated vasodepressive effects that were transient with peak reductions in blood pressure observed 60 minutes during treatment phase, $P < 0.05$. SBE reduced blood pressure from a pre-treatment value of 109 ± 5 mmHg to a peak reduced value of 82 ± 3 mmHg. Unlike APE, the effects of SBE were reversed during recovery period.

Group B extracts (FTE and PAE)

Figure 17B shows the effects of group B extracts on mean arterial pressure in anaesthetised STZ-induced diabetic rats. Group B extracts demonstrated a similar transient vasodepressive pattern to group A extracts. FTE reduced blood pressure from a pre treatment value of 111 ± 2 mmHg to a post-treatment value of 97 ± 1 mmHg, after 30 minutes of treatment which was significant by comparison to the corresponding value of control group, $P < 0.05$. FTE reduced blood pressure to an optimum value of 86 ± 7 mmHg at 60 minutes during treatment. PAE reduced blood pressure from a pre-treatment value of 110 ± 3 mmHg to 90 ± 2 mmHg after 30 minutes of treatment. During recovery period, the effects of group B extracts were reversed.

3.2.3.2.1. Summary of mean arterial pressure effects of test extracts

Non-diabetic rats

- APE, SBE, FTE and PAE demonstrated acute vasodepressive effects
- Vasodepressive effects of APE and FTE were sustained into recovery period. SBE treated groups recovered.

STZ-induced diabetic rats

- APE, SBE, FTE and PAE demonstrated acute vasodepressive effects
- Effects of APE were sustained during recovery period. SBE, FTE and PAE recovered.

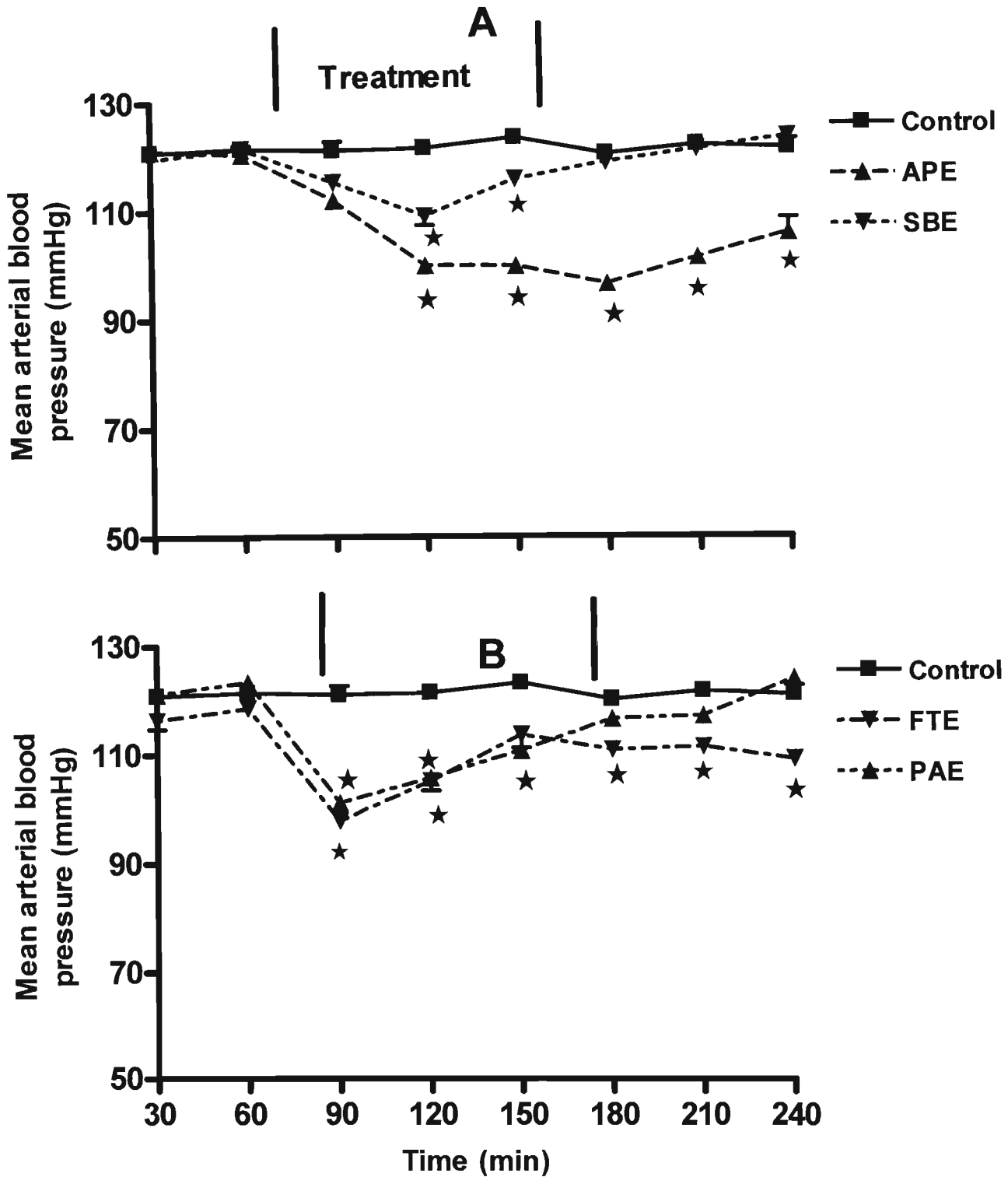

Figure 16. Effects of acute intravenous administration of APE and SBE (A) and FTE and PAE (B) on mean arterial pressure in anaesthetised non-diabetic rats. Values are mean ± S.E.M., n = 6. *significant values at $P<0.05$ vs. control group at corresponding time.

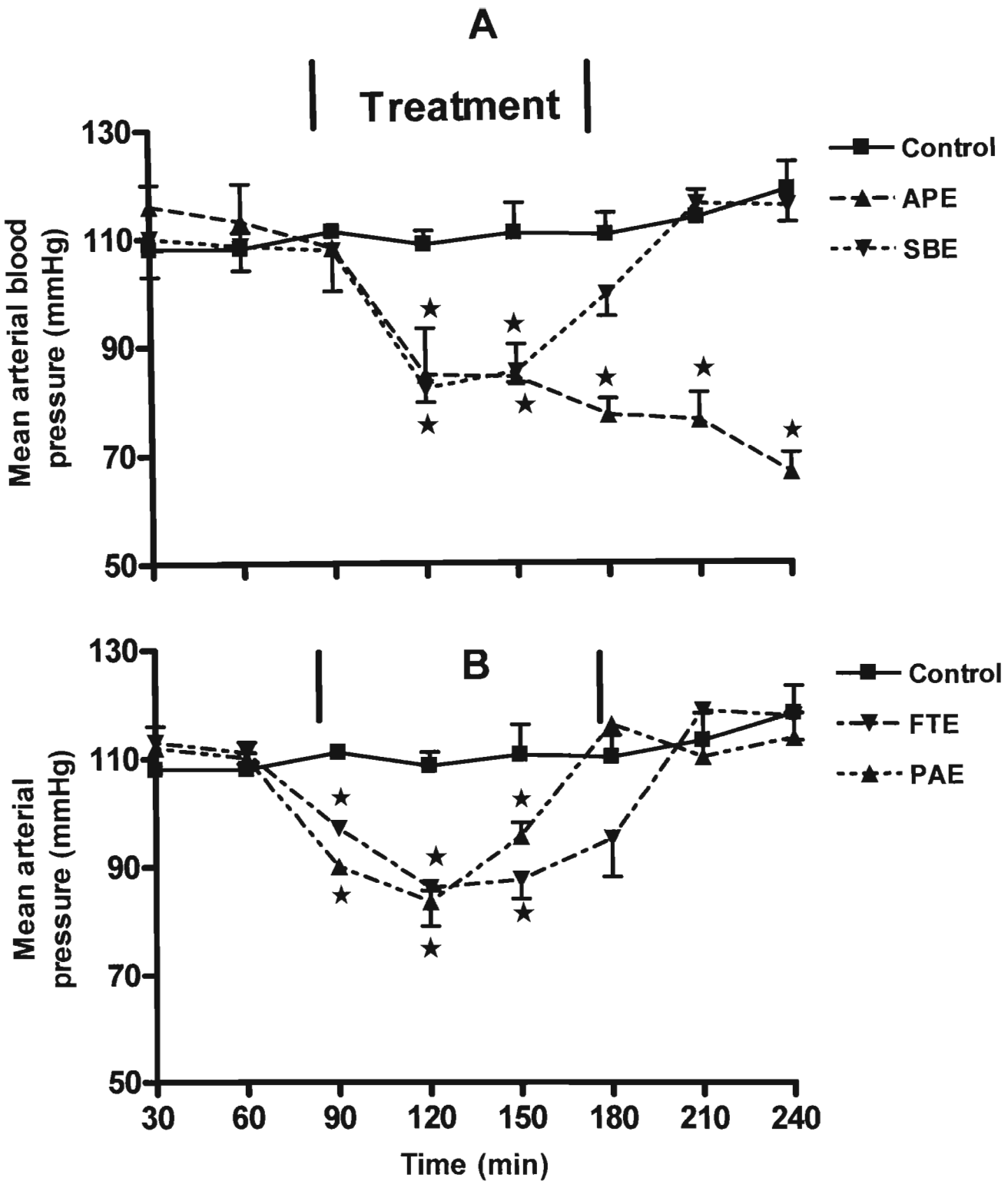

Figure 17. Effects of acute intravenous administration of APE and SBE (A) and FTE and PAE (B) on mean arterial blood pressure, in anaesthetised STZ-induced diabetic rats. Values are mean ± S.E.M., n = 6. *significant values at $P<0.05$ vs. control group at corresponding time

3.3. Chronic studies

3.3.1. Effects of test extracts on long term blood glucose concentration

3.3.1.1. *Non-diabetic rats*

Table 4 shows the effects of chronic administration of vehicle and test extracts on blood glucose in non-diabetic rats at the end of 6 weeks of treatment. Initial blood glucose concentrations were similar in all groups. Standard drugs, glibenclamide and metformin, significantly reduced ($P < 0.05$) blood glucose concentrations in non-diabetic rats, by comparison with the control group.

Group A extracts (APE and SBE)

Oral administration of group A extracts once a day for 6 weeks had marginal blood glucose lowering effects in non-diabetic rats (Table 4). Although, APE and SBE reduced blood glucose concentration in non-diabetic rats at the end of 6 weeks, these were not statistically different from corresponding control values.

Group B extracts (FTE and PAE)

Also shown in Table 4 are the effects of group B test extracts on blood glucose concentrations at the end of 6 weeks of treatment in non-diabetic rats. FTE demonstrated the most potent blood glucose lowering effects after 6 weeks of treatment, in comparison to corresponding control values, $P < 0.05$. Treatment with

PAE for 6 weeks reduced blood glucose in non-diabetic rats but levels did not reach statistical significance.

3.3.1.2. *STZ-induced diabetic rats*

Animals became diabetic 24 hours after induction by i.p. administration of STZ and this was sustained for the rest of the study period. Seven days after induction of diabetes, vehicle treated diabetic controls exhibited fasting glycaemic levels of 24.6 ± 1.5 mmol/l (n = 60), compared with 4.2 ± 0.1 mmol/l (n = 6), $P < 0.05$, in the corresponding no-diabetic control, and citrated buffer treated controls. STZ-induced diabetic rats exhibited marked polyuria, polyphagia and polydipsia. Furthermore, diabetic animals begun to exhibit severe wasting compared with the control animals.

Table 4 also summarises effects of chronic administration of test extracts on blood glucose in STZ-induced diabetic animals. Vehicle treated STZ-induced diabetic control rats exhibited 5-fold more glycaemia than vehicle treated non-diabetic rats. While metformin significantly reduced blood glucose concentrations, $P < 0.05$, in STZ-induced diabetic rats, glybenclamide demonstrated no effects.

Group A extracts (APE and SBE)

In contrast to non-diabetic rats, oral administration of APE and SBE (120 mg/kg) once daily, for 6 weeks markedly reduced blood glucose concentrations in STZ-induced diabetic animals at the end of 6 weeks of treatment.

Group B extracts (FTE and PAE)

FTE and PAE significantly reduced blood glucose concentrations in STZ-induced diabetic rats, $P < 0.05$, compared with STZ-induced diabetic control animals at the end of 6 weeks (Table 4).

3.3.1.3. Summary of effects of test extracts on chronic blood glucose concentration

Non-diabetic rats

- APE, SBE and PAE demonstrate mild hypoglycaemic effects that were not statistically significant.
- FTE reduced blood glucose concentrations.

STZ-induced diabetic rats

- APE, SBE, FTE and PAE reduced blood glucose concentration in diabetic rats.

3.3.2. Chronic effects of test extracts on mean arterial blood pressure

Table 4 also shows effects of chronic administration of test extracts on mean arterial pressure, after 6 weeks in non-diabetic rats. Blood pressure values remained unchanged in control non-diabetic rats at the end of 6 weeks in comparison to the first week.

3.3.2.1. *Non-diabetic rats*

Group A extracts (APE and SBE)

As shown in Table 4, APE and SBE reduced mean arterial pressure in non-diabetic rats. In comparison to control values, the effects of SBE were significant, $P < 0.05$, while those of APE were, however, not statistically significant.

Group B extracts (FTE and PAE)

Also shown in Table 4 are effects of group B test extracts on blood pressure in non-diabetic rats. Chronic administration of group FTE reduced mean arterial pressure in non-diabetic rats, $P < 0.05$. In contrast, PAE did not demonstrate vasodepressive significant vasodepressive effects in non-diabetic rats after 6 weeks.

3.3.2.2. *STZ-induced diabetic rats*

Table 4 also illustrates the effects of long term administration of test extracts on blood pressure in STZ-induced diabetic rats. STZ-induced diabetic control rats exhibited higher mean arterial pressure values than control non-diabetic animals.

Group A extracts (APE and SBE)

Long term treatment with group A test extracts significantly reduced blood pressure in STZ-induced diabetic rats by comparison to control diabetic rats, $P < 0.05$ (Table 4).

Group B extracts (FTE and PAE)

Like group A extracts, all group B test extracts (120 mg/kg) reduced blood pressure in diabetic rats in comparison to control values, $P < 0.05$.

3.3.2.3. Summary of effects on long term blood pressure

Non-diabetic rats

- APE, SBE and FTE reduced blood pressure in non-diabetic rats
- PAE had no effects on blood pressure in non-diabetic rats

STZ-induced diabetic rats

- APE, SBE, FTE and PAE were vasodepressive in STZ-induced diabetic rats.

Table 4

Weekly blood glucose concentration (mmol/l) and mean arterial pressure (mmHg) in separate groups of control and treated non-diabetic and STZ-induced diabetic rats. Treated animals were administered with test extracts (p.o.) daily for 6 weeks (n = 8 in each group).

	Parameter			
	Blood [glucose] (mmol/L)		**Mean arterial pressure (mmHg)**	
Group/treatment	week 1	week 6	week 1	week6
Non-diabetic rats				
Control	5.4±0.1	5.5±0.1	117±6	117±2
Glibenclamide	5.3±0.2	3.4±0.1*	117±4	116±6
Metformin	5.2±0.4	3.5±0.3*	118±3	112±3*
APE	5.2±0.3	5.0±0.3	118±2	116±2
SBE	5.3±0.2	4.9±0.1	117±3	98±2*
FTE	5.3±0.2	3.8±0.2*	116±2	96±3*
PAE	5.4±0.3	5.0±0.1	116±1	118±2
STZ-induced diabetic rats				
Control	24.6±1.6	25±1	114±2	123±2
Glibenclamide	24.3±2.2	24±3	115±3	122±4
Metformin	24.6±3.1	11±3*	114±4	108±4*
APE	24.8±1.5	19±2*	113±3	111±8*
SBE	26.4±2.3	18±1*	114±1	117±3*
FTE	24.5±3.2	19±2*	115±3	114±6*
PAE	23.6±2.6	16±2*	114±2	110±2*

3.3.3. Effects of test extracts on long term renal function

3.3.3.1. *Non-diabetic rats*

Effects of chronic administration of test extracts on Na^+ and K^+ excretion and urine flow rates per 24h in non-diabetic rats are shown are in Figures 18 and 19. Animals were treated once daily at 09h00 for 6 weeks. Control animals were administered with deionised (3 ml/kg).

Group A extracts (APE and SBE)

Figure 18 illustrates effects of chronic administration of group A extracts (120 mg/kg) once daily for 6 weeks on Na^+ and K^+ excretion and urine flow rates per 24h, in non-diabetic rats. APE significantly reduced Na^+ and K^+ excretion rates in non-diabetic rats. APE reduced urine flow rate in non-diabetic rats but this was not statistically significant. SBE failed to show any effects on renal Na^+ and K^+ excretion rates, and urine flow rates in non-diabetic rats compared with control animals.

Group B extracts (FTE and PAE)

Effects of chronic treatment of non-diabetic rats with group B extracts on Na^+, K^+ excretion and urine flow rates per 24h are shown in Figure 19. None of group B extracts had any effects on Na^+ and K^+ excretion and urine flow rates, in non-diabetic rats during 6 weeks of treatment.

3.3.3.2. *STZ-induced diabetic rats*

Effects of long term administration of test extracts on renal Na^+ and K^+ excretion, and urine flow rates per 24h in STZ-induced diabetic rats are shown in Figures 20 and 21. Control diabetic rats had reduced urinary Na^+ excretion rates compared with control non-diabetic rats. Na^+ excretion rate in control STZ-induced diabetic animals was approximately half that of non-diabetic control rats, while K^+ excretion rates was five times lower. STZ-induced diabetic rats were polyuric, exhibiting seven times more urine flow rate, compared with non-diabetic control groups.

Group A extracts (APE and SBE)

Figure 20 shows effects of long term treatment of group A extracts to STZ-induced diabetic rats on Na^+, K^+ and urine flow rates per 24h. APE reduced Na^+ and K^+ excretion and urine flow rates in STZ-induced diabetic rats, $P < 0.05$ (Figure 20). In contrast, chronic treatment of STZ-induced diabetic rats with SBE did not have any effects on Na^+ and K^+ excretion, and, urine flow rates.

Group B extracts (FTE and PAE)

Figure 21 illustrates the effects of long term treatment of group B extracts to STZ-induced diabetic rats on Na^+ and K^+ excretion and urine flow rates per 24h. Group B extracts had no effects on Na^+ and K^+ excretion, and urine flow rates.

3.3.3.3. Summary of effects on renal function

Non-diabetic rats

- APE depressed Na^+ and K^+ excretion rates in non-diabetic rats but its effects on urine flow rate were not significant
- SBE, FTE and PAE had no effects on chronic Na^+ and K^+ excretion, and urine flow rates.

STZ-induced diabetic rats

- APE depressed Na^+ and K^+ excretion and urine flow rates.
- SBE, FTE and PAE had no effects on Na^+ and K^+ excretion, and urine flow rates in STZ-induced diabetic rats.

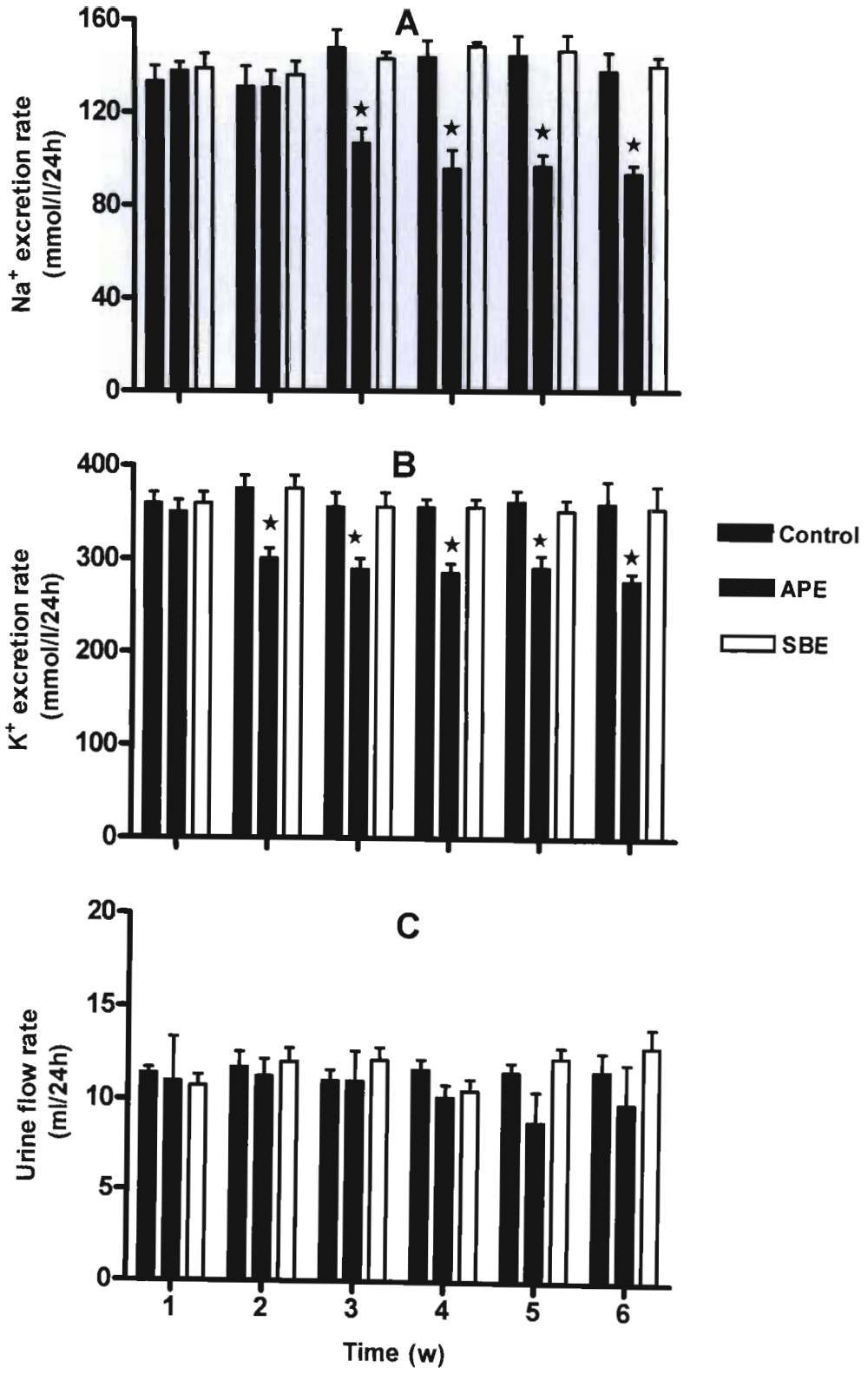

Figure 18. Na^+ (A) and K^+ (B) excretion, and, urine flow (C) rates in non-diabetic rats, chronically administered with APE and SBE at 120 mg/kg once daily for 6 weeks. Values are mean ± S.E.M., n = 8. significant values at $P<0.05$ vs. control group at corresponding time.

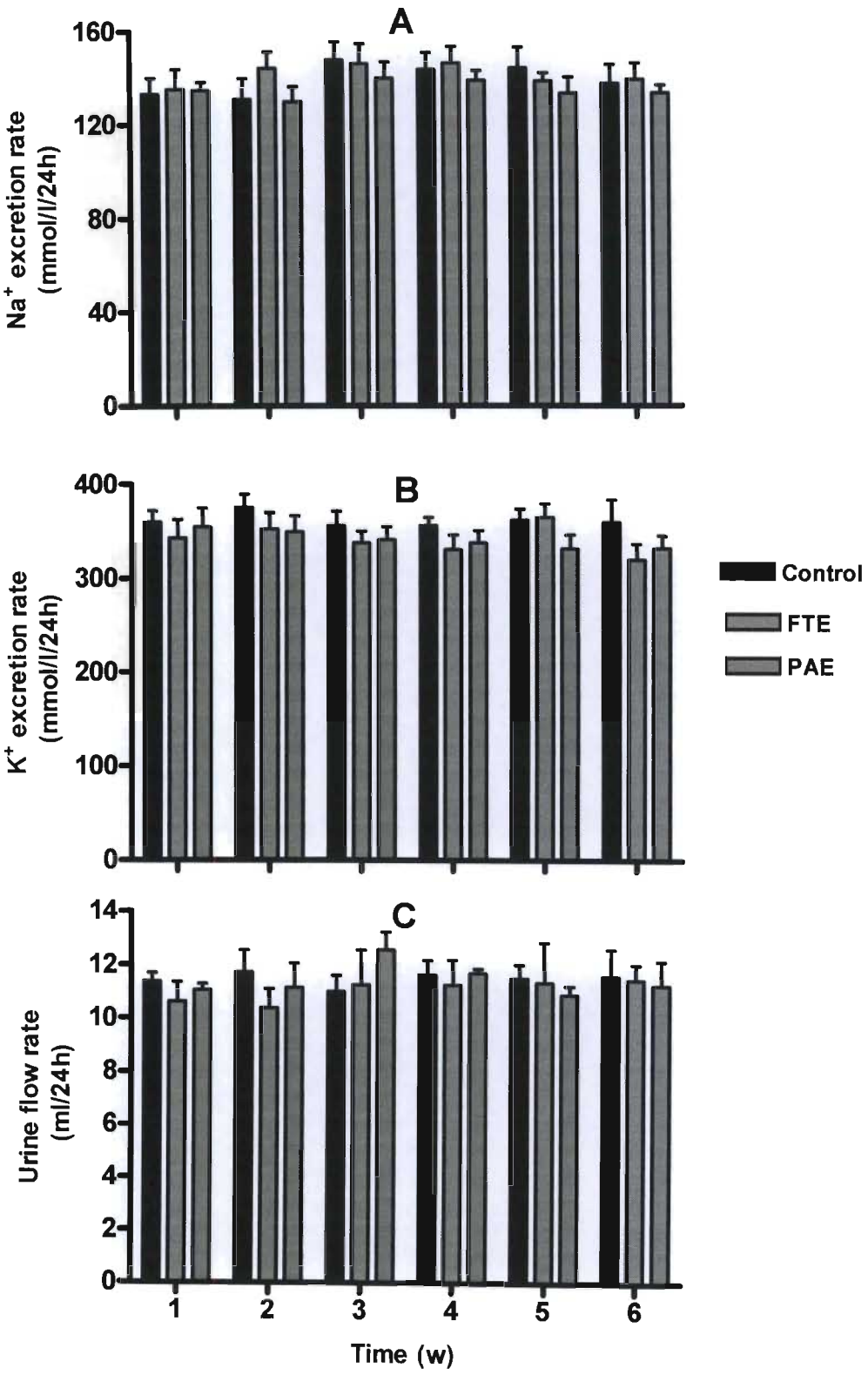

Figure 19. Na^+ (A) and K^+ (B) excretion, and, urine flow (C) rates in non-diabetic rats, chronically administered with FTE and PAE at 120 mg/kg once daily for 6 weeks. Values are mean ± S.E.M., n = 8. *significant values at $P<0.05$ vs. control group at corresponding time.

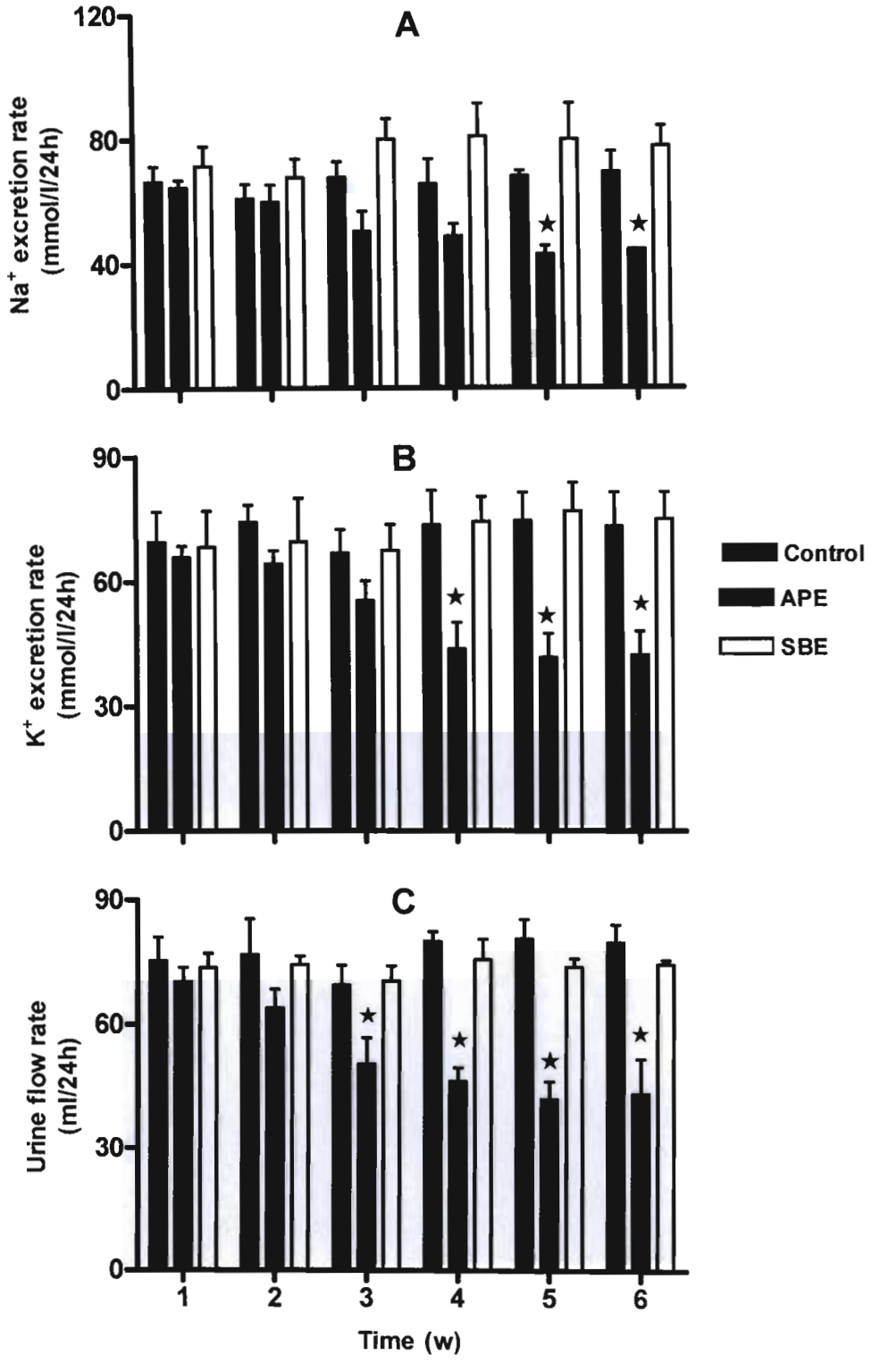

Figure 20. Na^+ (A) and K^+ (B) excretion, and, urine flow (C) rates in STZ-induced diabetic rats, chronically administered with APE and SBE at 120 mg/kg once daily for 6 weeks. Values are mean ± S.E.M., n = 8. ★significant values at $P<0.05$ vs. control group at corresponding time.

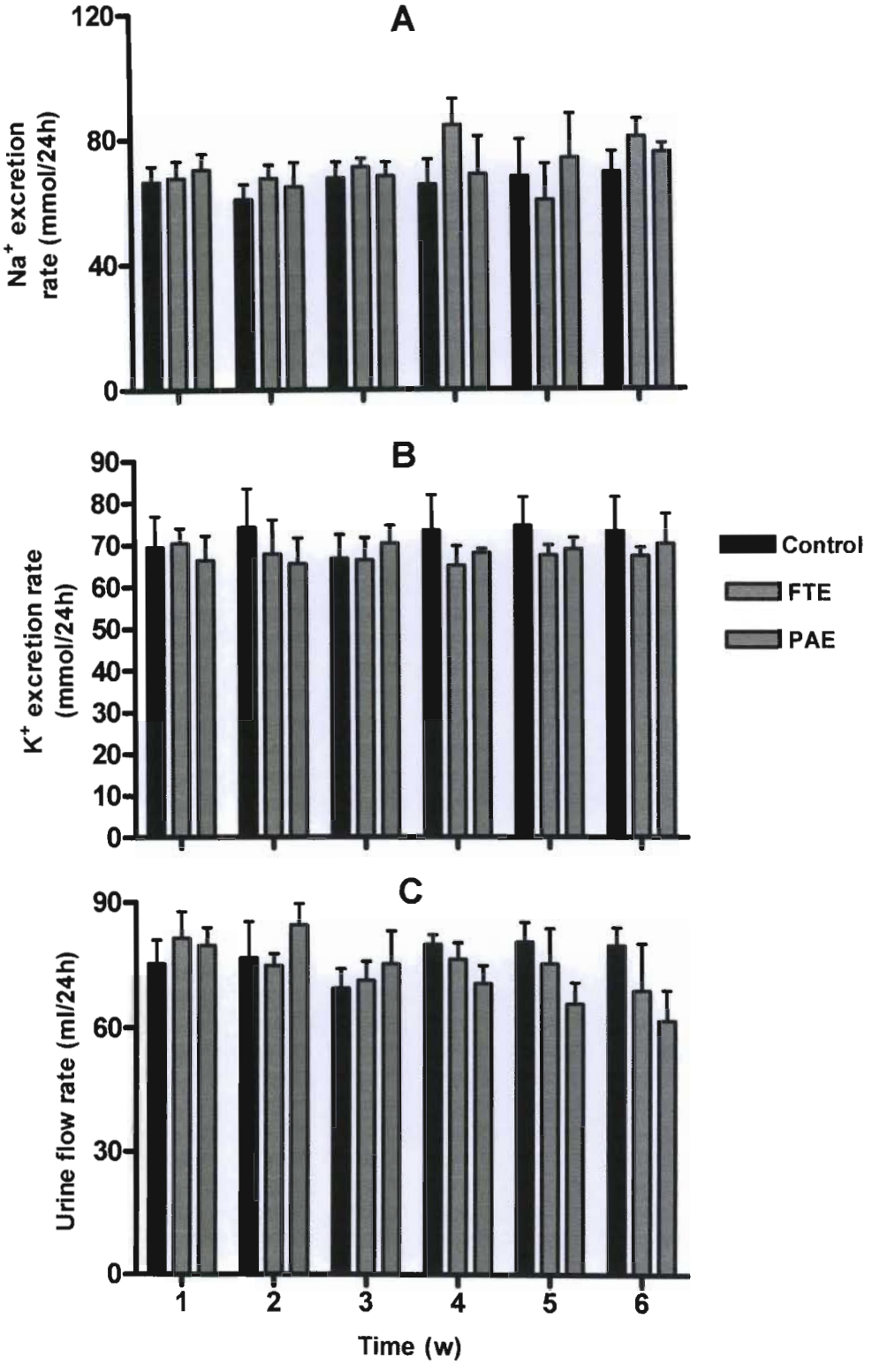

Figure 21. Na^+ (A) and K^+ (B) excretion, and, urine flow (C) rates in STZ-induced diabetic rats, chronically administered with FTE and PAE at 120 mg/kg once daily for 6 weeks. Values are mean ± S.E.M., n = 8. *significant values at $P<0.05$ vs. control group at corresponding time.

3.3.4. Effects of test extracts on plasma creatinine and urea concentrations

Effects of chronic treatment of test extracts on plasma concentrations of creatinine and urea in non-diabetic and STZ-induced diabetic rats are depicted in Table 5.

3.3.4.1. *Non-diabetic rats*

Group A extracts (APE and SBE)

As shown in Table 5, APE significantly increased plasma creatinine concentration by comparison to respective control values, $P < 0.05$. APE demonstrated no effects on urine and plasma concentrations of urea in non-diabetic rats. SBE reduced plasma creatinine concentrations which was statistically significant by comparison with control rats, $P < 0.05$, but had no effect on plasma urea levels.

Group B extracts (FTE and PAE)

Group B extracts significantly reduced plasma creatinine concentrations in non-diabetic rats, by comparison with control values, $P < 0.05$. FTE demonstrated no effects on plasma urea concentrations whereas PAE significantly reduced plasma urea concentrations, $P < 0.05$, by comparison with control values.

3.3.4.2. *STZ-induced diabetic rats*

Table 5 shows the effects of chronic treatment of test extracts on plasma creatinine and urea concentrations in STZ-induced diabetic rats. STZ-induced diabetic control rats exhibited increased plasma creatinine and urea concentrations in comparison to non-diabetic control rats.

Group A (APE and SBE) and B (FTE and PAE) extracts

Like in non-diabetic rats, APE significantly increased plasma concentrations of creatinine in STZ-induced diabetic rats, but demonstrated no effects on plasma urea. The rest of test extracts significantly reduced plasma creatinine, and plasma urea concentrations in STZ-induced diabetic rats, $P < 0.05$.

3.3.4.3. Summary of effects on creatinine and urea concentrations in plasma and urine

Non-diabetic rats

- APE increased plasma creatinine concentration but did not alter plasma urea concentration
- SBE and FTE reduced plasma creatinine concentrations but showed no effects on plasma urea concentration.
- PAE reduced plasma creatinine and urea concentrations.

STZ-induced diabetic rats

- APE increased plasma creatinine concentration, but had no effects on plasma urea concentrations.
- SBE, FTE and PAE reduced plasma creatinine, and plasma urea concentrations.

3.3.5. Effects of test extracts on plasma sodium and potassium levels

3.3.5.1. *Non-diabetic rats*

Also shown on in Table 5 are the terminal Na^+ sodium and K^+ levels after 6 weeks in non-diabetic rats.

Group A extracts (APE and SBE)

As shown in the Table 5, none of group A extracts had any effect on plasma Na^+ and K^+ levels in non-diabetic rats.

Group B extracts (FTE and PAE)

None of group B extracts influenced plasma Na^+ and K^+ levels in non-diabetic rats (Table 5).

3.3.5.2. *STZ-induced diabetic rats*

Also shown in Table 5 are the terminal plasma Na^+ and K^+ concentrations after 6 weeks in STZ-induced diabetic rats. There was no difference in the plasma concentrations of $Na^+$and K^+ between non-diabetic and STZ-induced diabetic control groups.

Group A (APE and SBE) and B (FTE and PAE) extracts

None of test extracts had any influence on plasma Na^+ and K^+ concentrations in STZ-induced diabetic rats.

Table 5

Influence of long term treatment of test extracts to non-diabetic and STZ-induced diabetic rats on terminal plasma concentrations of creatinine (μmol/l), urea (mmol/l), Na^+ (mmol/l) and K^+ (mmol/). Data are expressed as mean ± S.E.M., n = 8 rats in each group. ★Significant values at $P< 0.05$ compared to corresponding control values.

Group/Treatment	Creatinine	Urea	Na^+	K^+
Non-diabetic				
Control	55±3	9±1	142±2	3.6±0.3
APE	68±6★	9±1	143±3	4.5±0.4
SBE	32±4★	10±1	142±2	4.2±0.2
FTE	36±2★	8±0.7	143±2	3.2±0.1
PAE	36±2★	5±0.2★	144±1	4.7±1.5
STZ-induced diabetic				
Control	86±6	223±1.0	142±1	4.0±0.2
APE	97±5★	165±0.3★	142±3	3.0±0.1
SBE	46±12★	122±0.8★	143±2	4.0±0.1
FTE	73±9★	111±0.6★	143±2	4.1±0.2
PAE	65±8★	105±1.0★	143±3	3.6±0.1

3.3.6. Effects of test extracts on water and food intake, and weight changes

3.3.6.1. *Non-diabetic rats*

Table 6 depicts water and food intake, and percentage body weight changes in non-diabetic and STZ-induced diabetic rats at the end of 6 weeks.

Group A extracts (APE and SBE)

APE had no effect on water and food intake in non-diabetic rats. However, APE significantly increased, $P < 0.05$, percentage weight gain in non-diabetic rats. Chronic treatment of SBE to non-diabetic rats had no effect on water intake, but significantly reduced, $P < 0.05$, food intake in non-diabetic rats in comparison to non-diabetic control rats. SBE also markedly increased percentage body weight gain which was statistically significant, $P < 0.05$.

Group B extracts (FTE and PAE)

As shown in Table 6, chronic treatment of FTE to non-diabetic rats for 6 weeks stimulated increased water intake which was statistically significant, $P < 0.05$, in comparison to non-diabetic control rats, but had no effect on food intake. However, chronic administration of FTE increased body weight gain in non-diabetic rats. In contrast, PAE had no effects on water and food intake, and percentage weight gain in non-diabetic rats.

3.3.6.2. *STZ-induced diabetic animals*

Table 6 shows the effects of chronic administration of test extracts on water and food intake, and body weight gain at the end of 6 weeks, in STZ-induced diabetic rats. Control STZ-induced diabetic rats exhibited polypphagia evidenced by increased food intake by nearly 100% over control non-diabetic rats. Diabetic animals also exhibited polydipsia (Table 6). In comparison to control non-diabetic rats, water intake was elevated by nearly 400% in control diabetic rats. Vehicle treated control diabetic rats exhibited severe wasting by comparison with vehicle treated non-diabetic rats. These characteristics in STZ-induced diabetic rats were sustained for the duration of the study.

Group A extracts (APE and SBE)

As shown in Table 6, chronic treatment of APE extracts significantly reduced water intake in STZ-induced diabetic rats $P < 0.05$, in comparison to control STZ-induced diabetic rats. Moreover, APE reduced food intake in diabetic rats in comparison to control values, $P < 0.05$. Chronic administration of APE reduced body wasting and promoted a body weight gain of nearly 1%.

After 6 weeks of daily treatment with SBE, water and food intake significantly reduced in STZ-induced diabetic rats in comparison to vehicle treated diabetic rats, $P < 0.05$. SBE promoted a modest but significant increase in body weight gain in STZ-induced diabetic rats

Group B extracts (FTE and PAE)

FTE reduced both water and food intake. These effects were statistically significant, $P < 0.05$, compared to control STZ-induced diabetic rats. FTE also significantly promoted body weight gain in STZ-induced diabetic rats, $P < 0.05$, by comparison with non vehicle treated diabetic control group. (Table 6).

PAE mediated significant reduction in water intake, $P < 0.05$, by comparison with control STZ-induced diabetic rats, but had no effects on food intake in these animals (Table 7). PAE also significantly ameliorated wasting, $P < 0.05$, in diabetic rats.

3.3.6.3. Summary of effects of test extracts on water and food intake, and weight changes

Non-diabetic rats

- APE had no effect on water and food intake, but increased body weight.
- SBE had no effect on water intake but reduced food intake while promoting body weight gain.
- FTE increased water but not food intake. FTE increased body weight
- PAE had no effect on water and food intake and body weight

STZ-induced diabetic rats

- APE, SBE and FTE reduced water and food intake, and increased body weight.
- PAE reduced water intake, but had no effects on food intake. PAE modestly increased body weight.

Table 6

Effects of long term treatment of test extracts on body weight, fluid and food intake after 6 weeks. Data are expressed as mean ± S.E.M., $n = 8$ rats in each group,$^{\star}P<$ 0.05, compared to control values at week 6, respectively

	Parameter				
	Water intake (ml/24h)		Food intake (g/24h)		Body wt (%)
Group/Treatment	**wk1**	**wk6**	**wk1**	**wk6**	**wk6**
Non-diabetic					
Control	23±3	24±1	27±3	28±1	14±2
Glibenclamide	24±2	22±2	26±2	20±1*	37±3*
Metformin	24±4	26±1	28±3	21±1*	45±2*
APE	23±1	23±3	25±4	26±3	30±1*
SBE	23±2	25±1	26±1	23±1*	25±2*
FTE	22±1	30±1*	27±4	30±1	27±2*
PAE	24±3	25±2	25±2	27±0.4	13±2
STZ-induced diabetic					
Control	77±4	93±2	38±2	45±2	-22±2
Glibenclamide	73±6	92±4	37±4	47±1	-19±2
Metformin	72±5	31±2*	39±1	31±2*	21±4*
APE	74±2	62±2*	42±1	34±1*	1±0.1*
SBE	69±3	62±3*	39±3	31±2*	-6±3*
FTE	71±5	55±3*	40±2	37±3*	-3±1*
PAE	75±2	66±4*	37±2	39±2	-12±1*

Wk1, wk6 denote week 1 and week 6, respectively

3.3.7. Terminal insulin levels

3.3.7.1. *Non-diabetic rats*

Table 6 shows the effects of chronic administration of test extracts on insulin release in non-diabetic rats after 6 weeks. The pattern shown in Table 6 is generally similar to that of acute effects in Table 3. As shown in the table glibenclamide significantly increased insulin release in comparison to control non-diabetic rats, $P < 0.05$. Metformin demonstrated no effects on insulin release in non-diabetic rats.

Group A (APE and SBE) and B (FTE and PAE) extracts

None of test extracts had any long tem effects on insulin release in non-diabetic rats.

3.3.7.2. *STZ-induced diabetic rats*

Table 8 also shows the effects of chronic administration of group A test extracts on long insulin release in STZ-induced diabetic rats. Insulin concentrations in STZ-induced diabetic rats were markedly low in comparison to non-diabetic control rats Glibenclamide and metformin had no effects in comparison to control STZ-induced diabetic rats.

Group A (APE and SBE) and B (FTE and PAE) extracts

As shown in the Table 8, none of test extracts had any effects on plasma insulin release in STZ-induced diabetic rats.

3.3.7.3. Summary of effects of test extracts on terminal plasma insulin

Non-diabetic rats

- None of test extracts had any effects on terminal plasma insulin in non-diabetic rats.

STZ-induced diabetic rats

- None of test extracts had any effects on terminal plasma insulin in STZ-induced diabetic rats.

3.3.8. Effects on liver glycogen

3.3.8.1. *Non-diabetic rats*

A summary of hepatic glycogen levels are shown in Table 7 and Figure 22. The figure shows representative pas stained hepatic micrographs comparing density of glycogen stores in hepatic tissues of treated and untreated animals. Results of Pas staining showed glycogen and other periodate-reactive carbohydrates a magenta colour while cellular nuclei became blue.

Group A extracts (APE and SBE)

Chronic treatment of APE to non-diabetic rats significantly increased hepatic glycogen compared with control values, $P < 0.05$. In contrast, SBE induced a slight increase in hepatic glycogen levels in non-diabetic rats, which was not significant.

Group B extracts (FTE and PAE)

As shown in Table 7, FTE and PAE significantly, ($P < 0.05$), raised hepatic glycogen concentrations in non-diabetic rats by comparison with control rats.

3.3.8.2. *STZ-induced diabetic animals*

Non-diabetic animals generally exhibited high levels of hepatic glycogen compared with diabetic animals (Table 7). Animals treated with standard drug metformin exhibited higher hepatic glycogen levels than groups treated with test extracts. Compared with control non-diabetic rats, STZ-induced diabetic control rats had reduced hepatic glycogen levels.

Group A (APE and SBE) and B extracts (FTE and PAE)

All test extracts, significantly increased hepatic glycogen in STZ-induced diabetic rats, by comparison with STZ-induced diabetic control group, $P < 0.05$. The deeper the magenta colour intensity in Figure 22, the higher the glycogen levels. Based on this, Figure 22 shows that groups treated with test extracts (Figure 22C) generally had improved glycogen storage than those treated with vehicle (Figure 22B).

3.3.8.3. Summary of effects of test extracts on hepatic glycogen

Non-diabetic rats

- APE, FTE and PAE increased hepatic glycogen concentration.
- SBE had no effects on hepatic glycogen concentration.

STZ-induced diabetic rats

- APE, SBE and FTE increased hepatic glycogen concentration.
- PAE induced a modest but significant increase in hepatic glycogen concentration.

Table 7
Effects of chronic administration of test extracts, at 120 mg/kg, on terminal plasma insulin levels (ng/ml) and glycogen levels (mg/100 g, liver tissue). Data are expressed as mean ± S.E.M., $n = 8$ rats in each group. ★Significant values at $P< 0.05$ compared to corresponding control values.

	Parameter	
Group/Treatment	**Plasma insulin (ng/ml)**	**hepatic [glycogen] (mg/100g liver tissue)**
Non-diabetic		
Control	14.7±0.3	800±20
Glibenclamide	18.8±0.1★	1200±15★
Metformin	14.4±0.2	1100±31★
APE	14.7±0.2	1020±15★
SBE	14.9±0.4	850±20
FTE	14.6±0.1	1040±10★
PAE	14.2±0.2	930±40★
STZ-induced diabetic		
Control	2.3±0.1	110±13
Glibenclamide	3.3±0.3	115±21
Metformin	2.2±0.1	600±20★
APE	2.5±0.1	500±30★
SBE	2.4±0.1	400±15★
FTE	2.5±0.1	540±30★
PAE	2.4±0.1	320±14★

A

B

C

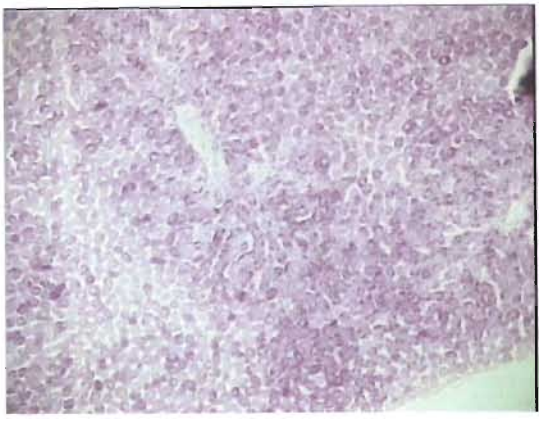

Figure 22. Liver micrographs pas stained showing magenta coloured glycogen stores (A) vehicle treated normal rats, (B) vehicle treated diabetic (C) extract treated diabetic (magnification x20).

3.4. Cell viability studies

An *in vitro* study illustrating the percentage viability of MDBK and LLCPK-1 cells, exposed to graded concentrations (100 – 1000 µg/ml) of test extracts for varied incubation periods (24, 48 and 72 hours), relative to vehicle treated controls, is shown in Figures 23 to 26.

Group A extracts (APE and SBE)

APE induced marked cell growth promotion in MDBK cell lines but cell death in LLCPK-1 lines (Figure 23). Cell growth in LLCPK-1 seemed to be strongly dependent on incubation time, whereby increased cell viability (up to 200% relative to untreated cells) was observed in cells incubated for 72 hours. MDBK cells exposed for 24 hours demonstrated mild cell death. While lower APE concentrations exposed to LLCPK-1 cells at minimal incubation period (24 hours) promoted cell proliferation, higher doses at higher incubation periods exhibited severe cytotoxicity, inflicting LLCPK-1 cell death by up to 97%, $P < 0.05$.

SBE demonstrated cytotoxicity in both cell lines (Figure 24). However the cytotoxic effects were moderate in MDBK cells (10.4 to 49.4% cell death) compared with LLCPK-1 cells where it was more severe (22.7 to 63.9%).

Group B extracts (FTE and PAE)

Effects of exposure of MDBK and LLCPK-1 cells to FTE are shown in Figure 25. FTE demonstrated promotion of cell growth for both types of cell lines. This growth was, however, modest in both MDBK and LLCPK-1, and appeared to be dose-dependent.

In contrast to FTE, PAE induced reduction in cell viability of both MDBK and LLCPK-1 cell lines (Figure 26). However, the effects were more marked in MDBK cells, 35.3% to 74.2%. MTT demonstrated that all test extract except FTE were cytotoxic towards LLCPK-1 cell lines but had mild cytotoxic effects on MDBK cells, except PAE.

3.4.1. Cell viability results summary

- APE increased cell growth in MDBK cell lines but promoted cell death in LLCPK-1 cell lines.
- SBE and PAE were cytotoxic to both cell lines
- FTE promoted cell growth in both cell lines

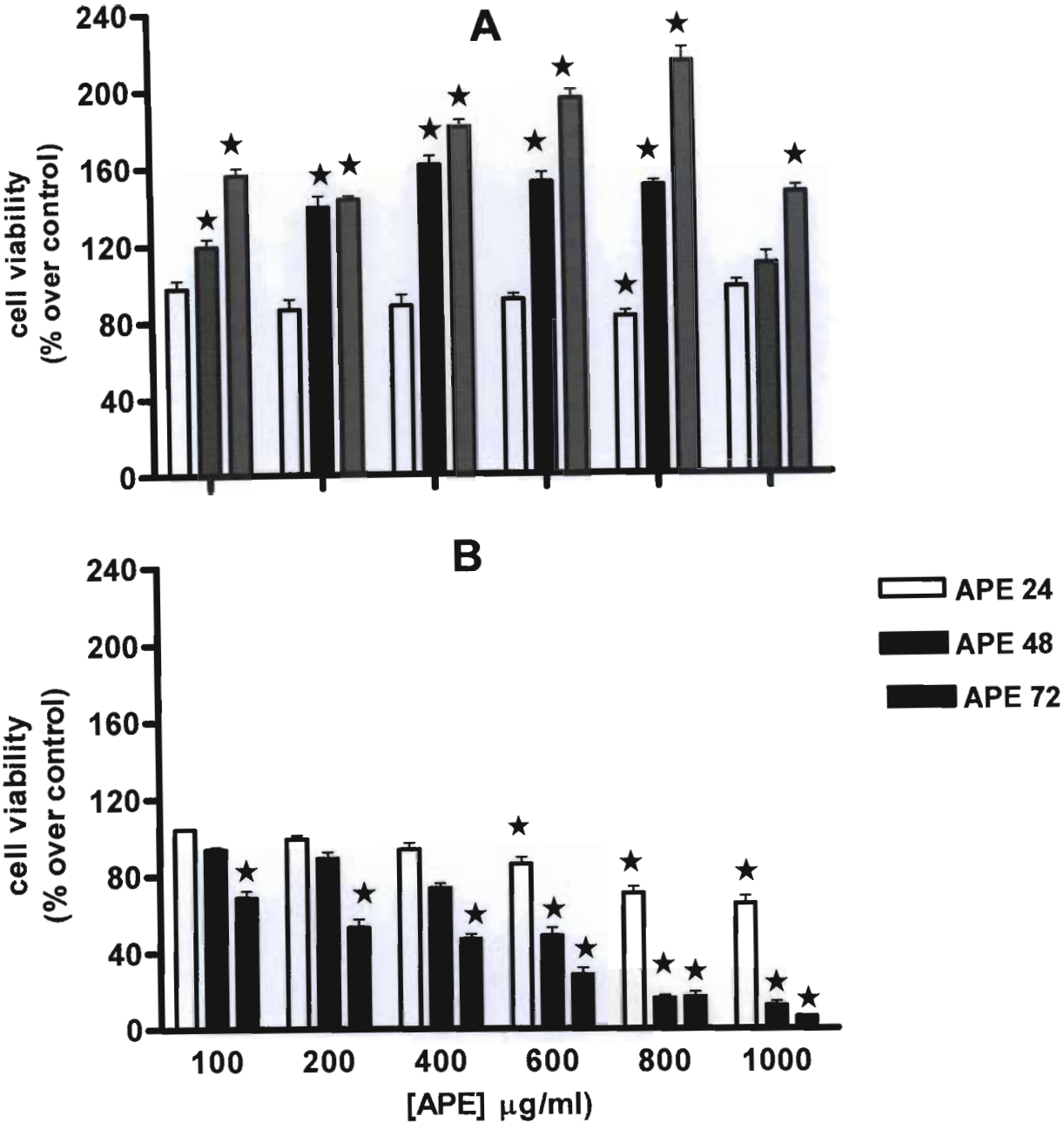

Figure 23. Effects of exposure of (A) MDBK and (B) LLCPK-1 cells for 24, 48 and 72 hrs to graded concentrations (100 -1000 µg/ml) of APE on cell viability. Cells were cultured in 96 micro-well plates and incubated at 37°C. Percentage cell viability was expressed relative to vehicle treated cells n= 6 replicates; *significant, $P<0.05$, by comparison to untreated group

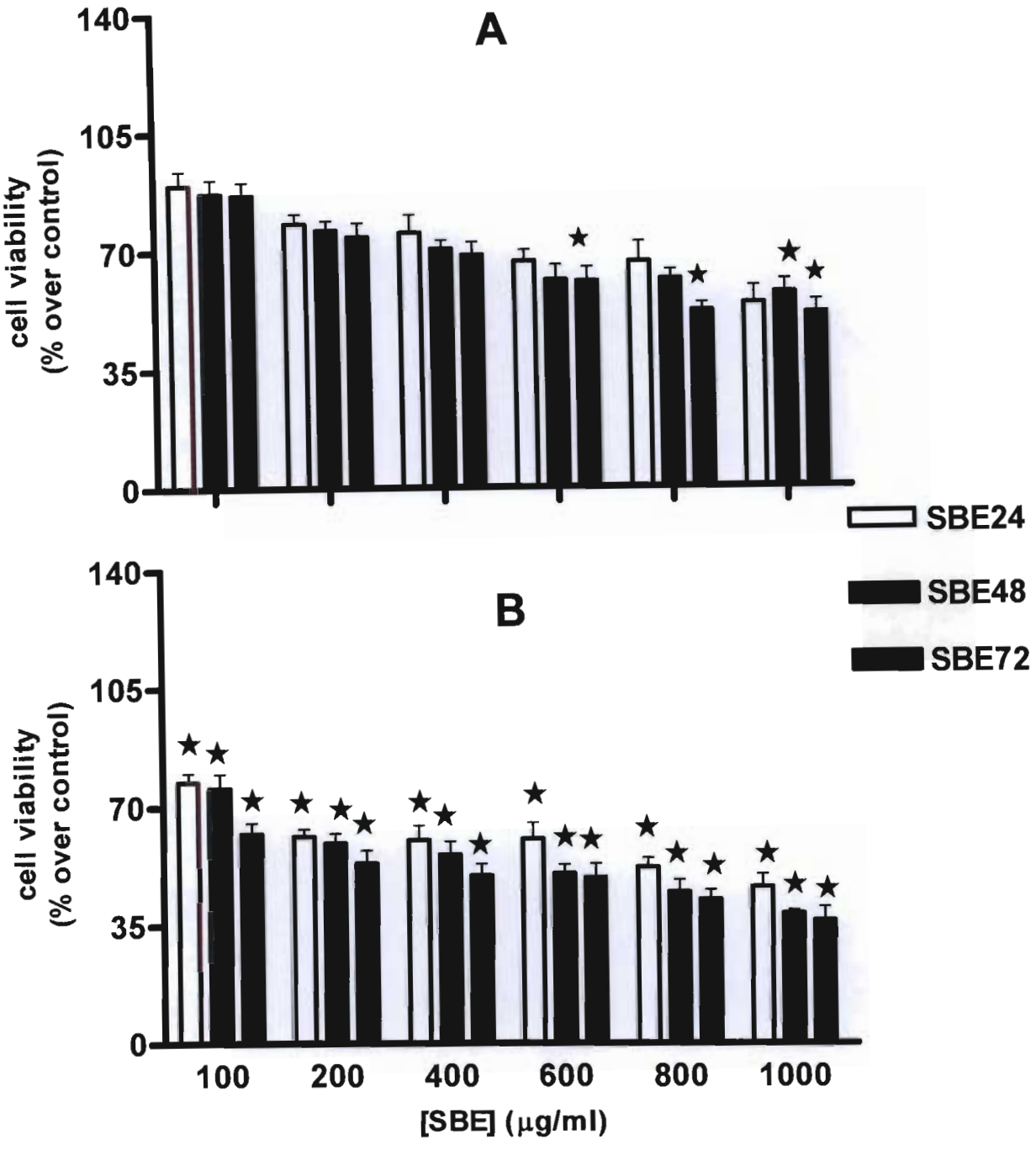

Figure 24. Effects of exposure of (A) MDBK and (B) LLCPK-1 cells for 24, 48 and 72 hrs to graded concentrations (100 -1000 µg/ml) of SBE on cell viability. Cells were cultured in 96 micro-well plates and incubated at 37°C. Percentage cell viability was expressed relative to vehicle treated cells. n= 6 replicates; *significant, $P<0.05$, by comparison to untreated group

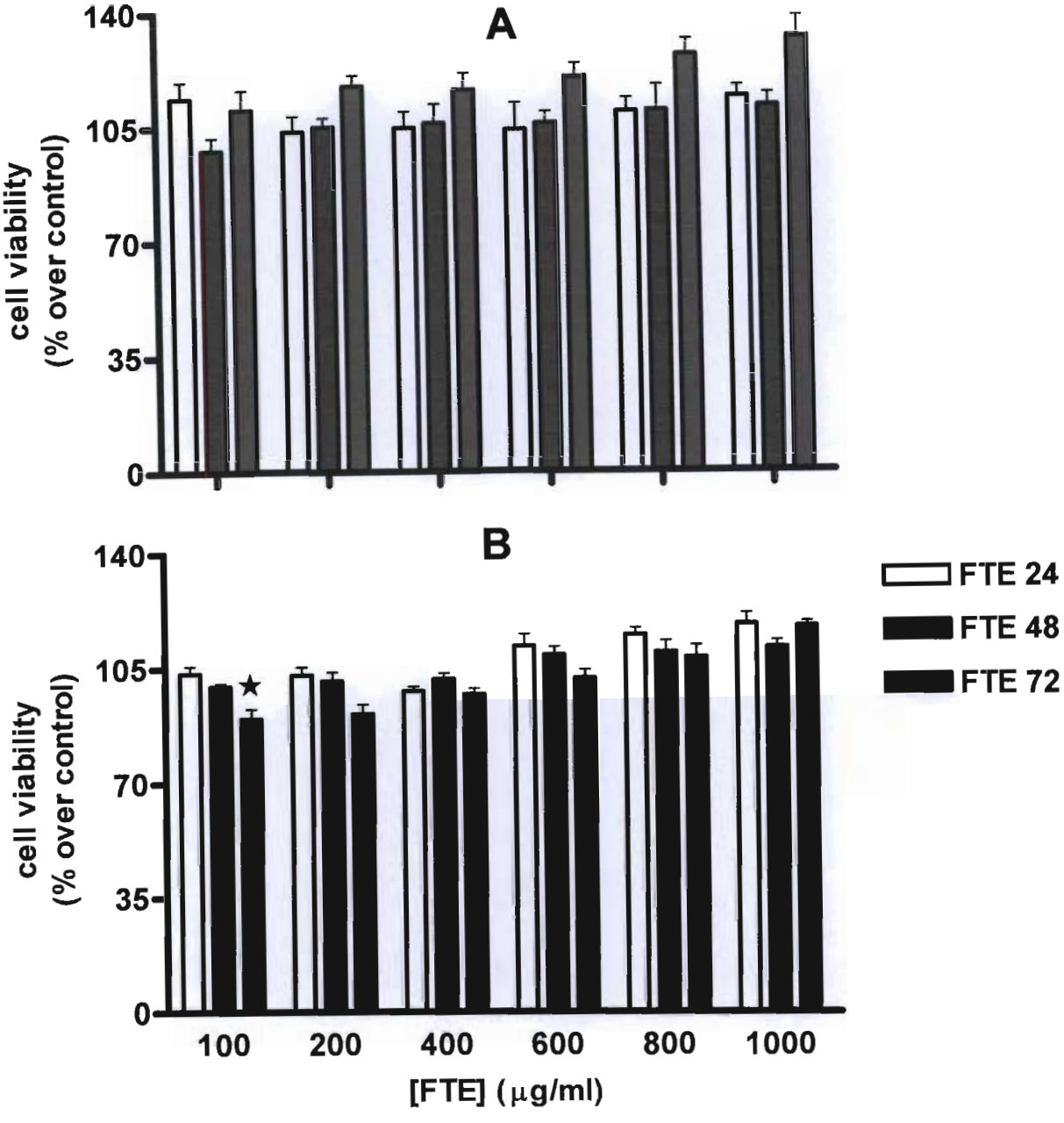

Figure. 25. Effects of exposure of (A) MDBK and (B) LLCPK-1 cells for 24, 48 and 72 hrs to graded concentrations (100 -1000 µg/ml) of FTE on cell viability. Cells were cultured in 96 micro-well plates and incubated at 37°C. Percentage cell viability was expressed relative to vehicle treated cells n= 6 replicates; *significant, $P<0.05$, by comparison to untreated group

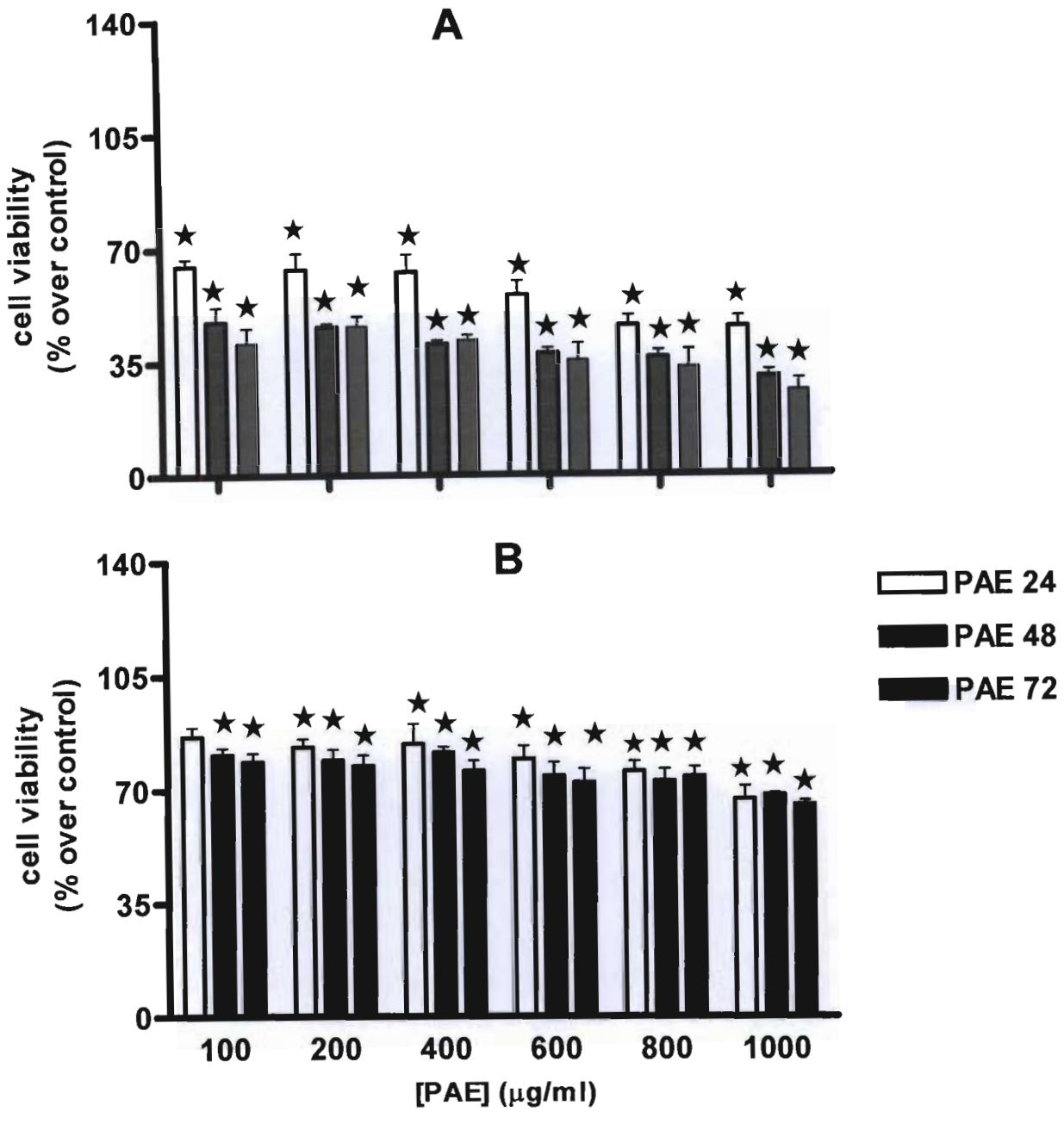

Figure. 26. Effects of exposure of (A) MDBK and (B) LLCPK-1 cells for 24, 48 and 72 hrs to graded concentrations (100 -1000 µg/ml) of PAE on cell viability. Cells were cultured in 96 micro-well plates and incubated at 37°C. Percentage cell viability was expressed relative to vehicle treated cells n= 6 replicates; *significant, $P<0.05$, by comparison to untreated group

3.5. Summary of all results

- APE, SBE, FTE and PAE possess acute and long term hypoglycaemic effects in STZ-induced diabetic rats
- APE, SBE, FTE and PAE increased hepatic glycogen concentrations in STZ-induced diabetic rats
- APE, SBE, FTE and APE had no effect on insulin release in non-diabetic and STZ-induced diabetic rats
- APE, SBE, FTE and PAE exhibited short and long term vasodepressive effects in diabetic rats
- APE increased plasma creatinine concentration, while SBE, FTE and PAE reduced plasma creatinine, concentrations in diabetic rats.
- APE promoted MDBK cell proliferation, but was cytotoxic to LLCPK-1 cells
- SBE and PAE were cytotoxic to MDBK and LLCPK-1 cell lines
- FTE promoted growth of both cell lines

CHAPTER 4

4.0 DISCUSSION

4.1. Background

The present study investigated the effects of ethanolic extracts of *Hypoxis hemerocallidea (*APE), *Sclerocarya birrea* (SBE), *Ficus thonningii* (FTE) and *Persea Americana* (PAE) on blood glucose, renal function and mean arterial blood pressure in non-diabetic and STZ-induced diabetic rats. Results demonstrated that all test extracts possess short and long term hypoglycaemic effects in non-diabetic and STZ-induced diabetic rats. While some plants demonstrated adverse effects on renal function, others appeared to be renoprotective. By exhibiting cardio-renoprotective effects, some of these test extracts may possess therapeutic potential in the amelioration of cardiovascular and renal complications in diabetes.

4.2. Hypoglycaemic effects

Both group A (APE and SBE) [Figure 7] and B (FTE and PAE) [Figure 8] extracts demonstrated hypoglycaemic effects in non-diabetic rats. We suggest that the mechanisms of these hypoglycaemic effects were mediated independent of insulin release. This is evidenced in Table 3, where none of the extracts stimulated insulin release in non-diabetic rats. While the insulin secretagogue glibenclamide and insulin increased plasma insulin concentrations in non-diabetic rats, metformin, like the test extracts, demonstrated no effects on insulin release (Table 3). This suggests that the mechanisms of hypoglycaemic effects of group A and B extracts closely mimic that of

metformin. Metformin is a biguanide that lowers blood glucose without stimulating insulin release (British National Formulary, 1995). It mediates hypoglycaemic effects by sensitizing target tissues cells to insulin action and blocking hepatic gluconeogenesis (Pari and Satheesh, 2006). It also increases expression or activities of GLUT-4 transporters which also enhances glucose uptake by muscles (Simó and Hernández, 2002). Other possible mechanisms of hypoglycaemic effects of test extracts, however, cannot be ruled out. For example, reduced peak blood glucose concentrations in non-diabetic groups treated with test extracts might suggest that test extracts mediate rapid peripheral tissue utilisation of glucose. The rapid reduction of glucose levels in extract treated groups may suggest a synergistic effect of test extract to endogenous insulin effects since non-diabetic rats had intact functional β-cell mass. Moreover, the absence of insulin stimulation by test extracts suggests possible mediatory effects via a decrease in the secretion of counteregulatory hormones and increased glycolytic enzymatic activities, in addition to increased glucose utilisation in the peripheral tissues (Kumar, Banu, Murugesan and Pandian, 2006).

By comparison with non-diabetic control group, some non-diabetic treated groups attained maximal blood glucose concentrations 15 minutes later than control group, after glucose challenge (Figures 7B and 8). Although brief, this may be a result of delayed absorption of glucose into blood from the gut. Ortiz-Andrade, Garc´ıa-Jim´enez, Castillo-Espa˜na, Ram´ırez- Avila, Villalobos-Molina and Estrada-Soto, (2006), stated that delaying absorption of glucose after a meal is one of the effective ways to control postprandial hyperglycaemia. The presence of tannins in food blocks its digestibility and consequently, delays its absorption. Subsequently blood glucose concentration is reduced (Aganga and Mosase, 2001). Phytochemical studies by Eloff,

(2001), and, Osadebe Okide and Akabogu (2004) have shown the presence of tannins in *S. birrea* bark and *P. americana* leaf extracts, respectively.

The demonstration of acute hypoglycaemic effects of test extracts APE, SBE, FTE and PAE in STZ-induced diabetic rats (Figures 10 and 11) is a significant finding in the present study. Since these animals are devoid of functional β-cells, these results eliminate insulin involvement in the mediatory roles of test extracts. Indeed, these observations are supported by results shown in Table 3, which show that none of test extracts in the present study stimulated insulin release in both non-diabetic and STZ-induced diabetic rats. The lack of insulin release in diabetic animals, induced by STZ destruction of pancreatic β-cells, could account for sustained elevated glycaemic levels and late hypoglycaemic effects of test extracts in these animals.

In the present study, glibenclamide demonstrated hypoglycaemic effects in non-diabetic rats (Figure 7) but exhibited no effects in STZ-induced diabetic animals (Figure 9). This observation corroborated that pancreatic β-cells were destroyed by STZ administration. This is further supported by absence of effects on plasma insulin levels in diabetic rats (Table 3). Moreover, this offers further evidence that hypoglycaemic effects of test extracts were independent of insulin release. Glibenclamide is a potassium channel blocker that stimulates insulin release in the pancreatic β-cells and belongs to a class of hypoglycaemic agents called sulphonylureas (Babichev, 1999).

Group B test extracts (FTE and PAE) demonstrated hypoglycaemic effects in both non-diabetic and STZ-induced diabetic rats. These effects were dose dependent in

STZ-induced diabetic rats but not in non-diabetic rats (Figures 8 and 10). The reasons for the discrepancy remain to be determined. The dose-dependent effects of PAE are consistent with the findings of Antia, Okokon and Kon, (2005) in alloxan-induced diabetic rats.

Previous studies have demonstrated that extracts of *S. birrea* and *H. hemerocallidea* have potent hypoglycaemic effects (Ojewole, 2003; Ojewole, 2006). Other studies have shown that aqueous extracts of *P. americana* possess antihyperglycaemic and antihyperlipidaemic effects (Gallagher, Flatt, Duffy and Abdel-Wahab, 2003). However, these studies did not establish the effects of extracts on plasma insulin and hepatic glycogen levels. Therefore, findings of the present study will help unravel mechanisms by which the extracts from these plants mediate hypoglycaemic effects.

SBE and FTE demonstrated long term hypoglycaemic effects in non-diabetic rats after 6 weeks (Table 4), while effects of APE and PAE were not statistically significant. The lack of statistical significance of long term hypoglycaemic effects of some test extracts (APE and PAE) in non-diabetic animals (Table 4) may be best explained by the trend shown in short term acute studies in non-diabetic animals (Figures 7 and 8). In these studies, hypoglycaemic effects of these test extracts at the median dose of 120 mg/kg, were of short duration, lasting between 1 and 2 hours and, therefore, may explain failure to detect lower glucose levels as these would have returned to baseline at time of measurement in chronic studies. Such rapid effects, however, suggest use to in the management of acute hyperglycaemic episodes.

As evident in Table 7, none of test extracts increased plasma insulin levels in non-diabetic animals. This suggests that the long term hypoglycaemic of both group A and B extracts in non-diabetic rats is independent of insulin release, and probably similar to that of metformin. In long term studies, the present study monitored postprandial glucose as opposed to fasting glycaemia. This is because postprandial glucose presents a better reflection of glycaemia in diabetes than fasting glycaemia (Baron, 1998). Moreover, it is postprandial glucose concentration in diabetes, which is strongly associated with diabetic complications (Gadsby, 2002).

Group A (APE and SBE) and B (FTE and PAE) test extracts exhibited significant chronic hypoglycaemic effects in STZ-induced diabetic rats after 6 weeks (Table 4) without affecting plasma insulin levels (Table 7). Like in non-diabetic rats, these hypoglycaemic effects may be mediated via mechanisms similar to that of metformin as previously explained.

In the present study, all the test extracts promoted hepatic glucose conversion into glycogen in STZ-induced diabetic rats compared with the control group (Table 7). Therefore, the long term hypoglycaemic effects of the test extracts might partly be due to conversion of blood glucose into stored glycogen in the liver and muscle. The mechanism(s) by which test extracts enhance conversion of glucose to glycogen remain to be established. However, these effects might be due to stimulating activities of enzymes involved in glycogen synthesis such as hepatic glycogen synthase (Arambewela *et al.*, 2005). These authors pointed out that increased glycogen storage suggests increased glucose uptake from blood and its subsequent conversion into glycogen. Hypoglycaemic investigations by Ghosh *et al.*, (2004), on *Ficus*

bengalensis revealed mediatory effects via conversion of glucose into glycogen. These findings, of glycaemic effects of a plant species belonging to the same genus as *F. thonningii* adds credence to the present results. Therefore, this is the first study that has linked observed hypoglycaemic effects of the present medicinal plants to glycogen synthesis. Previous studies have established hypoglycaemic effects of other medicinal plants as being mediated via similar mechanisms (Musabayane, *et al.*, 2005). The control of the synthesis and breakdown of glycogen in the liver is central to the regulation of blood glucose levels (Chakrabarti *et al.*, 2005). Moreover, Chakrabarti *et al.*, (2003), asserted that assessment of hepatic glycogen level may be considered as the best marker for evaluating the antidiabetic activity of any drug, as it indicates that peripheral free glucose is being stored in the liver in the form of glycogen. In diabetes mellitus, glycogen synthesis is compromised (Emilien *et al.*, 1999), resulting in persistent hepatic gluconeogensis which results in hyperglycaemia. The lack of capacity to store glycogen in STZ-induced diabetic rats observed in the present study is consistent with findings by many investigators (Singh, Vats, Suri, Shyam, Kumria, Ranganathan, Sridharan, 2001; Ramachandran, Kandaswamy, Narayanan, Subramanian, 2003; Adisakwattana, Roengsamran, Hsu and Yibchok-anun, 2005).

The hypoglycaemic effects observed for some of the plant extracts may be partly attributed to the presence of flavonoids in the test extracts. Previous studies by Jung *et al.*, 2006, showed that flavonoids possess potent antihyperglycaemic effects. Moreover, Jung and colleagues, (2006), demonstrated that flavonoids, hesperidin and naringin promoted increased mRNA expression of hepatic glucokinase in the *db/db* mice, a significant enzyme in the metabolism of glucose. The presence of bioactive

compounds such as flavonoids in test extracts is responsible for most long term antihyperglycaemic effects because of their unique antioxidant effects (Argolo, *et al.*, 2005; Czinner, Hagymasi, Blazovics, Kery Szoke and Lemberkovics). Using ethanol as a solvent of extraction, Aslan *et al.*, 2006, were able to isolate more total phenols including flavonoids from *Helichrysum plicatum* ssp. *plicatum* capitulums extracts, compared with water extraction. In addition the ethanolic extract demonstrated the most potent hypoglycaemic effects than the aqueous extract.

Other groups of chemical compounds with known antidiabetic effects include triterpenes (Perez and Vargas, 2002), flavonoids (Singab *et al.*, 2005; Jung *et al.*, 2006) and saponins (Li, Qu, Wang, Wan and Tian, 2002). The present study did not evaluate phytochemical composition of test extracts, but other investigators have established the presence of these groups of compounds in Ficus species, (Chiang *et al.*, 2005), *P. americana*, (Ramos *et al.*, 2004) and *S. birrea* (Ogbobe, 1992).
H. hemerocallidea has been shown to possess potent antioxidant effects. Furthermore, studies have revealed that it is inhibitory on cycloxygenase-2 catalysed prostaglandin synthesis (Steenkamp, Gouws, Gulumian, Elgorashi and van Staden, 2005). Hypoxoside and rooperol, two major compounds isolated from *H. hemerocallidea* were recently shown to possess potent antioxidant effects by Laporta *et al.*, 2007. Since hypoglycaemic activities may be associated with antioxidant properties (McCune and Johns, 2002; Sabu and Kuttan, 2002), the long term hypoglycaemic effects of APE in the present studies might be partly attributed to these properties.

The present studies have established that group A and B extracts possess hypoglycaemic effects in non-diabetic and STZ-induced diabetic rats. These effects

may be mediated by mechanisms that are extra-pancreatic, involving glycogen synthesis, and probably mimicking those of metformin. Food intake is an important behavioural component in blood glucose control in diabetes mellitus. Results illustrated in Table 6 show that the some test extracts decreased food intake in diabetic animals. Control STZ-induced diabetic rats exhibited hyperphagia by comparison with treated diabetic groups and normal animals. These observations were consistent with those of Namkoong, Kim, Jang, Han, Park, Koh, Lee, Kim, Park, Park and Lee, (2005). Further studies are needed to confirm this, but we speculate that this phenomenon may be mediated, partly, by reduced levels of hormone leptin (Sindelar *et al.*, 1999). Leptin is an adipocyte hormone that regulates reduction of food intake in part by inhibiting hypothalamic release of neuropeptide Y (NPY) (Yahya, Xiao, Chance, and Sheriff, 2006). The reduced fat stores as a result of excessive lipid catabolism to generate energy in the diabetic state, may be responsible for depleted leptin levels (Havel, Uriu-Hare, Liu, Stanhope, Stern, Keen and Ahŕen, 1998), and hence hyperphagia in diabetes, via increased expression of hypothalamic NPY. Havel *et al.*, (1998), also observed a decrease in leptinaemia 24 hrs after induction of diabetes attributed to changes in glycaemic levels and not due to wasting.

It is also tempting to speculate that, perhaps, in the present study the extracts could have direct inhibitory effects on AMPK activities or could act via inhibition of ghrelin activities. Studies have revealed that AMPK plays a pivotal role in the regulation of energy whereby its inhibition reduces food intake (Andersson *et al.*, 2004). AMPK acts mainly in the hypothalamus and its actions are partly influenced by ghrelin, a stomach derived hormone peptide. Further studies are clearly needed to support these speculations.

4.3. Renal effects

In acute renal studies, APE and PAE significantly depressed sodium and potassium excretion, and urine flow rates in non-diabetic and STZ-induced diabetic rats (Figures 12, 13, 14 and 15). Increased sodium retention may suggest increased reabsorption probably mediated via activation of Na^+, K^+-ATPase on the proximal tubular cells. About 60% sodium reabsorption occurs in the proximal tubular cells (Nabel, 2003).

In long term studies, APE was consistently suppressive on electrolyte and urine flow rates in non-diabetic and STZ-induced diabetic rats (Figures 18 and 20). These results coupled with increased plasma creatinine concentrations (Table 5) may suggest that APE compromised renal function in non-diabetic and STZ-induced diabetic rats. Significant increase in serum creatinine and urea level is indicative of renal function impairment in diabetic animals (Shinde and Goyal, 2003). Moreover, *in vitro* studies demonstrated that APE was significantly cytotoxic to proximal tubular cells despite promoting growth in distal cells (Figure 23). In contrast SBE, FTE and PAE did not affect electrolyte excretion and urine flow rates in long term studies in non-diabetic and STZ-induced diabetic rats. Although SBE and PAE reduced cell viability to renal cells in *in vitro* studies, they reduced plasma creatinine concentrations in *in vivo* studies (Table 5). This suggests that they may possess renoprotective effects.

Low urinary sodium and fluid excretion rates observed in STZ-induced diabetic animals in the present study is evidence of renal derangement 1 week after diabetes induction (Figures 20A and 21A). A number of studies have established that STZ-induced diabetes results in renal derangement leading to fluid and electrolyte retention

(Musabayane, Ndhlovu and Balment, 1995). Generally, STZ-induced diabetic animals exhibited pronounced antinatriuresis compared with non-diabetic animals. This was because in long term studies, increased diuresis results in reduced blood volume. This modulates sympathetic stimulation of renin and, subsequently, angiotensin II production whose effects are to promote sodium and fluid retention (Francois and Coffman, 2004). Since aldosterone mediates sodium retention and is produced in the presence of angiotensin II, its levels are expected to rise in diabetes mellitus. Moreover, hypovoleamia caused by excessive fluid excretion can induce secretion of vasopressin (Bunag, Tomita and Sasaki, 1982), to stimulate water retention. Indeed, studies have confirmed that vasopressin levels are elevated in diabetes mellitus (Ahloulay, Schmitt, Déchaux and Bankir, 1999).

Some test extracts demonstrated cytotoxic as well as renoprotective effects in the *in vivo* studies. For example while promoting cell death in LLCPK-1 cells (Figure 23B), APE demonstrated cell proliferation in MDBK cells (Figure 24A). The cytotoxic effects of APE on LLCPK-1 are supported by *in vivo* findings on plasma urea and creatinine after 6 weeks of chronic treatment, which showed an increase in plasma creatinine concentrations in non-diabetic rats (Table 5). Other than APE, the present findings show that all test extracts reduced plasma levels of both urea and creatinine compared with vehicle treated diabetic animals. This finding suggests the test extracts under investigation may be renoprotective.

It is known that weak acidic compounds tend to decrease mitochondrial activity by causing mitochondrial depolarization (Ignatius and Claes, 1996). Since, SBE is known to contain many acidic compounds (Braca *et al.*, 2003; Somova, Shode and Mipando,

2004), the decrease in cell viability observed in this study may be due to acidic compounds. In the *in vivo* situation, these acidic compounds may not be significant in renal function. Indeed, SBE treatment did not have any significant effect on renal fluid and electrolyte handling in non-diabetic and STZ-induced diabetic rats (Figures 18 and 20). Moreover, SBE decreased plasma creatinine concentrations in *in vivo* studies.

Our cell culture data clearly support this finding as MTT reduction is increased in the presence of FTE, especially at the higher concentration (Figure 25). Our data also confirm the lack of toxicity of FTE on renal cells derived from two species, *viz* porcine and bovine. The findings of the present study suggest that FTE is a useful agent in increasing glucose uptake by renal cells and glucose studies are needed to verify these. Our data indicate that FTE has potential to reduce plasma levels of creatinine in patients with diminished renal function and reduce cardiovascular complications and renal complications as well.

In vitro studies indicated reduced cell viability which is suggestive of cytotoxicity of test extracts to the cell lines. It is, therefore, interesting that although PAE reduced viability of both cell lines (Figure 26), results of *in vivo* studies demonstrated that PAE was not renotoxic as evidenced by reduced plasma concentrations of creatinine, in animals chronically treated with PAE (Table 5). Thus, we speculate that PAE may contain cytotoxic compounds which caused reduced cell viability *in vitro*, although these effects were inadequate to cause significant injury in *in vivo* investigations. Indeed, some extracts of *P americana* have been reported to contain bioactive compounds that are cytotoxic (Abe *et al.*, 2005). The cell culture provided an

experimental model to assess cytotoxicity and metabolic activities of test extracts on the two cell lines. Initially the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay was used as a measure of cell viability and proliferation, with the mitochondrial succinate reductase system being the major contributor to MTT reduction (Slater, Sawyer and Straeuli, 1963). However, recent evidence shows that most MTT reductions occur extra-mitochondrially with MTT salt crossing the intact plasma membrane to be reduced intracellulary (Bermas and Dobrucki, 2002). Other investigators have shown that most of the cellular reductions of MTT are dependant on microsomal enzymes and not only on succinate dehydrogenase (Berridge and Tan, 1993; Berridge, Tan, McCoy and Wang, 1996; Mann, Gerstein, Pogue, Bosch and Yusuf, 2001). This microsomal requires NADH and NADPH and is not affected by respiratory inhibitors (Berridge and Tan, 1993). This clearly indicates that cellular reduction of MTT is related more to the glycolytic rate, and thus NADH production, than to respiration, and is therefore primarily a measure of the rate of glycolytic NADH production (Bertuzzi, Bensi, Mayer, Niebylski, Armario and Gauna, 2003).

The present study has demonstrated that apart from APE, all the test extracts may have cardio-renal protective properties. It is noteworthy however, that the doses of test extract used in *in vitro* studies cannot easily be extrapolated in *in vivo* studies. Liang and Knox, (2000) advised caution when interpreting data from isolated tubular cell cultures because during *in vivo* studies tubular cells are exposed to complex neurohormonal microenvironments as well as various biophysical forces that are, to a large extent, absent in *in vitro* settings.

4.4. Antihypertensive effects

Figures 16 and 17 show that acute treatment with group A (APE and SBE) and B (FTE and PAE) test extracts reduced mean arterial blood pressure in anaesthetised non-diabetic and STZ-induced diabetic rats. There are many factors that could suppress elevation of blood pressure. Firstly, the presence of flavonoids in the test extracts may play a vasodepressive role by inhibiting effects of angiotensin II. Actis-Gorretta *et al.*, (2003), showed that flavonoids inhibited angiotensin I converting enzyme effects, thereby preventing formation of angiotensin I to angiotensin II. Interaction of Angiotensin II with its AT_1-receptors mediates vasoconstriction and inactivation of a vasodilator, bradykinin (Coppey *et al.*, 2006). Furthermore, flavonoids impair vascular smooth muscle cell growth which is necessary for atherogenesis (Moon *et al.*, 2003), and subsequently, hypertension and other cardiovascular diseases. Studies by Ajay, Achike, Mustafa and Mustafa, (2006), showed that quercetin, a flavonoid, highly prevalent in human diet improved vasorelaxation in isolated rat arterioles from animals with established diabetes. According to Ajay *et al.*, (2006), the vasorelaxant effects of the flavonoid were due to their antioxidant effects. Morello, Vellecco, Alfieri, Mascolo and Cicala, (2006), showed that galangin, a flavonoid demonstrated vasorelaxant properties on isolated rat thoracic artery whose effects were mediated via nitric oxide dependent and independent mechanisms. Indeed, antihypertensive effects of *H. hemerocallidea* have been attributed to reduction of peripheral resistance (Ojewole, Kamadyaapa and Musabayane, 2006). As aforementioned *P. americana* has been reported to mediate vasodilation via inhibition of calcium mobilisation through voltage dependent channels (Owolabi *et al.*, 2005).

As pointed out by Hall, (2003), the kidney function is particularly crucial in long term regulation of hypertension, hence the observed acute vasodepressive effects of test extracts suggest significant mediation via reduction of peripheral resistance, probably by vasodilation of the resistance vessels, in spite of acute sodium retention. Song *et al*, (2004), observed that administration of rosiglitazone, a thiazolidinedione, resulted in blood pressure decrease. However, this was accompanied with fluid and electrolyte retention. Since, antinatriuresis and antidiuresis are frequently associated with hypertension, Song, *et al.*, (2004), attributed vasodepressive effects of rosiglitazone, partly to vasodilation. The vasodepressive property of rosiglitazone was reported in earlier studies by Komers and Vrana, (1998).

P. americana has been reported to mediate vasodilation via inhibition of calcium mobilisation through voltage dependent channels (Owolabi *et al.*, 2005).

Long term antihypertensive effects of test extracts were significant in STZ-induced diabetic rats (Table 4). Since the test extracts demonstrated potential to lower blood glucose concentrations, the long term antihypertensive effects of extracts maybe secondary to long term hypoglycaemic effects of test extracts. Studies have shown that efficient control of blood glucose is associated with a reduction in mean arterial pressure (Richards, Donnelly, Nicholls, Ikram, Hamilton and Espiner, 1989).

Chronic hyperglycaemia evokes an increase in intracellular calcium concentration which increases vessel smooth muscle contractions and therefore, peripheral vascular resistance. Furthermore, increased glycaemic levels activate the sodium glucose co-transporters in the kidney tubules. These transporters mediate active glucose uptake

from blood while reabsorbing sodium (Sowers and Epstein 1995). An increase in blood sodium levels mediates water retention which results in increase in blood volume and consequently, hypertension. According to Coppey *et al.*, (2006), elevated glycaemia evokes increases in tissue angiotensin II, which mediates oxidative stress, endothelial insult, and disease pathology including vasoconstriction, thrombosis, inflammation, and vascular remodelling.

As in diabetes, reactive species generation is an important factor in the aetiology of hypertension (Vasdev *et al.*, 2002). Since presence of flavonoids, widely believed to possess antioxidant properties, are present in test extracts, long term control of blood pressure can be partly attributed to control of reactive species generation. Moreover Adeboye and colleagues, (1999), reported that *P. americana* leaf methanolic extract demonstrated hypotensive effects in Sprague-Dawley rats.

Hyperglycaemia stimulates increased tissue production of angiotensin II, which mediates endothelial damage and vasoconstriction (Coppey, Davidson, Rinehart, Gellet, Oltman, Lund, and Yorek, 2006). The increase in blood pressure in the untreated diabetic animals might be due to increase in peripheral vasoconstriction as a result of renin-induced angiotensin II production. Involvement of angiotensin II in hypertension in diabetes was supported by findings by Brands and Cloud, (2003), who stated that its increase early in diabetes is partly due to renin whose secretion is mediated by glucose through changes in nephron sodium chloride delivery and partly through osmotic induced diuresis. Indeed, STZ-induced diabetic rats exhibited a reduced urinary sodium excretion rate (Figures 20A and 21A) in comparison non-diabetic rats (Figures 18A and 19A). However, this was not reflected in plasma

concentrations of sodium and potassium (Table 5). Long term hypertension in STZ-induced diabetes observed in the present studies is consistent with findings by Hakim and Goya, 2000, Majithiya and Balaraman, 2006, Haidara *et al.*, 2004, and Ishihara *et al.*, 2000. Although animals exhibited severe diuresis and therefore, reduced blood volume, blood pressure increases have been observed even during diuretic conditions (Brands and Fitzgerald, 2001). However, hypertension in experimental STZ-diabetes is not a common occurrence (De Angelis *et al.* 2000; Maeda *et al.*, 1995; Fazan *et al.*, 1997). De Angelis and others, (2000) pointed out that the decline in blood pressure in STZ diabetes is attributed to reduced blood volume as a result of hyperglycaemia-induced diuresis while low heart rates are due to modifications in the functions of the sinoatrial node. Nonetheless, a number of factors favour development of hypertension in experimental STZ-diabetes. Firstly, hyperglycaemia induced - production of reactive species generation compromise bioavailability of nitric oxide, thereby depriving the vascular system of endogenous endothelium dependent relaxation. Secondly, hyperglycaemia mediates release of renin leading to subsequent production of angiotensin II which mediates vasoconstriction and therefore increases vascular resistance. Moreover, hypovoleamia caused by excessive fluid excretion can induce secretion of vasopressin (Bunag, Tomita and Sasaki, 1982), which is a potent vasoconstrictor.

CHAPTER 5

5.0. CONCLUSION

Hypoxis hemerocallidea (APE), *Sclerocarya birrea* (SBE), *Ficus thonningii* (FTE) and *Persea americana* (PAE) demonstrated hypoglycaemic effects substantiating their folkloric usage of these medicinal plants in the management and treatment of diabetes mellitus and its complications. The hypoglycaemic effects of the plant extracts are probably mediated via mechanisms that are independent of insulin release. The present study established hepatic conversion of blood glucose into glycogen as one of the mechanisms of hypoglycaemic effects.

Furthermore all test extracts exhibited vasodepressive effects. It is suggested that mechanisms of hypotensive effects of the group A (APE and SBE) and B (FTE and PAE) extracts are through reduction of peripheral resistance. This suggests that in addition to ameliorating hyperglycaemia, the extracts have potential to manage hypertension, a cardiovascular complication that frequently occurs as a result of diabetes mellitus. Although *Hypoxis hemerocallidea* was hypoglycaemic and hypotensive, it demonstrated toxic properties to the kidney function. However, *Sclerocarya birrea*, *Ficus thonningii* and *Persea americana* demonstrated cardio-renoprotective properties, in addition to hypoglycaemic effects.

5.1. Shortfalls of the present study

The present study had some limitations that created obstacles in unravelling the mechanisms of some observed biological effects of plant extracts. The present study did not monitor reactive oxygen species generation or levels in tissues, glycosylated haemoglobin, lipidaemia, enzymes of glucose homeostasis and glucosuric levels. Some plant extracts mediate their antihyperglycaemic effects via inhibition of renal glucose reabsorption in the kidney. Hence such mechanisms can only be established if urine glucose is monitored. Therefore, glucosuria should have been monitored, although monitoring of urine glucose levels is not usually recommended for glycaemic control. Renal hormones such as aldosterone and vasopressin should have been measured to determine the influence of test extracts on these. The present study did not assess presence of bioactive compounds in test extracts with known biological therapeutic effects e.g. flavonoids.

5.2. Recommendations for future studies

The recent study established that glycogen synthesis is the one of the mechanisms of the hypoglycaemic effects of the test extracts under investigation. Future studies should investigate mechanism(s) by which plants mediate hepatic glycogen storage. Hepatic glucokinase and other enzymes involved in glycogen synthesis should be measured to determine the effects of test extracts on these.

Further studies need to be carried out to isolate and investigate the individual active principles. Some medicinal plants are known to possess both hyper- and hypoglycaemic constituents. Therefore, isolation of the individual compounds would optimize the therapeutic effects of the plant.

Food intake is an important component in blood glucose control. Future studies need to be carried out to establish the cause of reduced food intake after administration of test extracts in the STZ-induced diabetic animals. Future studies should investigate effects of test extracts on ghrelin and AMPK.

Currently, studies are being undertaken to establish specific mechanisms of vasodepressive effects of test extracts.

CHAPTER 6

6.1 REFERENCES

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7.0. APPENDICES

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16 AUGUST 2005

MR. MM GONDWE (200200041)
HUMAN PHYSIOLOGY

Dear Mr. Gondwe

ETHICAL CLEARANCE NUMBER : HSS/05012A

I wish to confirm that ethical clearance has been granted for the following project:

"Hypoglycaemic effects of some medicinal plant extracts in streptozotocin-induced diabetic rats"

Yours faithfully

MS. PHUMELELE XIMBA
RESEARCH OFFICE

PS: The following general condition is applicable to all projects that have been granted ethical clearance:

THE RELEVANT AUTHORITIES SHOULD BE CONTACTED IN ORDER TO OBTAIN THE NECESSARY APPROVAL SHOULD THE RESEARCH INVOLVE UTILIZATION OF SPACE AND/OR FACILITIES AT OTHER INSTITUTIONS/ORGANISATIONS. WHERE QUESTIONNAIRES ARE USED IN THE PROJECT, THE RESEARCHER SHOULD ENSURE THAT THE QUESTIONNAIRE INCLUDES A SECTION AT THE END WHICH SHOULD BE COMPLETED BY THE PARTICIPANT (PRIOR TO THE COMPLETION OF THE QUESTIONNAIRE) INDICATING THAT HE/SHE WAS INFORMED OF THE NATURE AND PURPOSE OF THE PROJECT AND THAT THE INFORMATION GIVEN WILL BE KEPT CONFIDENTIAL.

cc. Faculty Officer
cc. Supervisor (Prof. CT Musabayane)
cc Co. Supervisors (Prof. J Ojewole and Prof. F Shode)

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LABORATORY STUDY

Effects of *Ficus thonningii* (Blume) [Morarceae] Stem-Bark Ethanolic Extract on Blood Glucose, Cardiovascular and Kidney Functions of Rats, and on Kidney Cell Lines of the Proximal (LLC-PK1) and Distal Tubules (MDBK)

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Previous observations indicate that *Ficus thonningii* (Blume) [Moraceae] stem-bark extracts may be useful in the control of diabetes mellitus. Accordingly, we investigated in some experimental animal paradigms the effects of *F. thonningii* stem-bark ethanolic extract (FTE) on renal and cardiovascular functions as complications of diabetes. Oral glucose tolerance tests were conducted in separate groups of non-diabetic and STZ-treated diabetic rats given glucose load (0.86 $g.kg^{-1}$, p.o.) after 18-h fast, followed by various FTE doses (60, 120, and 240 $mg.kg^{-1}$). Rats treated with deionized water (3 $mL.kg^{-1}$ p.o.), or metformin (500 $mg.kg^{-1}$ p.o.) acted as untreated and treated positive controls, respectively. Blood glucose was monitored at 15-min intervals for the first hour, and hourly thereafter for 3 h. Acute effects of FTE on kidney function and mean arterial blood pressure (MAP) were investigated in anaesthetized rats challenged with hypotonic saline after a 3.5-h equilibration for 4 h of 1 h control, 1.5 h treatment, and 1.5 h recovery periods. FTE was added to the infusate during the treatment period. Chronic effects of FTE were studied in individually caged rats treated daily with FTE (120 $mg.kg^{-1}$, p.o.) for five weeks. Cytotoxicity of FTE was assessed by dye-reduction colorimetric (MTT) assay on MDBK and LLCPK1 kidney cell lines exposed for 24 h, 48 h, and 72 h to graded concentrations of the extract. Myocardial contractile performance was evaluated on rat isolated atrial muscle strips. FTE, like metformin, decreased blood glucose levels in non-diabetic and STZ-diabetic rats. Both acute and chronic FTE treatments did not affect renal function. In vitro studies demonstrated that FTE increased MDBK cell metabolic activity by an average of 15% (72 h), and LLCPK1 mirrored the controls. Acute intravenous infusion of FTE reduced the MAP from 119 ± 1 mmHg to 98 ± 4 mmHg. The MAP also was reduced throughout the five-week experimental study period. FTE also produced concentration-dependent, negative inotropic and chronotropic effects on rat isolated, electrically driven left-, and spontaneously beating right-, atrial muscle preparations. Our experimental findings suggest that FTE possesses reno- and cardio-protective effects in diabetes mellitus.

Keywords *Ficus thonningii*, ethanolic extract, diabetes mellitus, renal and cardiovascular effects

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INTRODUCTION

We have previously reported that *Ficus thonningii* (Blume) [Moraceae] stem-bark ethanolic extract reduces blood glucose in non-diabetic and diabetic rats after five weeks exposure.[1] Because diabetes is often associated with impaired kidney function and cardiovascular disorders, appropriate goals in the control of diabetes mellitus using *F. thonningii* should include not only regulating blood glucose, but also the prevention or alleviation of these complications. Accordingly, the current study was designed to establish in some experimental animal paradigms the effects of *F. thonningii* bark ethanolic extracts

(FTE) not only on blood glucose, but also renal and cardiovascular functions. Thus, the main purpose of the present study was to investigate the effects of short-term (acute) and long-term (chronic) administration of FTE on renal fluid and electrolyte handling, as well as on blood pressure in male Wistar rats. Available evidence suggests that some herbal extracts may interfere with the concentrating and diluting mechanisms of tubular transport processes in the proximal tubule and distal tubule cells and/or on other protein components of tubular cell membranes.[2,3] Indeed, previous studies in our laboratories show that some crude plant extracts impair the renal handling of fluid and electrolytes.[4] Thus, we further speculated that FTE influences tubular reabsorption and secretion by altering tubular epithelial cells' viability. Therefore, the second objective was to assess the effects of FTE on cell viability on previously validated porcine proximal tubule and bovine distal tubule cell lines. Cell culture systems provide a good model for the evaluation of cytotoxicity of various compounds. We, therefore, employed the extensively used in vitro cell culture techniques of the proximal (LLC-PK1) and distal tubule (MDBK) cells[5–7] to study FTE-induced renal effects. The technique mimics the in vivo state, as these cell lines maintain similar biochemical function of levels of marker enzymes exhibited by freshly isolated cells.[8] We also assessed the effects of FTE on glomerular filtration rate (GFR), an indicator of renal tubular function.[9,10] Furthermore, the study investigated the influence of FTE on blood pressure, a parameter associated with the deterioration of kidney function in diabetic patients.[11] In an effort to shed more light on the plausible mechanism(s) through which FTE may affect blood pressure, we have also investigated the effects of FTE on myocardial contractility in vitro.

MATERIALS AND METHODS

Preparation of Plant Extract

Pieces of *Ficus thonningii* stem-barks were identified by Prof. H. Baijnath, the former Chief Taxonomist/Curator of the University of Durban-Westville's Department of Botany. A voucher specimen of the plant has been deposited in the University's Botany Departmental Herbarium. The stem-barks were air-dried at room temperature and milled into fine powder with a commercial blender. The powdered stem-bark was macerated in 95% ethanol for 24 h (with occasional shaking) and filtered. The filtrate was concentrated under reduced pressure in a rotary evaporator at 60 ± 1°C. The crude, powdery *Ficus thonningii* stem-bark ethanolic extract (FTE) was used throughout this study without further purification.

Animals

Male Wistar rats (250–300 g body weight) maintained under laboratory conditions of temperature, humidity, and 12 h light/12 h dark regime at the Biomedical Resource Unit, University of KwaZulu-Natal, were used. The rats were exposed to both food (Epol-diet 4700, Epol, South Africa) and water ad libitum. Ethical clearance was obtained for this study from the University of KwaZulu-Natal's Ethics committee.

Experimental Design

Oral glucose tolerance test (OGTT) studies were carried out in male, non-diabetic, and streptozotocin (STZ)-treated diabetic Wistar rats (250–300 g body weight). In vivo studies on renal function and blood pressure were carried out in non-diabetic and streptozotocin (STZ)-induced diabetic rats, while the in vitro effects of the plant's extract were conducted on kidney cell lines LLC-PK1 and MDBK. Effects of FTE on myocardial contractile performance were evaluated on rat isolated atrial muscle strips.

Induction of Diabetes Mellitus

Diabetes mellitus was induced in the diabetic group of rats by intraperitoneal injections of STZ (60 $mg.kg^{-1}$) in citrate buffer, pH 6.3. Vehicle (citrate buffer)-treated animals acted as controls. Animals that exhibited glucosuria after 24 h, tested by urine test strips (Rapidmed Diagnostics, Sandton, South Africa), were considered diabetic. Plasma glucose concentration of 25 $mmol.L^{-1}$ measured after one week was considered as a stable diabetic state before our experimental procedures.

Series 1: OGTT

The rats used were divided into the following groups for OGTT: non-diabetic control, treated non-diabetic, control STZ-treated diabetic, and treated STZ-diabetic rats ($n = 6$ in each group). Rats treated with deionized water served (3 $mL.kg^{-1}$, p.o.) served as control animals. All of the animals were starved for 18 h before being orally treated with glucose (0.86 $g.kg^{-1}$, body weight, p.o.), followed by FTE at various doses (60, 120, and 240 $mg.kg^{-1}$, p.o.). To establish whether FTE possesses pharmacological activities comparable to synthetic hypoglycemic drugs already in use, studies were conducted in separate groups of non-diabetic and STZ-diabetic rats orally treated with

metformin (500 mg.kg^{-1}, p.o.). Blood samples were collected from the tail veins of the animals at 15-min intervals for the first hour, and hourly thereafter for the subsequent 3 h for glucose measurements using Bayer's glucometer Elite® (Elite (Pty) Ltd, Health Care Division, South Africa).

Series 2: Renal Function

Acute Studies

Male Wistar rats were divided into groups of untreated control and treated rats (n = 6 in each group). Rats were anaesthetized by an intraperitoneal injection of Trapanal (sodium 5- ethyl-(1-methylbutyl)-2-thiobarbiturate, Byk Gulden, Konstanz, Germany) at a dose of 0.11 g.kg^{-1} and tracheotomized to maintain clear airway entry. The right jugular vein was cannulated with polyethylene tubing (i.d. 0.86 mm; o.d 1.27 mm, Portex, Hythe, Kent, UK) to allow intravenous infusion of 0.077 M NaCl. The urinary bladder of each rat was also cannulated with similar calibre polyethene tubing via an incision in the abdominal wall. The body temperature of each animal was maintained at 37 ± 1°C with a heated table.

The control group of animals (n = 6) was placed on a continuous infusion of 0.077 M NaCl at 9 mL·h^{-1} (Harvard syringe infusion Pump 22). Following an initial equilibration period of 3.5 h, eight consecutive urine collections were made into pre-weighed plastic vials at 30-min intervals over the subsequent 4 h for measurements of urine flow and Na^+ and K^+ excretion rates. The control group of rats was designed to check the stability of renal function.

Treated Group

Renal effects of the crude plant's extract (FTE) were studied in a group of rats following a 3.5 h equilibration period. FTE solution was prepared by using a modified method that has been previously described. [12,13] The extract was freshly dissolved in dimethyl sulfoxide (DMSO, 2 ml) and normal saline (19 ml) before use in each case. Urine samples were collected for 1 h (control period) for measurements of urine flow and Na^+ and K^+ excretion rates, following which the extract solution was infused at 0.06 µg·min^{-1} for 1.5 h (treatment period), resulting in a total dose of 18 g·kg^{-1} (for a 300-g rat). The animals were then switched back to the infusate alone for the last 1.5 h (recovery period).

Blood Pressure Measurements

Test groups of rats were surgically prepared as described for the renal studies, except that a heparinized cannula (Portex, i.d. 0.86 mm; o.d. 1.27 mm) was also inserted into the left common carotid artery to permit the recording of mean arterial blood pressure at 30-min intervals (Statham MLT 0380, Ad Instruments, compatible with the PowerLab System ML410/W, Australia).

Chronic Studies

Wistar rats (250–300 g body weight) were housed individually at the Biomedical Resource Unit, University of KwaZulu-Natal, in Makrolon polycarbonate metabolic cages (Techniplats, South Africa) that were cleaned daily. All animals were maintained on a 12 h dark/light cycle and allowed free access to water and food (Epol-diet 4700, Epol, South Africa). In those animals in which the effects of FTE were investigated, the rats were treated with FTE (120 mg.kg^{-1}, p.o.) daily for five weeks at 09h00. Control rats were similarly treated with distilled water (3 mL.kg^{-1}). Urine volume and total urinary outputs of Na^+ and K^+ were determined from 24 h samples for all groups.

Blood Pressure Measurements

Mean arterial blood pressure (MAP) was monitored every third consecutive day for five weeks at 09h00 using non-invasive tail cuff method with photoelectric sensors (IITC Model 31 Computerized Blood Pressure Monitor, Life Sciences, Woodland Hills, California, USA). The unit works with IITC hardware system to measure blood pressure and heart rate in conscious rats. The animals had been warmed in an enclosed chamber (IITC Model 303sc Animal Test Chamber, IITC Life Sciences, Woodland Hills, California, USA) for 30 min at ± 30°C before taking BP readings.

Terminal Studies

At the end of the five-week plants' extracts treatment period, blood glucose was measured from the tail veins of all groups of non-fasted animals using Bayer's glucometer Elite® (Elite (Pty) Ltd, Health Care Division, South Africa). Blood samples were also collected from all groups of animals by cardiac puncture into individual pre-cooled heparinized containers. Separated plasma was analyzed for Na^+, K^+, creatinine, and urea concentrations. Blood for insulin was collected into plain tubes, and the separated serum was stored in a Bio Ultra freezer (Mallkinckrodt, Ohio, USA) at –70°C until assayed.

Analytical Methods

Measurement of Electrolytes, Insulin, and Glomerular Filtration Rate

Urine volume was determined gravimetrically. Na^+ and K^+ concentrations were determined by ion activity using the Beckman Coulter (Synchron LX20 Clinical Systems, Fullerton, California, USA). Urea and creatinine analyses were performed using the Beckman Coulter instrument. Creatinine estimation employed the reaction of creatinine and sodium picrate to form creatinine picrate. Urea estimation employed the hydrolytic degradation of urea in the presence of urease. The methods used reagent kits from Beckman Coulter, Ireland, Inc., and measured using Beckman Coulter (Synchron LX20 Clinical Systems, Fullerton, California, USA). Glomerular filtration rate (GFR), as assessed by creatinine clearance, was calculated from measurements of urinary and plasma concentrations of creatinine and urine flow rate in the fifth week.

Plasma insulin concentrations were measured by Coat-A-Count procedure using a kit from Diagnostic Products Corporation, Los Angeles, USA. This is a solid-phase radioimmunoassay procedure based on insulin-specific antibody immobilized to the wall of a polypropylene tube. The lower limit of detection was 55 $pg.mL^{-1}$. Inter- and intra-assay coefficients of variation were 8.1% (n = 20) and 8.3% (n = 20), respectively.

Series 3: Cell Culture Studies

LLC-PK1 and MDBK cells were grown and maintained at 37°C in Eagle's Minimum Essential Medium (EMEM) (containing 0.1 mM Hepes buffer) supplemented with 5% heat inactivated foetal calf serum, 1% L-glutamine, and 1% penstrep-fungizone [complete culture medium (CCM)] (Delta Bioproducts, South Africa). Once the cells reached confluence, they were detached from the culture flask (75 cm^2) with 0.025% (w/v) trypsin and resuspended in CCM. Cell viability was determined in the presence of 0.2% (w/v) trypan blue in a haemocytometer. A 200-µl aliquot of the cell suspension (1.5×10^6 cells) was transferred into separate 96-well microtiter plates (Greiner Bio-one GmbH, Germany). Thereafter, the viability of cells incubated at 37°C for 24, 36, and 72 hours, containing various concentrations of FTE in separate wells, was assessed (0, 100, 200, 400, 600, 800, and 1000 µl mL^{-1}, n = 6 for each dilution). The wells were aspirated after each incubation and washed with Hank's balanced salt solution (HBSS). All supernatants were discarded. The cells were resuspended in 100 µl CCM containing 10 µl of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (i.e., 5$mg.ml^{-1}$ MTT salt in HBSS, Calbiochem, Darmstadt, Germany) and incubated for 4 h at 37°C. After 4 h, the plates were centrifuged (20 min, 2000 rpm at room temperature). The supernatant was removed, and any resulting formazan crystals were then solubilized with 100 µl dimethylsulphoxide (DMSO). After one hour, the optical density was determined spectrophotometrically using an ELISA plate reader (Bio-Tek Instruments) at 595 nm and a reference wavelength of 655 nm. Absorbance was expressed as percentage cleavage activity. Percentage cell viability was calculated as the mean absorbance of control cells/mean absorbance of treated cells.

Series 4: Isolated Atrial Muscle Strips

In order to throw some light on the plausible mechanism(s) by which FTE may influence blood pressure, we studied the effects of the extract on rat isolated atrial muscle strips. The Wistar rats used were sacrificed by stunning and exsanguination. The left and right atrial muscles of the animals were isolated and mounted as described by Ojewole.[14] The isolated left atrium of each rat was impaled on a thin platinum wire electrode and suspended under an applied resting tension of 1.0 g in a 30-ml Ugo Basile organ-bath containing Krebs-Henseleit physiological solution (of composition, in mmol/L NaCl, 118; KCl, 4.7; NaH_2PO_4, 1.28; $NaHCO_3$, 25.0; $MgCl_2$, 1.2; $CaCl_2$, 2.52; and glucose, 5.55, pH adjusted to 7.4) maintained at 34 ± 1°C and continuously aerated with carbogen (95% O_2 + 5% CO_2 gas mixture). Each left atrial muscle preparation was electrically driven with square wave pulses of 5 msec duration at a frequency of 3 Hz and supramaximal voltages of 5–10 volts, delivered by an SRI stimulator. The spontaneously beating right atrium of the animal was also set up under the same physiological, experimental conditions. Two isolated electrically driven left atrial muscle strips and two isolated spontaneously-beating right atrial muscle preparations, were always set up at a time (one used as the test and the other as the control) to allow for changes in the atrial muscle sensitivity. The atrial muscle preparations were left to equilibrate for 45–60 min (during which time the bathing physiological solution was changed every 15 min) before they were challenged with FTE or any of the reference drugs used. The test atrial muscle preparations were treated with sequentially applied, graded concentrations of the extract and/or reference drugs used, while the control atrial muscle strips were treated with volumes of distilled water equivalent to the volumes of bath-applied FTE (5–80 $g.mL^{-1}$). The electrically provoked and spontaneous contractions of the atrial muscles, as well as the FTE- (and reference drug)-induced

responses of the atrial muscle preparations, were recorded isometrically by means of Ugo Basile force-displacement transducers and pen-writing 'Gemini' recorders (model 7070).

DATA PRESENTATION

All data are expressed as means ±standard error of means (SEM). Cell viability was expressed as a percentage relative to control cells not exposed to any of the test compounds. Data obtained from test rat isolated atria treated with FTE alone, as well as those obtained from deionized water-treated control atrial strips, were pooled and compared with those of reference drugs. A statistical comparison of the differences between FTE and respective controls was performed with GraphPad InStat Software (version 3.00, GraphPad Software, San Diego, California, USA) using one-way analysis of variance (ANOVA; 95% confidence interval), followed by Tukey-Kramer multiple comparison test. A value of $p < 0.05$ was considered significant.

RESULTS

OGTT

Figure 1A compares the OGTT responses in acutely treated non-diabetic rats with respective control animals. Oral administration of various doses of FTE (60, 120, and 240 mg.kg^{-1}) decreased the blood glucose concentrations in a dose-dependent manner, with all doses exerting maximum effects after 60 min. The hypoglycemic effect of FTE was still significant by the end of the 4 h experimental period. A similar pattern of hypoglycemic effects was observed with metformin. Untreated control non-diabetic rats exhibited significantly high plasma glucose concentrations by comparison with treated animals. The plasma glucose concentrations of the control, non-diabetic rats increased to 6.8 ± 0.3 mmol.L^{-1} by 30 min from a baseline value of 4.2 ± 0.1 mmol.L^{-1} before slowly declining to 4.3 ± 0.1 mmol.L^{-1} (n = 6) after 4h, a value that was significantly elevated when compared to animals administered the highest dose of FTE at the corresponding period.

Similarly, oral administration of various doses of FTE (60, 120, and 240 mg.kg^{-1}) decreased blood glucose concentrations of STZ-treated diabetic rats in a dose-dependent manner by 45 min until the end of the 4 h experimental period (see Figure 1B). The glucose concentrations of STZ-treated diabetic control animals orally loaded with glucose did not significantly decline by the end of the 4-h experimental period. Metformin induced

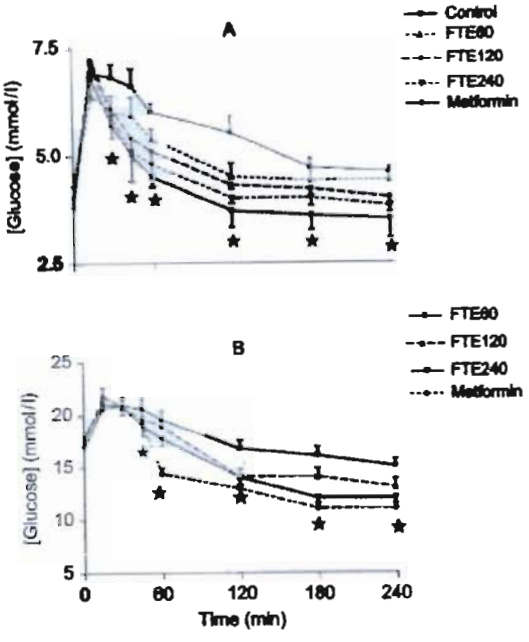

Figure 1. Comparison of OGTT responses in separate groups of (A) non-diabetic and (B) streptozotocin (STZ)-treated diabetic rats treated with graded doses of FTE with control animals treated with deionized water or positive controls treated with metformin. Values are presented as means, and vertical bars indicate SE of means (n = 6 in each groups). *$p < 0.01$ by comparison with control animals.

marked reductions in blood glucose concentrations by 45 min until the end of the 4-h experimental period.

In those animals in which FTE was chronically administered (120 mg.kg^{-1}, p.o.) daily for five weeks at 09h00, the mean plasma concentration of glucose was significantly decreased in non-diabetic and STZ-induced diabetic rats by the end of the experimental period in comparison with respective control animals at the corresponding time (see Table 1).

Renal Function Tests

Urine flow and Na^+ excretion rates ranged from 9 to 10 ml.h^{-1}and 619 to 660 µmol.h^{-1}, respectively, in vehicle-infused control animals during the 4 h post-equilibration period, values that compared with the infusion rate (9 mL.h^{-1} and 693 µmol.h^{-1}, respectively). K^+ excretion rate was also stable throughout the post-equilibration period, ranging from 226 to 256 µmol.h^{-1}. No significant changes in renal fluid flow and electrolyte excretion rates

Table 1
Plasma glucose, Na^+, K^+, urea and creatinine concentrations and GFR in non-diabetic and STZ-diabetic control and rats administered FTE every third consecutive day for five weeks (n = 6 in all groups)

Measure	Non-diabetic control	Non-diabetic FTE-treated	STZ-diabetic control	STZ-diabetic FTE-treated
Na^+ (mmol)	142 ± 2	143 ± 2	142 ± 1	143 ± 2
K^+ (mmol)	3.62 ± 0.34	3.21 ± 0.13	3.76 ± 0.21	3.10 ± 0.01
Urea (mmol)	15 ± 1	14 ± 2	33 ± 4	38 ± 5
Creatinine (μmol)	97 ± 4	85 ± 2*	102 ± 4	94 ± 7*
Glucose (mmol)	7.7 ± 0.2	5.5 ± 0.1*	36.6 ± 0.3	28.0 ± 0.5*
GFR ($ml.min^{-1}$)	2.64 ± 0.02	2.98 ± 0.03	0.7 ± 0.2	1.4 ± 0.3*

*$p < 0.01$ by comparison with respective control animals.

were observed in animals that were acutely treated with FTE. Compared with the control rats, the FTE-treated animals attained stable Na^+ excretion and urine flow rate, which approximated the infusion rates throughout the experimental period. Similarly, the mean weekly urine volume and the urinary Na^+ and K^+ outputs were not significantly different between animals chronically treated with FTE (120 $mg.kg^{-1}$, p.o.), and untreated rats. Urinary creatinine and urea outputs were not significantly different between the control and treated rats throughout the five-week experimental period. However, FTE treatment significantly reduced ($p < 0.01$) plasma creatinine concentration, unlike plasma urea concentration that was not altered (see Table 1). By the end of the five-week period, FTE administration significantly elevated ($p < 0.01$) the GFR value in STZ-treated diabetic rats, but the increase in non-diabetic rats did not achieve statistical significance.

Hemodynamic Studies

Acute infusion of hypotonic saline to control animals did not show any significant variations in the mean arterial blood pressure throughout the 4-h post-equilibration period. However, acute intravenous infusion of FTE at 120 $\mu g.h^{-1}$ for 1.5 h reduced the mean arterial blood pressure from a mean pre-treatment value of 119 ± 1 mmHg to 98 ± 4 mmHg (n = 6) by the end of treatment. The hypotensive effect of FTE persisted during the post-treatment period to a mean value of 110 ± 4 mmHg at the end of the experiment (see Figure 2A). The mean arterial blood pressure (MAP) changes due long-term (chronic) FTE treatments are shown in Figure 2B. Chronic treatment of the rats with FTE (120 $mg.kg^{-1}$ daily for five weeks, p.o.), caused significant decreases in MAP in non-diabetic and STZ-treated diabetic rats throughout the study period

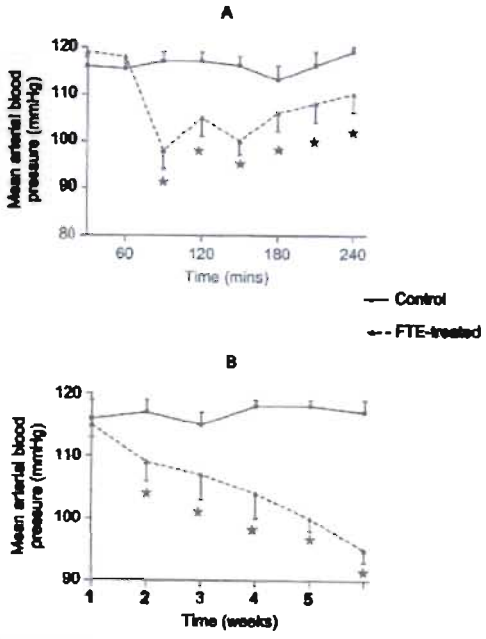

Figure 2. Effects of chronic FTE treatment on mean arterial blood pressures in (A) non-diabetic or (B) STZ-induced diabetic rats. Values are presented as means and vertical bars indicate SE of means (n = 6 in each group). *$p < 0.01$ by comparison with control animals.

in comparison with control rats at the corresponding period (Figure 2).

CELL CULTURE STUDIES

The MTT assay is a quantitative colorimetric method based on the reduced cleavage of the water soluble

monotetrazolium salt MTT to a purple formazan in metabolically active cells. The MTT salt is actively transported into metabolically viable cells. Both Figures 3A and 3B show the viability of LLC-PK1 and MDBK cells treated with various concentrations of FTE after 24, 48, and 72 hours. There was no toxicity noted in both cell lines after treatment with FTE. In Figure 3A, LLC-PK1 cell metabolism was increased with increased doses of FTE (600–1000 $\mu g.mL^{-1}$) for all incubation time periods. In contrast, the MDBK cells (Figure 3B) show increased metabolism for all concentrations of FTE. There was a significant increase in metabolism and cell viability after 72-h incubation, with significance being more pronounced at the highest concentrations (800 and 1000 $\mu g.mL^{-1}$).

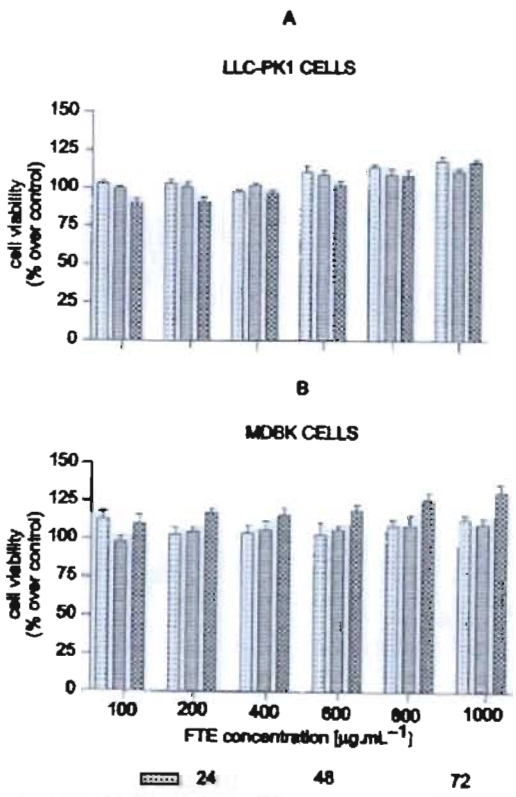

Figure 3. Viability of (A) LLC-PK1 and (B) MDBK cell lines treated with FTE. The cells were treated with 100, 200, 400, 600, 800, or 1000 µg FTE. Cell viability was determined by the Tryphan blue exclusion assay. Values for untreated control were taken as 100%. Each dose represents the mean of six treatments, while the vertical bars denote standard errors of the means.

Rat Isolated Atrial Muscle Strips

The cardiac effects of FTE are shown in Figure 4. This figure shows that FTE (5–80 $mg.mL^{-1}$) produced concentration-related, negative chronotropic (Figure 4A) and inotropic (Figure 4B) effects on rat isolated spontaneously beating right- and electrically driven left- atrial muscle strips, respectively. The cardio-depressant effects of FTE were not modified by bath-applied atropine (10^{-8}–10^{-6} M), suggesting that the cardio-inhibitory effects of the plant's extract are unlikely to be mediated through cholinergic mechanisms.

DISCUSSION

In the present study, we have evaluated the effects of short-term (acute) and long-term (chronic) oral treatments of *Ficus thonningii* stem-bark ethanolic extract (FTE) on cardiovascular systems and kidney functions of rats. The major findings of this study, apart from confirming our previous observations of the hypoglycaemic effects of oral administrations of the plant's extract,[1] show that FTE decreases blood pressure without significant influences on

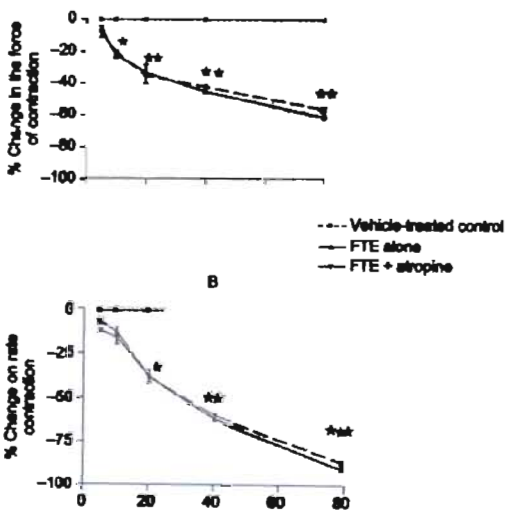

Figure 4. Effects of sequentially applied graded concentrations of FTE (5–80 $mg.mL^{-1}$) on the (A) rate and (B) force of contractions of rat isolated spontaneously beating right- and electrically driven left-atrial muscle strips, respectively. Each point represents the mean of 8–10 observations, while the vertical bars denote standard errors of the means. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus control.

renal function in normotensive rats. The management of diabetes mellitus without side effects is a global challenge, thus increasing the demand for natural products with antidiabetic activity.[15] Cardiovascular and renal complications are the major causes of mortality in diabetes mellitus.[16] We suggest that the use of FTE in the control of diabetes mellitus may be beneficial when hypertension and compromised renal function co-exist. This is significant considering the fact that diabetes mellitus is associated with cardiovascular complications and deterioration of kidney dysfunction in diabetic patients[17] and experimental animals.[18–20] Therefore, the management of diabetes with FTE has the potential to address both renal and cardiovascular protection. Conventionally, renoprotection is achieved through a reduction in blood pressure with antihypertensive regimens.[17, 21–23] Of note in the present study is the hypotensive effect of FTE without altering kidney function, in contrast to previous reports of impaired renal function following the administration of some hypoglycaemic plant extracts.[4,24] A significant increase in GFR as assessed by creatinine clearance and a concomitant decrease in plasma creatinine concentration was observed for the STZ-induced diabetic group treated with FTE over the five-week period. This finding is significant, given the fact that some antihypertensive agents (e.g., thiazide diuretics) and ß-blockers influence glycaemic control in a deleterious manner.[25] Evidence from biomedical literature suggests that some herbal extracts have protective effects against cardiovascular disease in diabetes.[26] We and several other authors have previously used creatinine clearance in rats to monitor GFR.[9,10,27,28] It is likely that chronic FTE treatment increased creatinine secretion as evidenced by increased MDBK cell metabolic activity. However, further studies are required to establish the mechanism(s) through which FTE reduces plasma creatinine levels. The cell culture studies provide an experimental model to assess cytotoxicity and metabolic activity of FTE in the two renal cell lines. Initially, the MTT assay was used as a measure of cell viability and proliferation, with the mitochondrial reduction by succinate reductase system being the major contributor to MTT reduction.[29] However, recent evidence shows that most MTT reduction occurs extra-mitochondrially with MTT salt crossing the intact plasma membranes to be reduced intracellularly.[30] Other investigators have shown that most of the cellular reductions of MTT are dependent on microsomal enzymes and not only on succinate dehydrogenase.[30–32] This microsomal reduction requires NADH and NADPH and is not affected by respiratory inhibitors.[31] This clearly indicates that cellular reduction of MTT is related more to the glycolytic rate, and thus NADH production, than to respiration, and is therefore primarily a measure of the rate of glycolytic NADH production.[32] Our cell culture data clearly support this finding, as MTT reduction is increased in the presence of FTE, especially at the higher concentrations. Our data also confirm the lack of toxicity of FTE on renal cells derived from two species, viz., pig and bovine. The findings of the present study suggest that FTE is a useful agent in increasing glucose uptake by renal cells and glucose uptake studies are needed to verify this phenomenon. Our data indicate that FTE has the potential to reduce plasma levels of creatinine in patients with diminished renal function and reduce cardiovascular and renal complications as well. The elevation of plasma creatinine is a risk factor for the development of cardiovascular[33] and end stage renal[34] diseases. FTE reduced mean arterial pressure in normotensive rats without significant effects on renal fluid and electrolyte handling, suggesting that the cardiovascular effects of FTE are mediated through influences on components of the cardiovascular system. We suggest that the cardioinhibitory effects of FTE may contribute, in part at least, to the hypotensive of the plant's extract. This hypothesis is supported by our findings from experiments on isolated guinea-pig atrial muscle strips which demonstrated significant negative chronotropic and inotropic, cardiodepressant effects of FTE. In conclusion, our experimental findings suggest that FTE possesses reno- and cardioprotective effects in diabetes mellitus.

ACKNOWLEDGMENT

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Cardiovascular Topics

Cardiovascular effects of *Persea americana* Mill (Lauraceae) (avocado) aqueous leaf extract in experimental animals

JAO OJEWOLE, DR KAMADYAAPA, MM GONDWE, K MOODLEY, CT MUSABAYANE

Summary

The cardiovascular effects of *Persea americana* Mill (Lauraceae) aqueous leaf extract (PAE) have been investigated in some experimental animal paradigms. The effects of PAE on myocardial contractile performance was evaluated on guinea pig isolated atrial muscle strips, while the vasodilatory effects of the plant extract were examined on isolated portal veins and thoracic aortic rings of healthy normal Wistar rats *in vitro*. The hypotensive (antihypertensive) effect of the plant extract was examined in healthy normotensive and hypertensive Dahl salt-sensitive rats *in vivo*.

***P americana* aqueous leaf extract (25–800 mg/ml) produced concentration-dependent, significant ($p < 0.05$–0.001), negative inotropic and negative chronotropic effects on guinea pig isolated electrically driven left and spontaneously beating right atrial muscle preparations, respectively. Moreover, PAE reduced or abolished, in a concentration-dependent manner, the positive inotropic and chronotropic responses of guinea pig isolated atrial muscle strips induced by noradrenaline (NA, 10^{-9}–10^{-5} M), and calcium (Ca^{2+}, 5–40 mM). PAE (50–800 mg/ml) also significantly reduced ($p < 0.05$–0.001) or abolished, in a concentration-dependent manner, the rhythmic, spontaneous, myogenic contractions of portal veins isolated from healthy normal Wistar rats. Like acetylcholine (ACh, 10^{-8}–10^{-4} M), the plant extract (25–800 mg/ml) produced concentration-related relaxations of isolated endothelium-containing thoracic aortic rings pre-contracted with noradrenaline. The vasorelaxant effects of PAE in the isolated, endothelium-intact aortic rings were markedly inhibited or annulled by N^{ω}-nitro-L-arginine methyl ester (L-NAME, 10^{-4} M), a nitric oxide synthase inhibitor. Furthermore, PAE (25–400 mg/kg iv) caused dose-related, transient but significant reductions ($p < 0.05$–0.001) in the systemic arterial blood pressure and heart rates of the anaesthetised normotensive and hypertensive rats used.**

The results of this laboratory animal study indicate that PAE caused bradycardia, vasorelaxation and hypotension in the mammalian experimental models used. The vasorelaxant action of PAE was endothelium dependent, and was, therefore, possibly dependent on the synthesis and release of nitric oxide (NO). The vasorelaxant effects of PAE appeared to contribute significantly to the hypotensive (antihypertensive) effects of the plant extract. However, the findings of this study tend to suggest that *P americana* leaf could be used as a natural supplementary remedy in essential hypertension and certain cases of cardiac dysfunctions in some rural Africa communities.

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In our current pharmaco-chemical exploration of African medicinal plants, we have examined in our laboratories some of the frequently used South African medicinal plants for their chemical constituents and pharmacological actions,[1-10] in an attempt to establish a scientific basis for their folkloric, ethnomedical uses. One of such commonly used African medicinal plants is *Persea americana* Mill (family: Lauraceae).

P americana, otherwise known as the avocado pear, Mexican avocado and so on, is a medium-sized, single-stemmed, terrestrial, erect, perennial, deciduous tree 15–20 m in height. Although a native of Central America (Mexico),

P americana is now found in most tropical and subtropical countries of the world. The branches are fissured and grey, but the twigs are green and smooth. The 15–25-cm long and 10–20-cm broad leaves with well-developed petioles are spirally arranged, often clustered near the branch ends, narrowly to broadly elliptical or obovate, and are usually pointed at the tip.[11]

The greenish-yellow flowers are borne on branched, compact panicles, which are shorter than the leaves. The often pear-shaped, one-seeded fruits are variable in size and shape according to the variety, up to 18 cm long and usually shiny and green, or brownish when ripe. The flesh is soft, oily, greenish or yellow surrounding one large, loose round seed.[11] The avocado is now cultivated commercially as a fruit crop in many countries of the world. In many parts of Africa, the fruits of the avocado are much sought after by humans and some animals as valuable foodstuff. Besides the oil, avocado fruit pulp contains carbohydrates and more protein than many other fruits, while its contents of vitamins A and B are high.[11,12]

In addition to the nutritional value of its fruit, the leaves and other morphological parts of *P americana* possess medicinal properties and are widely used in traditional medicines of many African countries. For example, the fruit pulp is eaten as an aphrodisiac and as an emmenagogue in South Africa,[12] while a hot-water extract of the leaves is taken orally as a diuretic and for hypertension in many West African countries.[11] In some other parts of the world, various morphological parts of *P americana* have been employed for a wide range of human ailments. Products of the plant have been effectively used for the management, control and/or treatment of amenorrhoea, anaemia, insomnia, hyperlipidaemia, hypertension, diabetes mellitus, diarrhoea, dysentery, gastritis, peptic ulcers, bronchitis, cough, hepatitis, and so forth.[11,12]

Previous studies on the avocado have shown that leaf extracts of *P americana* possess a catalogue of pharmacological activities, including analgesic, anti-inflammatory, anti-diabetic, hypoglycaemic, hypotensive and antihypertensive properties.[13-16] The present study was prompted by the claim of some traditional health practitioners in KwaZulu-Natal that decoctions and infusions of avocado leaves are effective remedies for the management and/or control of hypertension and certain cardiac disorders.

The aim of the present study was, therefore, to investigate the cardiac, vascular and antihypertensive (hypotensive) effects of *P americana* aqueous leaf extract in experimental animal paradigms, with a view to providing a pharmacological justification (or otherwise) for the ethnomedical uses of the plant leaf in the management, control and/or treatment of essential hypertension and certain cardiac dysfunctions in some rural African communities.

Materials and methods

The experimental protocol used in this study was approved by the ethics committee of the University of Durban-Westville and conforms to the *Guide to the Care and Use of Animals in Research and Teaching*.[17]

Plant material and preparation

Fresh leaves of *P americana* were collected from a playground behind Willowpark Centre along Umbilo Road in Durban, between January and June 2003. The leaves were identified by Prof H Baijnath, the former chief taxonomist/curator of the Department of Botany, University of Durban-Westville, as those of *P americana* Mill (family: Lauraceae). A voucher specimen of the plant has been deposited in the Botany Departmental Herbarium.

Room air-dried leaves (1 kg) of *P americana* were milled in a Waring commercial blender. The powdered leaf was macerated in distilled water and extracted twice, on each occasion with 2.5 l of distilled water at room temperature for 48 hours, with occasional shaking. The combined distilled water extracts were concentrated to dryness at 60 ± 1°C in a rotary evaporator. Freeze drying and solvent elimination under reduced pressure finally gave 21.50 g (2.15% yield) of a light-brown, powdery aqueous leaf extract. This crude extract was used in our study without further purification. Aliquot portions of the residue from the aqueous extract were weighed and dissolved in distilled water for use on each day of our experiments.

Animal material

Healthy male Dunkin-Hartley guinea pigs (*Cavia porcellus*) weighing 300–450 g, and healthy young adult male Wistar rats (*Rattus norvegicus*) weighing 250–300 g were used. The animals were kept under laboratory conditions of temperature, humidity and light and were allowed free access to food (standard pellet diet) and water *ad libitum*. All the animals were fasted for 16 hours, but allowed free access to water before the commencement of our experiments. Guinea pig isolated atrial muscles were used for the *in vitro* evaluation of the effects of the aqueous extract on myocardial contractility, whereas rat isolated portal veins and thoracic aortic rings were used to examine the vasorelaxant effects of the extract. Normotensive (normal) Wistar, and hypertensive Dahl salt-sensitive rats were used for the *in vivo* investigation of the hypotensive (antihypertensive) effect of the aqueous extract.

Isolated muscle experiments

Guinea pig muscle strips

The guinea pigs were sacrificed by stunning and exsanguination. The left and right atrial muscles of the animals were isolated and mounted as previously described by Ojewole.[18]

The isolated left atrium of each guinea pig was impaled on a thin platinum wire electrode and suspended under an applied resting tension of 1.0 g in a 30-ml Ugo Basile organ-bath containing Krebs-Henseleit physiological solution (composition in mmol/l, pH adjusted to 7.4: NaCl, 118; KCl, 4.7; NaH_2PO_4, 1.28; $NaHCO_3$, 25.0; $MgCl_2$, 1.2; $CaCl_2$, 2.52; glucose, 5.55) maintained at 34 ± 1°C and continuously aerated with carbogen (95% O_2 + 5% CO_2 gas mixture). Each left atrial muscle preparation was electrically driven with square wave pulses of 5-ms duration at a frequency of 3 Hz and a supramaximal voltage of 5–10 V, delivered by

an SR1 stimulator. The spontaneously beating right atrium of the animal was also set up under the same physiological experimental conditions and allowed to beat spontaneously. Two isolated electrically driven left atrial muscle strips and two isolated spontaneously beating right atrial muscle preparations were always set up at a time (one as the test, and the other as the control) to allow for changes in the atrial muscle sensitivity.

The atrial muscle preparations were left to equilibrate for 45–60 min (during which time the physiological bath solution was changed every 15 min) before they were challenged with PAE or any of the reference drugs used. The test atrial muscle preparations were treated with sequentially applied graded concentrations of PAE and/or reference agonist drugs used, whereas the control atrial muscle strips were treated with volumes of distilled water (0.1–0.6 ml) equivalent to the volumes of bath-applied PAE solution used. The electrically provoked and spontaneous contractions of the atrial muscles, as well as the PAE- and reference agonist drug-induced responses of the atrial muscle preparations were recorded isometrically by means of Ugo Basile force-displacement transducers and pen-writing Gemini recorders (model 7070).

Rat portal veins

The rats were sacrificed by stunning and exsanguination. The abdomen of each rat was quickly opened by midline incision, and the intestines were pulled aside. The portal vein of each rat, with an *in situ* length of approximately 2 cm, was cleaned of extraneous connective and fatty tissues and then removed from the animal. Each isolated portal vein was suspended under an applied resting tension of 0.5 g in a 30-ml Ugo Basile organ bath containing Krebs-Henseleit physiological solution. Two isolated venous tissue preparations (one control and the other PAE- or reference drug-treated test) were always set up in order to make allowances for changes in the venous tissue sensitivity. Control venous muscle strips were treated with distilled water only (the vehicle in which PAE and reference drugs were dissolved). The venous tissue preparations were allowed to equilibrate for 45–60 min (during which time the physiological bath solution was changed every 15 min) before they were challenged with PAE or any of the reference drugs used. The plant extract- (25–800 mg/ml) and reference drug-induced responses of the venous smooth muscle preparations were recorded isometrically by means of Ugo Basile force-displacement transducers and pen-writing Gemini recorders (model 7070).

Rat thoracic aorta rings

The rats were sacrificed by decapitation. The descending thoracic aorta of each normotensive rat was quickly and carefully excised and placed in a Petri dish filled with ice-cold Krebs-Henseleit physiological solution. The aorta was cleaned of extraneous fat and connective tissues and cut into rings approximately 3–4 mm in width. All dissecting procedures were carefully done to protect the functional endothelium from inadvertent damage. In some aortic rings, the endothelial layer was mechanically removed by gently rubbing the luminal surface three times with distilled water-moistened cotton wool, followed by six times with a small, plastic tubing. A pair of rat isolated aortic rings, one with intact functional endothelium, and the other one with endothelium denuded, were always set up in parallel for appropriate comparison.

Each of the isolated endothelium-containing and endothelium-denuded aortic rings was suspended under an applied resting tension of 1.0 g in a 30-ml Ugo Basile organ-bath containing Krebs-Henseleit physiological solution maintained at 36 ± 1°C and continuously aerated with carbogen (95% O_2 + 5% CO_2). The aortic tissue preparations were left to equilibrate for 45–60 min (during which time the physiological bath solution was changed every 15 min) before they were challenged with graded concentrations of PAE or any of the reference drugs used. At the end of the equilibration period, the aortic ring preparations were initially contracted with bath-applied noradrenaline (10^{-5} M).

Endothelial integrity and successful removal of the functional endothelium was assessed by the presence or absence, respectively, of relaxant response to acetylcholine (10^{-5} M). ACh-induced relaxation ≤ 5% was taken as satisfactory removal of the functional endothelial layer. Such endothelium-denuded aortic muscle preparations were used in this study. After the subsequent wash-out and equilibration period of 30 min, cumulative dose-response curves were obtained with noradrenaline in aortic rings with and without endothelium.

Subsequently, 20-min pretreatment of the aortic muscle preparations with graded concentrations of the plant extract (25–800 mg/ml) was carried out before the next cumulative additions of noradrenaline (10^{-10}–10^{-5} M) to the bath fluid. After the addition of each NA concentration, a plateau response was obtained before the addition of the next higher dose in all cases of cumulatively applied noradrenaline concentrations. Consecutive dose-response curves were taken at 30-min intervals, during which time the physiological bath solution was changed three to five times until the tension developed returned to basal level.

Following 20-min incubation of the aortic ring preparations with the plant extract (25–800 mg/ml), the arterial relaxant effect of PAE was examined on endothelium-containing and endothelium-denuded aortic ring preparations pre-contracted with sequentially applied or cumulatively administered noradrenaline (10^{-10}–10^{-5} M). The effect of the vehicle in which PAE and the reference drugs used were dissolved (distilled water), was also tested. After each challenge, the aortic rings were washed three to five times with fresh physiological solution and allowed to equilibrate for 30 min before they were challenged again with any of the reference drugs or PAE. The contractile and/or relaxant effects of all the reference drugs, as well as PAE-induced relaxations of the isolated aortic ring preparations were recorded isometrically by means of Ugo Basile force-displacement transducers and pen-writing Gemini recorders (model 7070).

Whole-animal experiments

Normotensive Wistar and hypertensive Dahl salt-sensitive rats weighing 250–300 g were used. Before the commence-

ment of our experiments, the salt-sensitive rats were placed on 4% saline water and normal food (standard pellet diet) for six to eight weeks (during which time the arterial blood pressure of the animals rose to between 170/130 and 190/140 mmHg). Salt-sensitive rats with arterial blood pressure ≥ 170/120 mmHg were considered to be hypertensive and used in this study.

Each of the normotensive and hypertensive rats was anaesthetised with intraperitoneal injection of 0.11 g/kg of Trapanal® [sodium 5-ethyl-(1-methylbutyl)-2-thiobarbiturate]. The right femoral vein was cannulated with a small polythene cannula for the administration of the plant extract and reference drugs. In order to minimise blood coagulation, heparin (500 units/kg) was intravenously administered to the animal, and flushed in with 0.2 ml of 0.9% w/v sodium chloride solution. The left carotid artery of each rat was also cannulated and connected to a four-channel Grass polygraph for systemic arterial blood pressure recording. The trachea of each rat was cannulated for artificial respiration, but the animal was allowed to breathe spontaneously. The rat's body temperature was maintained at 36 ± 1°C with an incandescent lamp placed over the abdomen.

After 20 min stabilisation period, systemic arterial blood pressure (systolic, diastolic and mean arterial pressures) and heart rate of each rat were measured and recorded. The effects of PAE and the reference drugs [acetylcholine (0.5–4.0 µg/kg iv) and noradrenaline (0.5–4.0 µg/kg iv)] on systemic arterial blood pressure and heart rates (calculated from the ECG limb lead II recording at a fast paper speed of 25 mm/sec) were recorded by means of a four-channel Grass polygraph recorder (model 79D). In some of the rats, the hypotensive (depressor) effect of PAE (25–400 mg/kg iv) was examined after atropinisation [pretreatment of the rats with atropine sulphate (1.5 mg/kg ip) 18–24 hours before use]. Because PAE and other drugs used in this study were dissolved in distilled water, rats treated with distilled water (2 ml/kg iv) alone were used as control animals under the same experimental conditions.

Compounds and drugs used

The following compounds and drugs were used: *P americana* aqueous leaf extract, acetylcholine chloride (Sigma, England); (-)-noradrenaline hydrochloride (Sigma, England); atropine sulphate (Sigma, England); N$^{\omega}$-nitro-L-arginine methyl ester (L-NAME) (Sigma, England); Trapanal® [sodium 5-ethyl-(1-methylbutyl)-2-thiobarbiturate] (Byk Gulden, Konstanz, Germany); (±)-propranolol hydrochloride (Sigma, England); calcium chloride and potassium chloride (Sigma, England). The drugs were dissolved in distilled water each day at the beginning of our experiments. Drug concentrations and doses quoted in the text refer to the salts, except PAE, and denote final organ bath concentrations in the *in vitro* experiments.

Data analysis

Data obtained from test guinea pig isolated atria, rat isolated portal vein, aortic ring strips, and anaesthetised normotensive and hypertensive rats treated with PAE alone, as well as those obtained from distilled water-treated control isolated atria, portal veins, aortic rings and anaesthetised rats, were pooled and expressed as means (± SEM). Statistical comparison of the differences between PAE- and reference drug-treated test means, and distilled water-treated control means, was performed with GraphPad InStat Software (version 3.00, GraphPad Software, San Diego, California, USA) using one-way analysis of variance (ANOVA; 95% confidence interval), followed by Tukey-Kramer multiple-comparison tests. Values of $p \leq 0.05$ were taken to imply statistical significance.

Results

Isolated muscle experiments

Guinea pig muscle preparations

Sequential administrations to the bath fluid of relatively low to high concentrations of PAE (25–800 mg/ml) significantly reduced ($p < 0.05$–0.001) or abolished the force of contractions of guinea pig isolated electrically driven left atrial muscle preparations in a concentration-related manner (Fig. 1). The negative inotropic effect of PAE on these muscle strips was not affected by prior exogenous administration of atropine to the bath fluid.

At the same concentration range, the plant extract also significantly reduced ($p < 0.05$–0.001) or abolished the rate of contractions of guinea pig isolated spontaneously beating right atrial muscle preparations in a concentration-dependent manner (Fig. 2). However, the negative chronotropic effect of PAE on these muscle strips was not antagonised by atropine which reduced or abolished the negative chronotropic effect of acetylcholine on six other spontaneously beating right

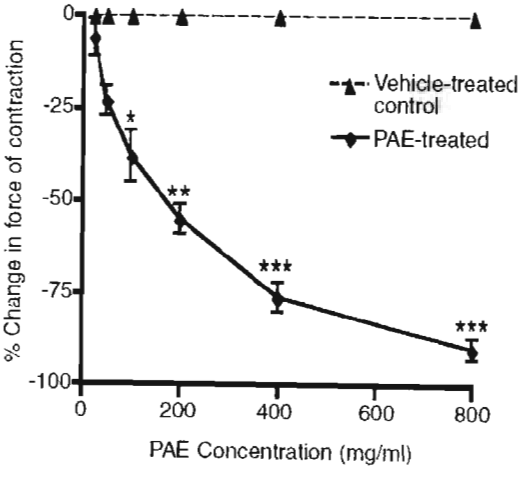

Fig. 1. Effects of graded concentrations of PAE (25–800 mg/ml) on guinea pig isolated electrically driven left atrial muscle strips. Vehicle (distilled water)-treated control preparations received the same volume of PAE solution only. Each point represents the mean of eight observations, while the vertical bars denote standard errors of the means. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ vs vehicle-treated control.

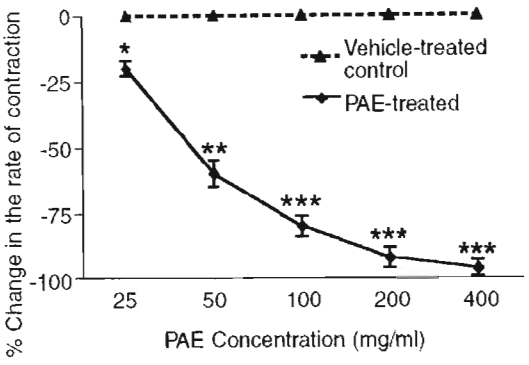

Fig. 2. Effects of graded concentrations of PAE (25–400 mg/ml) on guinea pig isolated spontaneously beating right atrial muscle strips. Vehicle (distilled water)-treated control preparations received the same volume of PAE solution only. Each point represents the mean of eight observations, while the vertical bars denote standard errors of the means. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ vs vehicle-treated control.

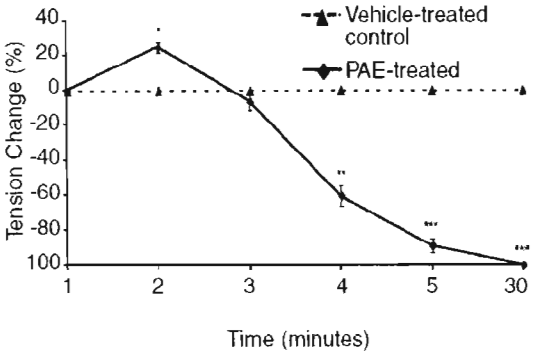

Fig. 3. Effects of PAE (800 mg/ml) on rhythmic myogenic spontaneous contractions of rat isolated portal veins. Vehicle (distilled water)-treated control preparations received the same volume of PAE solution only. Each point represents the mean of eight to 10 preparations, while the vertical bars denote standard errors of the means. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ vs vehicle-treated control.

atrial muscle preparations examined. PAE significantly reduced ($p < 0.05$–0.001) or abolished, like propranolol, the positive inotropic and chronotropic effects of noradrenaline on all eight other isolated atrial muscle strips tested. The plant extract also significantly ($p < 0.05$–0.001) inhibited or abolished calcium-induced positive inotropic and chronotropic responses on all other nine atrial muscle strips examined.

Rat portal veins

Sequential administrations to the bath fluid of relatively low to high concentrations of PAE always induced concentration-dependent, biphasic effects on the amplitude and frequency of the rhythmic myogenic contractions of the rat isolated portal veins. The biphasic effects produced by PAE always consisted of an initial slight but significant ($p < 0.05$) contraction (stimulation) of short duration, followed by a secondary, longer-lasting and significant ($p < 0.05$–0.001) relaxation (inhibition) of the venous muscle preparations (Fig. 3). At the same concentration range, the plant extract also inhibited or abolished in a concentration-dependent manner, contractions of the venous muscle preparations induced by noradrenaline or potassium.

Rat aortic ring strips

Cumulative additions of graded concentrations of noradrenaline to the bath fluid provoked concentration-dependent contractions of both endothelium-containing and endothelium-denuded normotensive rat isolated aortic ring strips, with a maximum of 3.76 ± 0.30 g tension developed. Acetylcholine provoked concentration-related, significant relaxations ($p < 0.05$–0.001) of endothelium-containing aortic ring preparations pre-contracted with bath-applied noradrenaline, but did not significantly relax ($p > 0.05$) endothelium-denuded aortic ring preparations pre-contracted with bath-applied noradrenaline.

Like acetylcholine, PAE produced concentration-dependent, significant relaxations ($p < 0.05$–0.001) of the endothelium-containing aortic ring preparations pre-contracted with noradrenaline (Fig. 4), but did not relax endothelium-denuded aortic ring preparations pre-contracted with bath-applied noradrenaline. Moreover, the plant extract shifted cumulatively administered noradrenaline concentration-response curves to the right in a non-parallel and non-competitive fashion, and suppressed NA-induced maximal contractions of endothelium-containing aortic ring muscle preparations. Ten minutes' prior incubation of the endothelium-intact aortic ring tissues with L-NAME, a nitric oxide synthase inhibitor, inhibited or abolished PAE- or ACh-induced

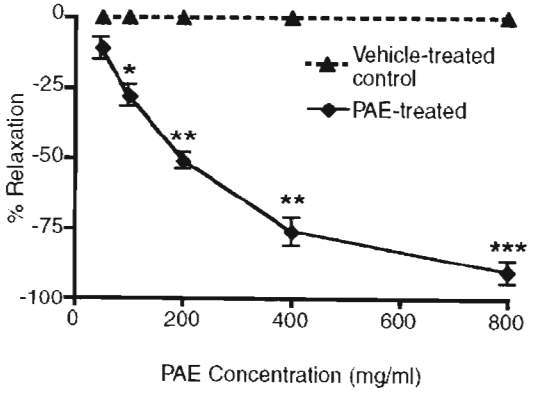

Fig. 4. Arterial relaxant effects of graded concentrations of PAE (50–800 mg/ml) on noradrenaline (10^{-5} M)-induced contractile responses of endothelium-intact aortic rings from normal rats. Vehicle (distilled water)-treated control preparations received the same volume of PAE solution only. Each point represents the mean of eight observations, while the vertical bars denote standard errors of the means. *$p < 0.05$; **$p < 0.01$, ***$p < 0.001$ vs vehicle-treated control.

TABLE 1. EFFECTS OF PAE ON SYSTEMIC ARTERIAL BLOOD PRESSURE AND HEART RATES OF NORMOTENSIVE RATS. EACH VALUE REPRESENTS THE MEAN (± SEM) OF OBSERVATIONS FROM EIGHT RATS

Cardiovascular parameter	*Before treatment: control values*	*After treatment: PAE (25–400 mg/kg iv)*				
		25	*50*	*100*	*200*	*400*
Systolic BP (mm Hg)	124.5 ± 4.6	112.5 ± 4.4	91.6 ± 4.6*	73.4 ± 4.1**	58.5 ± 4.0***	42.6 ± 3.4***
Mean BP (mm Hg)	111.4 ± 4.1	98.8 ± 4.7	84.3 ± 4.8*	66.5 ± 4.0**	51.4 ± 4.1***	38.4 ± 3.1***
Diastolic (mm Hg)	94.3 ± 4.0	80.4 ± 4.2	68.5 ± 4.0*	56.4 ± 4.3**	44.3 ± 4.3***	31.4 ± 3.0***
Heart rate (beats/min)	396.6 ± 18.6	364.8 ± 18.2	332.3 ± 15.4*	302.8 ± 14.4**	283.5 ± 12.3***	242.7 ± 11.5***

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ vs control

TABLE 2. EFFECTS OF PAE ON SYSTEMIC ARTERIAL BLOOD PRESSURE AND HEART RATES OF HYPERTENSIVE RATS. EACH VALUE REPRESENTS THE MEAN (± SEM) OF OBSERVATIONS FROM EIGHT RATS

Cardiovascular parameter	*Before treatment: control values*	*After treatment: PAE (25–400 mg/kg iv)*				
		25	*50*	*100*	*200*	*400*
Systolic BP (mmHg)	188.2 ± 6.4	173.6 ± 6.6	156.4 ± 6.3*	140.6 ± 6.0**	124.4 ± 4.8***	101.3 ± 4.8***
Mean BP (mmHg)	146.8 ± 6.1	132.4 ± 6.4	120.6 ± 5.8*	106.4 ± 5.2**	92.5 ± 4.0***	76.7 ± 4.4***
Diastolic (mmHg)	120.4 ± 6.3	106.4 ± 4.0	91.3 ± 5.1*	78.5 ± 4.4**	64.3 ± 4.2***	50.5 ± 4.0***
Heart rate (beats min)	424.6 ± 20.4	391.4 ± 18.6	356.5 ± 16.0*	318.2 ± 15.2**	286.4 ± 14.6***	236.4 ± 12.5***

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ vs control.

relaxations of the endothelium-containing aortic rings pre-contracted with noradrenaline. Ten minutes' prior incubation of the aortic ring tissues with atropine sulphate also inhibited or abolished acetylcholine-induced relaxations of the endothelium-containing aortic ring preparations pre-contracted with noradrenaline.

Whole animal experiments

Acute intravenous administrations of PAE into anaesthetised normotensive and hypertensive rats produced transient, dose-related, significant reductions ($p < 0.05$–0.001) in the systemic arterial blood pressure and heart rates of the rats (Tables 1, 2). The transient hypotensive (antihypertensive) effect of the plant extract persisted for 12–85 min, depending on the PAE dose administered. Furthermore, the plant extract dose-dependently inhibited or abolished the pressor effects of noradrenaline on systemic arterial blood pressure and heart rates of the animals. Pre-treatment of the normotensive and hypertensive rats with atropine sulphate abolished or markedly reduced the depressor effects of acetylcholine on systemic arterial blood pressure and heart rates of the animals. However, the depressor effects of PAE on blood pressure and heart rates were not affected by pre-treatment with atropine sulphate.

Discussion

The results of this study indicate that the aqueous leaf extract of *P. americana* possessed cardiodepressant, vasorelaxant and hypotensive (antihypertensive) effects in the experimental animal paradigms used. These observations are in agreement with the findings of some of the earlier investigators who have reported vasorelaxant[16] and hypotensive[15] effects of the leaf extract in experimental animal models.

Furchgott and Zawadzki[19] first described the involvement of the endothelium-derived relaxing factor (EDRF), which was subsequently determined to be nitric oxide or NO derivatives synthesised from guanidine groups of L-arginine.[20,21] Endothelium-dependent relaxation, which has been demonstrated in many vascular preparations, including some veins, arteries and microvascular vessels, occurs in response to stimulation by a variety of substances, such as acetylcholine, adenine nucleotides,[22] thrombin, substance P,[23] calcium ionophore A23187, bradykinin and histamine.[24] The vasodilatation effects of endothelium-dependent substances can be inhibited by several L-arginine analogues, such as N-monomethyl-L-arginine (L-NMMA) and N$^{\omega}$-nitro-L-arginine methyl ester (L-NAME).[20,25-27]

Endothelial nitric oxide plays a vital role in the control of vasomotor tone and structure.[22,28] On the other hand, vascular tone plays an important role in the regulation of arterial blood pressure. The development and maintenance of hypertension has been suggested to involve a reduced endothelium-dependent vasodilator influence on the vascular tissue.[28] Impairment of endothelium-dependent vascular relaxation in human and experimental hypertension has been observed by Luscher and Vanhoutte,[22] and the ability of nitric oxide to maintain vascular tone has been shown to be deficient in this condition.[29,23] Because NO is a potent vasodilator, a deficient production and/or release of endothelium-derived NO will result in diminished vasodilator tone, thus allowing vascular resistance to rise, and this, in turn, will lead to elevated blood pressure.[22,28]

Relaxation of vascular smooth muscle by NO involves a series of steps. Nitric oxide is formed in functional endothelium by the activation of nitric oxide synthase (NOS), which uses L-arginine as a substrate. Once formed, NO diffuses out of the endothelium, with some entering the underlying vascular smooth muscle where it binds to and activates soluble guanylate cyclase.[28] This enzyme catalyses the conver-

sion of guanidine triphosphate (GTP) to cyclic guanidine monphosphate (cGMP), which in turn, causes relaxation of the vascular smooth muscle cells.[29,28,30,31]

In pathological conditions of the cardiovascular system, there is a dysfunction in the integrity of the vascular endothelium with a subsequent reduction in the release, bioavailability and/or action of nitric oxide.[28] NO release and function have been shown to decrease in cardiovascular diseases, such as hypertension,[32] atherosclerosis[32] and congestive heart failure.[33] Therefore, the development of vasodilators which can restore the level and integrity of NO in the vascular system would potentially contribute to the treatment of these cardiovascular diseases.[28]

In the present study, the plant extract, like acetylcholine, caused concentration-dependent relaxation of the normotensive rat isolated endothelium-containing aortic ring preparations pre-contracted with noradrenaline. This vasorelaxant property would appear to have contributed, at least in part, to the antihypertensive (hypotensive) effect of the plant extract. The arterial muscle relaxant effect of the extract disappeared by removal of the functional endothelium.

Furthermore, pre-treatment of the endothelium-containing aortic ring preparations with L-NAME, a nitric oxide synthase inhibitor, inhibited or abolished the vasorelaxant effect of PAE. Taken together, these observations would appear to suggest that the vasorelaxant effect of the extract, like that of acetylcholine, was dependent on the formation and/or synthesis and release of endothelium-derived nitric oxide, since removal of the functional endothelial cells led to the absence of relaxant response to PAE in the endothelium-denuded aortic ring preparations. These observations are in agreement with the findings of Martin *et al.*,[34] Ignarro *et al.*,[35] Kang *et al.*,[36-38] Baisch *et al.*[39] and Yin *et al.*[28]

The present study also suggests that the endothelium-dependent vasorelaxant effect of PAE could be mediated via endothelial NO signaling in the aortic tissue preparations. However, the release of endothelial NO and the opening of potassium channels have also been implicated in the vasorelaxant effects of extracts from some other medicinal plants.[39-41]

Noradrenaline-induced contractions of blood vessels have been shown to be partly due to calcium release from intracellular storage sites and partly due to the influx of extracellular calcium into the cell via receptor-gated channels following alpha$_1$ (α_1)-adrenoceptor activation.[42] In the present study, endothelium-containing aortic rings pre-contracted with NA in Krebs-Henseleit solution with and without normal calcium concentrations were relaxed by exogenous additions of PAE or acetylcholine. Moreover, the non-parallel shift of the noradrenaline concentration-response curves to the right by the plant extract seems to suggest a mechanism of non-competitive α_1-adrenoceptor blockade. This hypothesis is in consonance with the work of Abreu *et al.*[43] on ethanolic extract of *Jatropha gossypiifolia* Linn in rats.

The findings of the present study indicated that PAE induced vasorelaxation in normotensive rat isolated portal veins and endothelium-containing aortic rings, and caused hypotension in anaesthetised, normotensive and hypertensive rats. Although α_1-adrenoceptor blockade may have partially contributed to the hypotensive effect of the plant extract, the experimental evidence obtained in the present study tends to suggest that vasorelaxation might largely have been responsible for the hypotensive action of the plant extract. This vasorelaxant effect of the extract was probably mediated through endothelium-dependent NO production and cGMP release, and not related to activation of vascular endothelial muscarinic receptors.

Although the precise mechanism of the hypotensive action of PAE could not be established in the present study, we excluded involvement of cholinergic mechanisms. However, a complicating factor in the interpretation of the data obtained in the hypotensive experiments was the bradycardia associated with the reduction in systemic arterial blood pressure of the rats. Firstly, the reduction in heart rate could, on its own, have been the cause of the hypotension. However, based on the results obtained from the rat isolated aortic rings, it would seem unlikely that the fall in arterial blood pressure produced by PAE was solely dependent on reduction in heart rate. Secondly, the observed transient, secondary reflex tachycardia accompanying the fall in arterial blood pressure would probably suggest that the plant extract did not affect central cardiovascular centres and/or brain cardiovascular receptors. The plant extract may, therefore, also have had a direct effect on the sinus node of the heart, or on the central nervous system control machinery of arterial blood pressure.

P americana has been reported to contain many bioactive chemical compounds, including polyphenolics, tannins, coumarins, flavonoids, triterpenoids, phytosterols (especially β-sitosterol), biotin, α-tocopherol, carotene, ascorbic acid, scopoletin, quercetin, oils, organic acids and inorganic substances such as calcium, magnesium, zinc and phosphorus.[11,12] However, our present state of knowledge of the chemical constituents of the leaf extract is limited. It is therefore impossible for us at this stage to identify with certainty the vasorelaxant and antihypertensive constituent/s of PAE. Although we speculate that one or more of the major chemical constituents of the plant [namely flavonoids, polyphenols, tannins, coumarins (especially scopoletin and other coumarins), triterpenoids and phytosterols] may possibly have accounted for the observed cardiodepressant, vasorelaxant and antihypertensive properties of the plant extract, there are no sufficient scientific data at present to justify this speculation. However, the experimental evidence obtained in the present study showed that *P americana* aqueous leaf extract produced significant cardiodepressant, vasorelaxant and hypotensive (antihypertensive) effects in the laboratory animal paradigms used.

In conclusion, the findings of the present laboratory animal study lend pharmacological support to the suggested anecdotal ethnomedical uses of *P americana* aqueous leaf extract as a natural supplementary remedy in the management, control and/or treatment of hypertension and certain cardiac disorders in some rural Africa communities.

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Hypoglycaemic effects of *Hypoxis hemerocallidea* (Fisch. and C. A. Mey.) [Hypoxidaceae] corm ethanolic extract in rats

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Previous studies in our laboratories suggest that *Hypoxis hemerocallidea* corm (African Potato) extract has a potential in the management of diabetes mellitus. Accordingly, we investigated the short-term effects of *Hypoxis hemerocallidea* corm ethanolic crude extract (APE) on blood glucose levels of Wistar rats (250-300 g b.wt). Oral glucose tolerance tests (OGTT) were conducted in five separate groups (n=6 in each group) of male Wistar rats (250-300 g b.wt). The animals were starved for 16 hours and administered with glucose (0.86 g. $mg.kg^{-1}$ b.wt) by gastric gavage, followed by graded doses of APE (60, 120 and 240 $mg.kg^{-1}$ p. o.). Blood glucose concentrations were monitored at 15 min intervals for the first hour, and hourly thereafter for the next 3 hours. A separate group of animals treated with metformin (500 mg/kg p. o.) served as the treated control. Animals treated with deionised water (3 ml/kg p. o.) served as the untreated control animals. Statistical significance was determined by using Graph Pad Prism (version 2.00) Software. The data were subjected to analysis of variance using a one-way design and Scheffe's multiple comparison test was used to assess any differences. A value of $p<0.05$ was considered significant. APE (60-240 $mg.kg^{-1}$ p. o.) decreased blood glucose concentrations of the rats in a dose-dependent manner; with low to moderate doses of APE exerting their maximum hypoglycaemic effects after 30 minutes. The median dose of APE (120 $mg.kg^{-1}$) decreased blood glucose levels from a peak of 6.9±0.2 $mmol.l^{-1}$ to 5.9±0.2 $mmol.l^{-1}$ by 30 minutes. Metformin (500 $mg.kg^{-1}$ p. o.) induced a sustained, highly significant ($P<0.001$) reduction in the blood glucose levels of the animals. Findings from the present study indicate a short-term hypoglycemic effect of oral administration of APE in rats, presumably in a mechanistic manner similar to that of metformin.

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The effects of *Sclerocarya birrea* [(a. rich.) hochst.] [Anacardiaceae] stem-bark aqueous extract on blood glucose, kidney and cardiovascular function in rats

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Available evidence suggests that *Sclerocarya birrea* stem-bark extract (SBE) which possesses hypoglycaemic activity may be useful in the treatment and management of diabetes mellitus. Since diabetes is associated with cardiovascular and kidney complications, we investigated the influence of SBE on blood glucose, blood pressure and renal function in some experimental animal paradigms. For OGTT tests, separate groups (n=6 in each group) of non-diabetic and streptozotocin-(STZ)-treated diabetic rats fasted for 18 hours were given glucose (0.86 g.kg^{-1} body weight, p.o.) followed by SBE at various doses (60, 120, 240 mg.kg^{-1}). Animals treated with deionised water (3 ml.kg^{-1}) or metformin (500 mg.kg^{-1}) served as untreated and positive controls, respectively. Blood glucose was monitored at 15 minute intervals for the first hour, and hourly thereafter for 3 hours. Acute renal effects were assessed by urine flow, Na^+ and K^+ excretion rates in anaesthetized rats challenged with hypotonic saline infusion at 30-min intervals for 4 h of 1 h control, 1½ h treatment and 1½ h recovery periods following equilibration period (3¼ h). SBE was added to the infusate during the treatment period. Renal effects were also monitored in individually-caged rats administered daily with SBE for 5 weeks. The hypotensive effect of SBE was examined in anaesthetized and conscious normotensive rats, while myocardial contractile performance was evaluated on guinea-pig isolated atrial muscle strips. SBE dose-dependently decreased blood glucose within the first 30 minutes in non-diabetic and STZ-diabetic rats. Similar effects were observed with metformin. Na^+ and K^+ excretion rates were not altered by acute or chronic treatment with SBE. The extract caused significant reduction in blood pressure in anaesthetized and conscious rats. SBE also produced concentration-dependent, significant (P<0.01) negative inotropic and chronotropic effects on guinea-pig isolated, electrically-driven left-, and spontaneously-beating right-, atrial muscle preparations, respectively. The current observations suggest that SBE has a cardioprotective role in management of diabetes mellitus.

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