CHAPTER ONE

1.0 Introduction

Diabetes mellitus (DM) is a disorder associated with persistent and chronic hyperglycaemia which results from lack of secretion of insulin, insulin effects, or both (WHO, 2017). The hyperglycaemia in diabetes mellitus is characterized with long-term failure and dysfunction of many different organs, most especially the blood vessels, kidneys, eyes and heart (American Diabetes Association, 2012). More than 400 million people have been diagnosed with diabetes and this value has been projected to increase in the nearest future (WHO, 2017). Therefore, the problems associated with increase prevalence of diabetes and the need to provide immediate treatments cannot be overemphasized.

The gut play important roles in regulating glucose homeostasis in diabetes mellitus and therefore, any factor that influences the activities of glucose transporters (SGLT and GLUT) in the enterocytes of small intestine will also alter the absorption and metabolism of glucose (Holst *et al*., 2016). Most complex carbohydrates reaching the small intestine become hydrolyzed to monosaccharides subunits such as glucose or galactose in order to be transported across the intestinal mucosa. The pathway of glucose absorption is across the intestinal brush-border membrane, which was predominantly mediated by sodium-glucose transporter (SGLT), a membrane protein that couples two Na^+ together with one molecule of glucose (Navale, 2016). The passive movement of glucose out of the basolateral surface of enterocytes is mediated by facilitated-diffusion glucose transporter (GLUT 2) which allows glucose to move from the intracellular compartment into the extracellular medium near the blood capillaries. Translocation of GLUT 2 from cytoplasmic vesicles into the basolateral membrane markedly increases the capacity of glucose uptake by the enterocytes (Thorens, 2010).

The liver also play a role in carbohydrate homeostasis and is essential in glucose regulation in humans in the fasted, hyperglycemic or diabetic states. It is constantly supplied with insulin which enhances glucose production as well as glucose consumption. It is the main site of the glucoregulatory action of glucagon and absorbes glucose before being delivered to muscle and adipose tissues (Sadava *et al*., 2011).

Hyperglycaemia-induced oxidative stress has been reported in the etiology and pathogenesis of both human and animal models of diabetes mellitus. With increased oxidative stress, most enzymatic antioxidants undergo degeneration with reduced ability to mop up free radicals (Campbell *et al*., 2006). The variations in the level of these enzymes make various tissues of the body susceptible to oxidative damage.

Furthermore, electrolytes contribute to many physiological processes including controlling fluid levels, acid-base balance (pH), nerve conduction, blood clotting and muscle contraction. However, electrolyte imbalance resulting from kidney failure, dehydration, fever, and vomiting has been suggested as one of the contributing factors toward diabetic complications and other endocrine disorders (Husain *et al*., 2009).

The Center for Disease Control in 2014 reported that diabetic nephropathy is the leading cause of chronic kidney disease and kidney failure in most of the developed and industrialized world and the number of cases are increasing rapidly. Diabetic nephropathy is caused by the abnormal deposition of matrix material in the glomerular mesangium, leading to a thickened, sclerotic glomerular lining of renal tubular cells (Hall *et al*., 2006). Studies have shown that glucose reacts with proteins in the blood, chemically forming permanent cross-linked protein complexes and therefore excessive accumulation of these complexes, or advanced glycosylated end products (AGEs) formation is believed to directly accelerate the vascular complications of diabetes and accumulation of waste products of metabolism which may be toxic at high concentrations.

The hyperglycaemia observed in diabetes mellitus induced tissue damage through several mechanisms which include the polyol pathway, intracellular production of AGE precursors, protein kinase C (PKC) activation pathway and hexosamine pathway (Lee *et al*., 1999). Protein kinase C (PKC) activation pathway is the major culprit involved in

hyperglycemia induced renal damage and therefore formed one of the main focus of this study. In this pathway, intracellular hyperglycemia increases the synthesis of a molecule called diacylglycerol, which is a critical activating co-factor for the classic isoforms of protein kinase-C, (Koya *et al*., 1998). When PKC is activated, it causes a variety of effects on gene expressions by increasing the level of transforming growth factor which catalysis the formation of cytoskeletal materials deposited within the glomeruli and renal tubular cell leading to nephropathy.

The use of traditional medicine as therapeutic agents for the treatment of diseases, particularly in the developing countries has continued to yield fruitful results. It has been reported that not less than 80% of the African population depends on traditional medicine to meet up with their primary health care needs and these herbal preparations are mostly used in their crude forms and contain active compounds that are responsible for their biological roles.

Diet, insulin, and oral hypoglycemic agents have remained the mainstays of therapy for diabetic patients for decades and many local plants also have been identified and tested for their anti-diabetic properties. Among the medicinal plants that are currently in use in Nigeria is the plant called *Parquetina nigrescens* (Family name- Asclepiadaceae). This plant has been used in the treatment anaemia because of its effects on erythrocyte cell membrane (Saba *et al*., 2010). It has also been used in the treatment of fever, inflammatory and painful disorders (Owoyele *et al*., 2008). Furthermore, aqueous extract of *Parquetina nigrescens* have been reported to cause significant antinociception (Olajide *et al*., 2003).

1.1 Statement of the problem

Diabetes mellitus is a debilitating disease treated or managed by using various antidiabetic agents. However, despite the various interventions against diabetes mellitus, the incidence has continued to increase globally most especially in Africa (Nigeria). Therefore, the need to provide alternative, cheap and relatively safe antidiabetic agents against diabetes mellitus cannot be overemphasized.

1.2 Justification

Diabetes mellitus is characterized with episodes of hyperglycaemia. Although, *Parquetina nigrescens* has been reported to reduce blood glucose, the mechanisms of its normoglycaemic effects have not been fully elucidated. This study therefore was designed to investigate the mechanisms of antidiabetic potential of methanol extract of *Parquetina nigrescens* (MEPN) and the effects of its bioactive components (phytol and squalene) in alloxan-induced diabetic Wistar rats. The effects of the Phytol and Squalene in preventing hyperglycaemia-induced renal damage was also investigated.

1.3. Research question

How does the methanol extract of *Parquetina nigrescens* and its constituents (phytol and squalene) exhibit anti-hyperglycemic effect in alloxan-induced diabetes in male Wistar rats?

1.4 Research hypothesis

The methanol extract of *Parquetina nigrescens*, phytol and squalene do not exhibit anti-diabetic effects in alloxan-induced diabetes in male Wistar rats.

1.5 Aims

This study was designed to;

1. Investigate the anti-diabetic effects of MEPN, Phytol and Squalene in alloxan-induced diabetic Wistar rats.
2. Investigate the anti-oxidative effects of MEPN, Phytol and Squalene in alloxan-induced diabetic Wistar rats
3. Assess the reno-protective effects of MEPN, Phytol and Squalene in alloxan-induced diabetic Wistar rats
4. Assess the role of Phytol and Squalene in the mechanism of hyperglycaemia-induced renal damage.

1.6 Research objectives

The specific objectives of this research were to investigate the effects of methanol extract of *Parquetina nigresens* (MEPN), Phytol and Squalene on;

Anti-diabetic studies

Body weight changes (BW), Fasting blood glucose level (FBG), Duodenal glucose absorption (DGA), Jejunal glucose absorption (JGA), Liver weight (LW), Liver glycogen (LG), Histo-architecture of the liver, Insulin concentration (IC) and Histo-architecture of the pancreas.

Anti-oxidative studies

Lactate dehydrogenase (LDH) activity, Glucose-6-phosphate dehydrogenase (G-6-PDH) activity,Catalase (CAT) activity, Superoxide dismutase (SOD) activity, Glutathione peroxidase (GPx) activity and Albumin (ALB) level

Reno-protective studies

Creatinine (CRT) level, Blood urea nitrogen (BUN) level, Histoarchitecture of the kidney, Sodium concentration, Potassium concentration, Calcium concentration Magnesium concentration, Bicarbonate concentration. Chloride concentration

Mechanism of hyperglycemia induced renal damage study

Diacylglycerol (DAG) , Protein kinase C-alpha (PKC-α) , Mitogen activated protein kinase-8 (MAPK-8), Transformin growth factor Beta (TGF-β), p16 expression in kidney tissue samples, p53 expression in kidney tissue samples, Bcl-2 expression in kidney tissue samples, COX-2 expression in kidney tissue samples

CHAPTER TWO

2.0 Literature Review

2.1 Diabetes mellitus

Diabetes is a derangement that manifest either due to insufficient insulin production or inability of cells to appropriately utilize the insulin produced (WHO, 2017). Increased hyperglycemia, a consequence of chronic diabetes, could results in serious defects in the nerve cells, blood vessels, kidneys, heart and the eyes. Studies have shown that a large percentage of the World's population (400 million people are suffering from diabetes (WHO, 2017).

The type I diabetes mellitus also referred to as insulin-dependent juvenile or childhood-onset diabetes is characterized by deficiency in insulin production within the pancreatic islet cells. Individuals with this type of diabetes need daily insulin injection in order to control glucose level of their blood and without access to insulin, they may not survive. The cause of this diabetes is unknown and presently, it cannot be prevented (WHO, 2017). Some of the signs are fatigue, weight loss, thirst, vision impairment, increased appetite and many others.

The type II diabetes is also referred to as the non-insulin dependent diabetes which results from inability of cells to utilize the insulin produced. It is the main cause of diabetes globally and the signs are similar to the type I diabetes, but usually less marked. The disease could remain undiagnosed for a long period after which complications may arise. For several years, it was diagnosed in adult only but, it has begun to occur in children. Glucose intolerance and hyperglycemia are intermediate conditions which could later manifest as diabetes most especially type II diabetes mellitus. People with glucose intolerance or

hyperglycemia are at increased risk of developing strokes and heart attacks. Gestational diabetes mellitus is another type of diabetes which occur during pregnancy with an increased risk of developing type II diabetes. The disorder manifest when blood glucose levels are above normal, but still below those diagnosis of diabetes.

Women with gestational diabetes are at increased risk of developing complications during pregnancy and delivery. This condition is usually diagnosed through prenatal screening, rather than the signs and symptoms that are reported.

2.2. The Liver

The liver is an important organ in human. It has several functions some of which include detoxification of metabolites, digestion of food, hormone synthesis, degradation of used red blood cells. It also regulates blood glucose level through glycogenolysis. (Breakdown of liver glycogen) most especially during fasting so that more glucose is available in the circulation (Abdel *et al*., 2010). The absence of liver in the body could affect many of its functions and over the years, dialysis had been used as the main form of treatment for liver failure however, this cannot take care of its function for a long period of time (Lopes, 2020). Therefore liver transplantation is still the major long-term treatment available for the liver failure.

2.2.1 Structure of the liver

The liver is a wedge-shaped organ located at the right upper quadrant of the abdomen. It has four lobes which overlies the gall bladder that produces bile for fat emulsification (Contran *et al*., 2005). It is supplied by two major vessels, the hepatic artery from the aorta which carries oxygenated blood and the hepatic portal vein which carries digested food nutrients from the intestine. These vessels narrowed to form sinusoid which terminates on lobules. Lobules consist of many hepatocytes with high metabolic functions. The liver is

Covered by a thin layer of fibrous peritoneum called Glisson's capsule which provides it with structural support (Haubrich, 2001).

2.2.3 Glycogen

This is a complex polysaccharide which stores excess glucose in the blood of animals. It is analogous to starch which stores excess glucose in plants. Glycogen is mainly stored in the liver although it may also be found in muscles, kidneys and uterus in smaller amount. In muscles, most of the glycogen are converted to glucose by the muscle cells when needed (Campbell *et al*., 2006).

2.3 The Pancreas

The pancreas has three different parts which include the head, neck and the body. It is supplied by superior, inferior mesenteric artery and vein, splenic, and coelic artery and veins. It is about 15 cm long and located in the abdominal cavity behind the stomach. It is a mixed gland having both endocrine and exocrine cells which secrete hormones. The endocrine cells include the alpha cells which secrete glucagon, beta cells which secrete insulin, delta cells which secrete somatostatin and gamma cells which secrete polypeptide YY. The glucagon produced by alpha cells increase circulating blood glucose by increasing the breakdown of glycogen through the process of glycogenolysis (Stanojevic, 2016). However, the insulin produced by the beta cell increases the uptake of glucose by peripheral cells. Both the somatostatin and polypeptide YY regulate insulin and glucagon production (Schoenwolf *et al*., 2009). The exocrine pancreas secretes digestive enzymes through the intralobular duct and ampulla of vater into the duodenum. These digestive enzymes are very rich in bicarbonate ion which neutralizes the acidity of the chyme on entering the duodenum (Ionescu-Tirgoriste, 2015). The digestive enzymes also contain amylase which is the primary enzyme involved in carbohydrate digestion. It also contains lipase, phospholipase A_2 and cholesterol esterase which is involved in lipid digestion. The pancreatic juice also contains other inactive enzyme like chymotrypsinogen which is activated and converted to trypsin which is necessary for protein digestion. Autoactivation of these enzymes in the pancreas could result in inflammation and self-digestion of the pancreatic tissues (Ross *et al*., 2021). Inflammation of the pancreas is called pancreatitis and it may be diagnosed by

traces of bile, lipase, amylase and trypsin enzymes in the blood. Excessive alcohol intake and scorpion bites may cause inflammation of the pancreas and pain of the visceral organ which is often referred to the posterior or back of the body. When the immune system attack the islet cells of the pancreas, insulin may be deficient and this is vital for glucose uptake therefore, this may results in type 1 diabetes which is often treated mainly by using insulin injection (Roep, 2021). The type 2 diabetes is often a combination of insulin deficiency and insulin resistance and it's managed through diet and lifestyle modifications. Biguinides like metformin could also be used to manage this condition since they are oral hypoglycemic agents.

2.4 The Kidney

The kidneys are complex organs, and they are vital in maintaining normal body functions. A human being's survival depends, to a large degree, on the crucial functions and processes performed by the kidneys. The renal system affects all parts of the body by keeping body fluids in balance and other organ systems functioning normally. Renal and urologic disorders may strike anyone at any age and at any time. An estimated 20 million Americans are affected with renal disorders each year (Wallace, 1998).

The kidneys play a vital role in the excretion of waste products and toxins such as urea, creatinine and uric acid, regulation of extracellular fluid volume, serum osmolality and electrolyte concentrations, as well as the production of hormones like erythropoietin and 1,25 dihydroxy vitamin D and renin. The functional unit of the kidney is the nephron, which consists of the glomerulus, proximal and distal tubules, and collecting duct. Assessment of renal function is important in the management of patients with kidney disease or pathologies affecting renal function (Nwose, 2019). Tests of renal function have utility in identifying the presence of renal disease, monitoring the response of kidneys to treatment, and determining the progression of renal disease. According to the National Institutes of Health, the overall prevalence of chronic kidney disease (CKD) is approximately 14%. Worldwide, the most common causes of CKD are hypertension and diabetes (Damiati, 2019).

For the assessment of renal function, specimen collection requirements are dependent on the procedure or test requested. Generally, for serum creatinine and blood urea nitrogen

(BUN) levels, no additional patient preparation is required, and a random blood sample suffices (Gai, 2019). However, the effect of recent high protein ingestion may increase serum creatinine and urea levels to a significant extent. Also, hydration status can have a considerable impact on BUN measurement (Kamianowska, 2019). For timed urine collections such as the 24-hour urine creatinine clearance, it is essential that urine be collected accurately over the required period as under or over collection will affect final results. Hence, a 5 to 8-hour timed collection is preferable to a 24-hour collection.

2.5.0 Oxidative Stress and Its Effects: Genetic, Physiological, and Biochemical Mechanisms

Oxidative stress occurs when the balance between antioxidants and ROS are disrupted because of either depletion of antioxidants or accumulation of ROS. When oxidative stress occurs, cells attempt to counteract the oxidant effects and restore the redox balance by activation or silencing of genes encoding defensive enzymes, transcription factors, and structural proteins (Hawkins, 2019). Ratio between oxidized and reduced glutathione (2GSH/GSSG) is one of the important determinants of oxidative stress in the body. Higher production of ROS in body may change DNA structure, result in modification of proteins and lipids, activation of several stress-induced transcription factors, and production of pro-inflammatory and anti-inflammatory cytokines (Pizzino *et al*., 2017).

2.5.1 Effects of oxidative stress on DNA

ROS can lead to DNA modifications in several ways, which involves degradation of bases, single- or double-stranded DNA breaks, purine, pyrimidine or sugar-bound modifications, mutations, deletions or translocations, and cross-linking with proteins (Cadet, 2017). Most of these DNA modifications are highly relevant to carcinogenesis, aging, and neurodegenerative, cardiovascular, and autoimmune diseases. Tobacco smoke, redox metals, and non-redox metals, such as iron, cadmium, chrome, and arsenic, are also involved in carcinogenesis and aging by generating free radicals or binding with thiol groups. Formation of 8-OH-G is the best-known DNA damage occurring via oxidative stress and is a potential biomarker for carcinogenesis (Ock, 2012).

2.5.2 Base modifications introduced by reactive oxygen species.

Promoter regions of genes contain consensus sequences for transcription factors. These transcription factor–binding sites contain GC-rich sequences that are susceptible for oxidant attacks (Cadet, 2013). Formation of 8-OH-G DNA in transcription factor binding sites can modify binding of transcription factors and thus change the expression of related genes as has been shown for AP-1 and Sp-1 target sequences (Ghosh, 1999) Besides 8-OH-G, 8,5′-cyclo-2′-deoxyadenosine (cyclo-dA) has also been shown to inhibit transcription from a reporter gene in a cell system if located in a TATA box (Marietta, 2002). The TATA-binding protein initiates transcription by changing the bending of DNA. The binding of TATA-binding protein may be impaired by the presence of cyclo-dA. Oxidative stress causes instability of microsatellite (short tandem repeats) regions. Redox active metal ions, hydroxyl radicals increase microsatellite instability (Jackson, 1998). Even though single-stranded DNA breaks caused by oxidant injury can easily be tolerated by cells, double-stranded DNA breaks induced by ionizing radiation can be a significant threat for the cell survival (Caldecott, 2003). Methylation at CpG islands in DNA is an important epigenetic mechanism that may result in gene silencing. Oxidation of 5-MeCyt to 5-hydroxymethyl uracil (5-OHMeUra) can occur via deamination/oxidation reactions of thymine or 5-hydroxymethyl cytosine intermediates (Cooke, 2003). In addition to the modulating gene expression, DNA methylation also seems to affect chromatin organization. Aberrant DNA methylation patterns induced by oxidative attacks also affect DNA repair activity.

2.5.3 Effects of oxidative stress on lipids

ROS can induce lipid peroxidation and disrupt the membrane lipid bilayer arrangement that may inactivate membrane-bound receptors and enzymes and increase tissue permeability (Girotti, 1985). Products of lipid peroxidation, such as MDA and unsaturated aldehydes, are capable of inactivating many cellular proteins by forming protein cross-linkages. 4-Hydroxy-2-nonenal causes depletion of intracellular GSH and induces of peroxide production, activates epidermal growth factor receptor, and induces fibronectin production. Lipid peroxidation products, such as isoprostanes and thiobarbituric acid reactive substances, have been used as indirect biomarkers of oxidative stress, and increased

levels were shown in the exhaled breath condensate or bronchoalveolar lavage fluid or lung of chronic obstructive pulmonary disease patients or smokers. (Montuschi, 2000).

2.5.4 Effects of oxidative stress on proteins

ROS can cause fragmentation of the peptide chain, alteration of electrical charge of proteins, cross-linking of proteins, and oxidation of specific amino acids and therefore lead to increased susceptibility to proteolysis by degradation by specific proteases. Cysteine and methionine residues in proteins are particularly more susceptible to oxidation (Dean, 1985). Oxidation of sulfhydryl groups or methionine residues of proteins cause conformational changes, protein unfolding, and degradation. Enzymes that have metals on or close to their active sites are especially more sensitive to metal catalyzed oxidation. Oxidative modification of enzymes has been shown to inhibit their activities.

In some cases, specific oxidation of proteins may take place. For example, methionine can be oxidized to methionine sulfoxide and phenylalanine to o-tyrosine sulfhydryl groups can be oxidized to form disulfide bonds and carbonyl groups may be introduced into the side chains of proteins (Luo, 2009). Gamma rays, metal-catalyzed oxidation, HOCl, and ozone can cause formation of carbonyl groups.

2.5.5 Effects of oxidative stress on signal transduction

Reactive oxygen species (ROS) can induce expression of several genes involved in signal transduction. A high ratio for GSH/GSSG is important for the protection of the cell from oxidative damage (Gibson, 2012). Disruption of this ratio causes activation of redox sensitive transcription factors, such as NF-κB, AP-1, nuclear factor of activated T cells and hypoxia-inducible factor 1, that are involved in the inflammatory response. Activation of transcription factors via ROS is achieved by signal transduction cascades that transmit the information from outside to the inside of cell. Tyrosine kinase receptors, most of the growth factor receptors, such as epidermal growth factor receptor, vascular endothelial growth factor receptor, and receptor for platelet-derived growth factor, protein tyrosine phosphatases, and serine/threonine kinases are targets of ROS (Schieber, 2014). Extracellular signal-regulated kinases, JNK, and p38, which are the members of mitogen-activated protein kinase family and involved in several processes in cell including

proliferation, differentiation, and apoptosis, also can be regulated by oxidants (Milkovic, 2019).

Under oxidative stress conditions, cysteine residues in the DNA-binding site of c-Jun, some AP-1 subunits, and inhibitory κ-B kinase undergo reversible S-glutathiolation. Glutaredoxin and TRX have been reported to play an important role in regulation of redox-sensitive signaling pathways, such as NF-κB and AP-1, p38 mitogen-activated protein kinase, and JNK (Filomeni, 2002).

Nuclear factor kappa beta (NF-κB) can be activated in response to oxidative stress conditions, such as ROS, free radicals, and UV irradiation (Pande, 2005). Phosphorylation of IκB frees NF-κB and allows it to enter the nucleus to activate gene transcription. A number of kinases have been reported to phosphorylate IκBs at the serine residues. These kinases are the targets of oxidative signals for activation of NF-κB (Lingappan, 2018). Reducing agents enhance NF-κB DNA binding, whereas oxidizing agents inhibit DNA binding of NF-κB. TRX may exert 2 opposite actions in regulation of NF-κB: in the cytoplasm, it blocks degradation of IκB and inhibits NF-κB activation but enhances NF-κB DNA binding in the nucleus. Activation of NF-κB via oxidation-related degradation of IκB results in the activation of several antioxidant defense–related genes. NF-κB regulates the expression of several genes that participate in immune response, such as IL-1β, IL-6, tumor necrosis factor-α, IL-8, and several adhesion molecules NF-κB also regulates angiogenesis and proliferation and differentiation of cells (Elliott, 2001). AP-1 is also regulated by redox state. In the presence of H_2O_2, some metal ions can induce activation of AP-1. Increase in the ratio of GSH/GSSG enhances AP-1 binding while GSSG inhibits the DNA binding of AP-1 DNA binding of the Fos/Jun heterodimer is increased by the reduction of a single conserved cysteine in the DNA-binding domain of each of the proteins (Abate, 1990) while DNA binding of AP-1 can be inhibited by GSSG in many cell types, suggesting that disulphide bond formation by cysteine residues inhibits AP-1 DNA binding.

2.5.6 Enzymatic antioxidants

The major enzymatic antioxidants are SODs, catalase, and GSH-Px. In addition to these major enzymes, other antioxidants, including heme oxygenase-1, and redox proteins, such

as thioredoxins, peroxiredoxin, and glutaredoxins, have also been found to play crucial roles in the antioxidant defenses.

Since superoxide is the primary ROS produced from a variety of sources, its dismutation by SOD is of primary importance for each cell. All the three forms of SOD, that is, CuZn-SOD, Mn-SOD, and EC-SOD, are widely expressed in the human. Mn-SOD is localized in the mitochondria matrix. EC-SOD is primarily localized in the extracellular matrix, especially in areas containing high amount of type I collagen fibers and around pulmonary and systemic vessels. It has also been detected in the bronchial epithelium, alveolar epithelium, and alveolar macrophages (Kinnula, 2005). Overall, CuZn-SOD and Mn-SOD are generally thought to act as bulk scavengers of superoxide radicals. The relatively high EC-SOD level with its specific binding to the extracellular matrix components may represent a fundamental component of matrix protection.

Hydrogen peroxide (H_2O_2) that is produced by the action of SODs or the action of oxidases, such as xanthine oxidase, is reduced to water by catalase and the GSH-Px. Catalase exists as a tetramer composed of 4 identical monomers, each of which contains a heme group at the active site. Degradation of H_2O_2 is accomplished via the conversion between 2 conformations of catalase-ferricatalase (iron coordinated to water) and compound I (iron complexed with an oxygen atom). Catalase also binds NADPH as a reducing equivalent to prevent oxidative inactivation of the enzyme (formation of compound II) by H_2O_2 as it is reduced to water (Kirkman, 1999)

Enzymes in the redox cycle responsible for the reduction of H_2O_2 and lipid hydroperoxides (generated as a result of membrane lipid peroxidation) include the GSH-Pxs. The GSH-Pxs are a family of tetrameric enzymes that contain the unique amino acid selenocysteine within the active sites and use low-molecular-weight thiols, such as GSH, to reduce H_2O_2 and lipid peroxides to their corresponding alcohols. Four GSH-Pxs have been described, encoded by different genes: GSH-Px-1 (cellular GSH-Px) is ubiquitous and reduces H_2O_2 and fatty acid peroxides, but not esterified peroxyl lipids (Arthur, 2000). Esterified lipids are reduced by membrane-bound GSH-Px-4 (phospholipid hydroperoxide GSH-Px), which can use several different low-molecular-weight thiols as reducing equivalents. GSH-Px-2 (gastrointestinal GSH-Px) is localized in gastrointestinal epithelial cells where it serves to reduce dietary

peroxides (Chu, 1993) GSH-Px-3 (extracellular GSH-Px) is the only member of the GSH-Px family that resides in the extracellular compartment and is believed to be one of the most important extracellular antioxidant enzyme in mammals. Of these, extracellular GSH-Px is most widely investigated in the human lung.

In addition, disposal of H_2O_2 is closely associated with several thiol-containing enzymes, namely, TRXs (TRX1 and TRX2), thioredoxin reductases (TRRs), PRXs (which are thioredoxin peroxidases), and glutaredoxins. Two TRXs and TRRs have been characterized in human cells, existing in both cytosol and mitochondria. In the lung, TRX and TRR are expressed in bronchial and alveolar epithelium and macrophages. Six different PRXs have been found in human cells, differing in their ultrastructural compartmentalization. Experimental studies have revealed the importance of PRX VI in the protection of alveolar epithelium. Human lung expresses all PRXs in bronchial epithelium, alveolar epithelium, and macrophages (Kinnula, 2005). PRX V has recently been found to function as a peroxynitrite reductase, which means that it may function as a potential protective compound in the development of ROS-mediated lung injury.

Common to these antioxidants is the requirement of NADPH as a reducing equivalent. NADPH maintains catalase in the active form and is used as a cofactor by TRX and GSH reductase (EC 1.6.4.2), which converts GSSG to GSH, a co-substrate for the GSH-Pxs. Intracellular NADPH, in turn, is generated by the reduction of $NADP^+$ by glucose-6-phosphate dehydrogenase, the first and rate-limiting enzyme of the pentose phosphate pathway, during the conversion of glucose-6-phosphate to 6-phosphogluconolactone. By generating NADPH, glucose-6-phosphate dehydrogenase is a critical determinant of cytosolic GSH buffering capacity (GSH/GSSG) and, therefore, can be considered an essential, regulatory antioxidant enzyme.

Glutathione S transaminase (GSTs), another antioxidant enzyme family, inactivate secondary metabolites, such as unsaturated aldehydes, epoxides, and hydroperoxides. Three major families of GSTs have been described: cytosolic GST, mitochondrial GST and membrane-associated microsomal GST that has a role in eicosanoid and GSH metabolism (Ma, 2017). Seven classes of cytosolic GST are identified in mammalian, designated Alpha, Mu, Pi, Sigma, Theta, Omega, and Zeta. During nonstressed conditions, class Mu and Pi

GSTs interact with kinases Ask1 and JNK, respectively, and inhibit these kinases (Cooper, 2018). It has been shown that GSTP1 dissociates from JNK in response to oxidative stress GSTP1 also physically interacts with PRX VI and leads to recovery of PRX enzyme activity via glutathionylation of the oxidized protein.

2.5.7 Non-enzymatic antioxidants

Non-enzymatic antioxidants include low-molecular-weight compounds, such as vitamins (vitamins C and E), β-carotene, uric acid, and GSH, a tripeptide (L-γ-glutamyl-L-cysteinyl-L-glycine) that comprise a thiol (sulfhydryl) group.

i Vitamin C (Ascorbic Acid)

Water-soluble vitamin C (ascorbic acid) provides intracellular and extracellular aqueous-phase antioxidant capacity primarily by scavenging oxygen free radicals. It converts vitamin E free radicals back to vitamin E. Its plasma levels have been shown to decrease with age (Mezzetti, 1996).

ii Vitamin E (α-Tocopherol)

Lipid-soluble vitamin E is concentrated in the hydrophobic interior site of cell membrane and is the principal defense against oxidant-induced membrane injury. Vitamin E donates electron to peroxyl radical, which is produced during lipid peroxidation. Alpha-tocopherol is the most active form of vitamin E and the major membrane-bound antioxidant in cell. Vitamin E triggers apoptosis of cancer cells and inhibits free radical formations.

iii Glutathione

GSH is highly abundant in all cell compartments and is the major soluble antioxidant. GSH/GSSG ratio is a major determinant of oxidative stress. GSH shows its antioxidant effects in several ways (Masella, 2005). It detoxifies hydrogen peroxide and lipid peroxides via action of GSH-Px. GSH donates its electron to H_2O_2 to reduce it into H_2O and O_2. GSSG is again reduced into GSH by GSH reductase that uses NAD(P)H as the electron donor. GSH-Pxs are also important for the protection of cell membrane from lipid peroxidation. Reduced glutathione donates protons to membrane lipids and protects them from oxidant attacks.

GSH is a cofactor for several detoxifying enzymes, such as GSH-Px and transferase. It has a role in converting vitamin C and E back to their active forms. GSH protects cells against apoptosis by interacting with proapoptotic and antiapoptotic signaling pathways (Masella, 2005). It also regulates and activates several transcription factors, such as AP-1, NF-κB, and Sp-1.

iv Carotenoids (β-Carotene)

Carotenoids are pigments found in plants. Primarily, β-carotene has been found to react with peroxyl (ROO•), hydroxyl (•OH), and superoxide ($O_2^{-\cdot}$) radicals (El-Agamey, 2004). Carotenoids show their antioxidant effects in low oxygen partial pressure but may have pro-oxidant effects at higher oxygen concentrations. Both carotenoids and retinoic acids (RAs) are capable of regulating transcription factors β-Carotene inhibits the oxidant-induced NF-κB activation and interleukin (IL)-6 and tumor necrosis factor-α production. Carotenoids also affect apoptosis of cells. Antiproliferative effects of RA have been shown in several studies. This effect of RA is mediated mainly by retinoic acid receptors and vary among cell types. In mammary carcinoma cells, retinoic acid receptor was shown to trigger growth inhibition by inducing cell cycle arrest, apoptosis, or both (Donato, 2005)

2.6 Diabetes Mellitus and Electrolyte Disorders

Diabetes mellitus is associated with disorders of electrolytes. The main substrate in carbohydrate, protein and lipid metabolism is glucose, an osmotically active substrate. The movement of glucose into the blood stream causes hyperglycaemia and if this persist, it may result in glucose intolerance (Stull, 2016). Glucose is osmotically active and therefore its movement causes imbalance in electrolyte either through increase or decrease in the level of these electrolytes. For instance, sodium ions are more concentrated intracellularly and any increase in circulating blood glucose will cause more Na^+ to leave the cell into the extracellular environment (Liamis, 2014). This diminishes the level of Na^+ in the cell causing hyponatremia. Na^+ and K^+ imbalance could also be due the loss through the renal tubule since glucose is excreted in urine (Glucosuria) (Sousa, 2016).

Hyperglycemia also induces hypomagnesaemia and hypocalcaemia. This occurs because magnesium ions are necessary for the synthesis of parathyroid hormone. Therefore, deficiency of parathyroid hormone affects calcium homeostasis (Arpaci, 2015). Parathyroid hormone is known to act by causing bone resorption, calcium reabsorption from the renal tubule and the small intestine. The defect in parathyroid hormone production may affect these processes leading to low calcium ions in the blood.

Bicarbonate ions are blood buffers and they act by removing excess hydrogen ions from the blood. In hyperglycaemic conditions, body cells do not utilize the excess glucose for energy production and therefore switch to other forms or source of energy such as lipids and proteins which even produce more energy than carbohydrates. In the process of breaking fat to release energy, the body produces acetic acid and ketones which release excess hydrogen ions into the blood thereby decreasing the pH of blood and causing metabolic acidosis (Chiasson, 2003).

2.7 Renal Function in Diabetic Nephropathy

Diabetic nephropathy is the major cause of end-stage renal disease, morbidity and mortality in many affected population. Kidney damage or dysfunction occurs in stages and is often associated with sclerosis and disturbances in glomerular filtration rate. Mortality is often prevented if the condition is diagnosed mostly at the early stage and treated.

According to International Diabetes Federation, the global prevalence of diabetes mellitus is said to increase in the nearest future and complications of diabetes are the major reasons why many individuals visit the hospital to present the condition. Some of the risk factors for diabetic nephropathy include hypertension, obesity, insulin resistance, increase adipotectin, free fatty acid, urea and albuminurea (Ramachandran *et al*., 2007). In order to manage nephropathy, the underlying cause must first be treated. For instance, medications useful for the treatment of hypertension must be administered in other to regulate blood pressure. When hyperglycaemia persist for a long period of time, it may activate the G-protein coupled receptor (a transmembrane receptor) found on the surface of the renal tubular cells. This receptor, have attached to it, a Gq trimeric subunits made up of alpha, beta and gamma subunits (Klag *et al.*, 1996). Stimulation of this receptor activate the alpha subunit which in turn activate phospholipase C (PLC) on membrane of renal cells. Phospholipase C breakdown the phospholipid phosphatidyl inositol 4,5 biphosphate (PIP_2) on the membrane to inositol 3 phosphate (IP_3) and diacylglycerol (DAG) which are second messengers. The IP_3 act by binding to receptors on the sarcoplasmic recticulum and releasing Ca^{2+} which phosphorylate protein kinase C while diacylglycerol acts by directly stimulating protein kinase C present within the cell. Upon activation of PKC, there is increase synthesis of cytoskeletal materials, some of which include type I and IV collagen, fibronectin, laminin, extracellular cell matrix.

All these materials are deposited within the glomerulus and tubule of the renal cells leading to sclerosis, mesangial expansion, decrease renal blood flow (RBF), decrease renal perfusion and ischemia causing the renal tissue to be susceptible to hypoxia and damage (Mohan *et al*., 2007). The cells of the efferent arterioles could sense decrease in renal blood flow to the glomerulus and secrete rennin. Once rennin is formed, it migrates to the liver where it converts angiotensinogen to angiotensin I. Another enzyme called angiotensin

converting enzyme in the lungs convert angiotensin I to angiotensin II. This causes vasoconstriction therefore, decreasing the synthesis of endothelial derived nitric oxide synthase, a potent vaso-dialator. Angiotensin II increases sympathetic stimulation of blood vessels and also stimulate the thirst centres in the brain to increase fluid intake. Angiotensin II increases the synthesis of a peptide hormone called antidiuretic hormone (ADH) or vasopressin (Javanmard, 2021). This enters the blood and move to the kidney where it acts and bind on principal cells and via the second messenger system, increases aquaporin water channels which fuse to renal cell membrane to increase reabsorption of water and electrolytes from the lumen of the kidney back into the blood (Lu, 2016). Angiotensin II also stimulate aldosterone production from the adrenal cortex which enters the blood and migrates to the kidney to stimulate sodium reabsorption. Aldosterone being a steroid hormone penetrate the renal tubular membrane and increase the synthesis of water channels and subsequently increase sodium and water reabsorption (Muñoz-Durango, 2016). Therefore, angiotensin II act in order to increase extracellular fluid volume, blood volume and blood pressure. That's why many patients with diabetic nephropathy are often presented with renal hypertension.

2.8 Apoptosis in the Pathophysiology of Diabetes Mellitus

Apoptosis is referred to as programmed cell death. It is a physiological cell death which is in contrast to necrosis, a cell death due to injury or disease. Apoptosis has been implicated in the etiology of diabetes mellitus (Sadeghi, 2016). Diabetes increases the production of reactive oxygen species and these increase the production of pro-apoptotic proteins in the cell cytoplasm. Pro-apoptotic proteins binds to the membrane of mitochondria to increase their permeability to cytochrome C of the electron transport chain which would activate cascade of caspases within the cytoplasm (Seervi, 2015). Caspases-3 is the final in the death signaling pathway and the main cause of cell death or apoptosis.

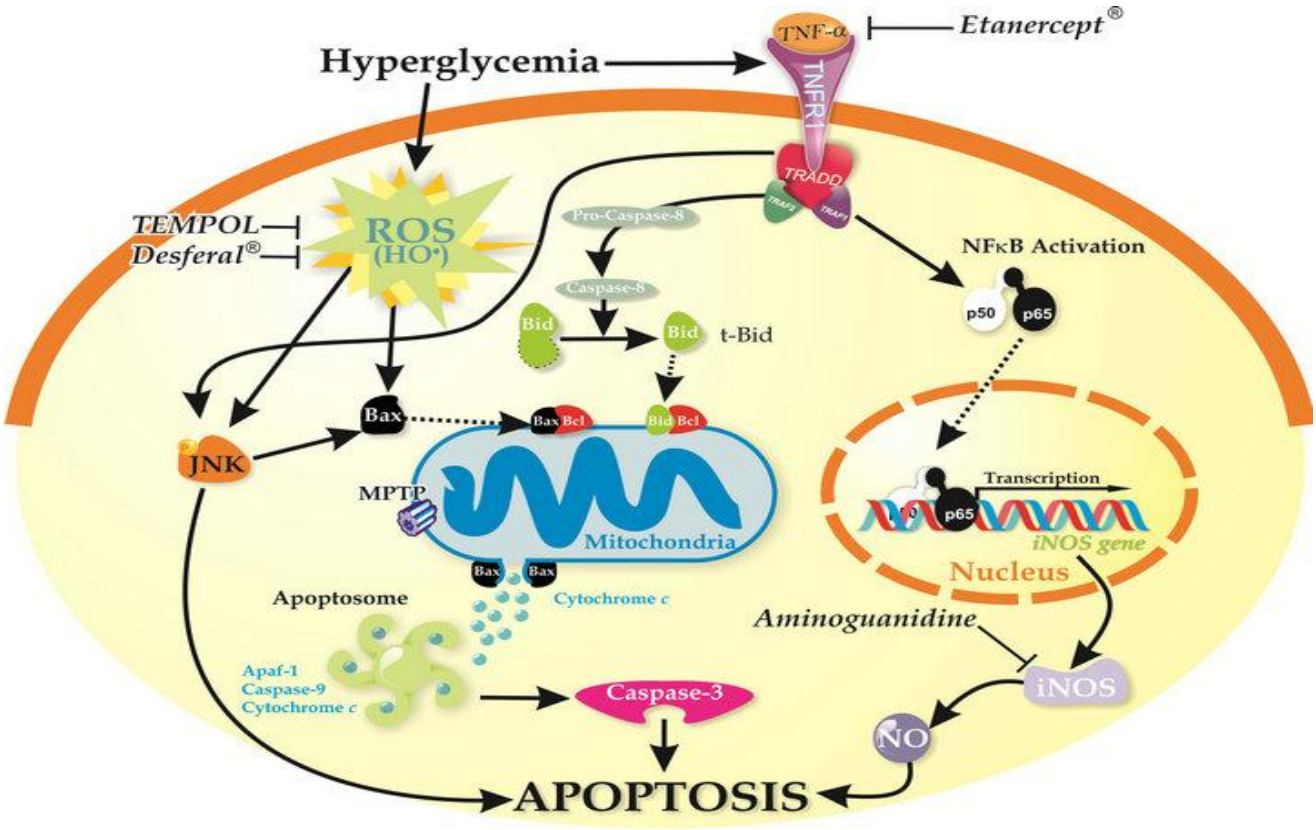

Fig 2.1. Hyperglycemia-induced apoptosis (Green *et al*., 2005)

2.9 Diabetic Nephropathy

Diabetic nephropathy is the leading cause of end-stage renal disease (ESRD) worldwide (Sulaiman, 2019). In patients with type I diabetes, 25–40% will develop diabetic nephropathy. Over 5% of newly diagnosed patients with type II diabetes will already have diabetic nephropathy and a further 30–40% will develop diabetic nephropathy with a high likelihood of progression to ESRD (Wang, 2019). With the continuous expansion of the population with type II diabetes, it is necessary to define the pathophysiologic mechanisms for this disorder in order to design specific therapies for prevention and reversal of diabetic nephropathy.

Several biochemical pathways have been proposed to explain the adverse effects of hyperglycemia. These include Activation of diacylglycerol (DAG)-protein kinase C (PKC) pathway, enhanced polyol pathway, increased oxidative stress, and overproduction of advanced glycation end products (Iwai, 2018).

2.9.1 Protein kinase C

PKC is a family of serine/threonine kinases that consist of isoforms. PKC isoforms are classified according to whether they contain domains that bind Ca2 þ or DAG, both of which positively regulate the kinase activity. Conventional PKC (a, b1, b2, and g) binds both Ca2 þ and DAG, novel PKC (d, e, Z, y, m) binds DAG, but not Ca2 þ, and atypical PKC (z, l) binds neither (Mochly-Rosen, 2012). Chronic activation of PKCs require sustained elevations of DAG, which involves the activation of phospholipase D/C or the de novo synthesis of DAG. In hyperglycemic and diabetic state, all of these pathways probably contribute to the activation of DAG-PKC cascade.

2.9.2 Mechanisms of hyperglycemia-induced PKC activation

Increased total DAG contents have been reported in a variety of tissues associated with diabetic vascular complications, including renal glomeruli, heart, aorta and retina from diabetic animal models and patients (Bhattacharjee, 2016). High glucose concentrations can cause increased de novo DAG synthesis through several different metabolic pathways. One proposed mechanism is that increased synthesis of DAG is caused by inhibition of the

glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase, thereby diverting upstream metabolites from glycolysis into pathways of glucose over utilization. This results in increased flux of dihydroxyacetone phosphate to DAG. There is also evidence that the increased levels of DAG can also be derived from the actions of phospholipase D from phosphatidylcholine (Momchilova, 1999). Another supportive evidence that the increased DAG levels are derived from pathways other than the activation of phospholipase C is the findings that palmatate and oleate are the major fatty acids in the elevated levels of DAG (Sahonero-Canavesi, 2015). Furthermore, there is evidence that oxidants and glycated products can also increase DAG levels and activate PKC.

2.9.3 PKC activation in diabetic nephropathy

Diabetic nephropathy is characterized by initial glomerular hyperfiltration, progressive accumulation of ECM in glomerular mesangium and tubulointerstitium, and progressive renal insufficiency. Hyperglycemia-induced metabolic, hemodynamic, and possibly inflammatory factors are thought to be mediating these injuries (Kawanami, 2016). Glomerular hyperfiltration Increased glomerular filtration rate is described in the kidney of diabetic patients and animal models. This alteration is likely to be the result of hyperglycemia-induced decreases in arteriolar resistance, leading to an elevation of glomerular filtration pressure (Wada, 2013). Multiple mechanisms have been proposed to explain the increases in glomerular filtration rate and filtration pressure, including an enhanced activity of angiotensin II and prostaglandin productions. Activation of DAG-PKC pathway may play a role in both the enhancement of angiotensin II actions and increases in vasodilatory prostaglandins. The enhanced production of prostaglandin E2 induced by diabetes and hyperglycemia could be the result of sequential activation of PKC and cytosolic phospholipase A2, a key regulator of arachidonic acid synthesis (Haneda, 2001). Increases in the activities of nitric oxide (NO) may also enhance glomerular filtration rate. Urinary excretion of NO2 and NO3, metabolites of NO, has been reported to be increased in diabetes of short duration, possibly due to enhanced expression of inducible NO synthase gene and increased production of NO in mesangial cells (Sifuentes-Franco, 2018). In addition, both increases in inducible NO synthase gene expression and NO production can be mimicked by PKC agonists and inhibited by PKC inhibitors when induced by

hyperglycemia, suggesting that NO production might be increased in diabetes through PKC-induced upregulation of inducible NO synthase. However, it has been reported that NO and its second messenger, cyclic guanosine monophosphate productions were decreased in diabetic rat glomeruli and PKC inhibitors restore the glomerular cyclic guanosine monophosphate production (Campo, 2010). Thus, it is possible that high glucose-induced PKC activation may regulate renal hemodynamics by increasing or decreasing NO production depending on the type of cells and duration of hyperglycemia. Lastly, recent studies have also shown that the expression of vascular endothelial growth factor (VEGF) is also increased in the glomeruli similar to the retina (Wang, 2012). PKC activation is also known to mediate several biological actions of VEGF including its effect to increase capillary permeability. As VEGF can also increase blood flow and capillary permeability, it is possible that increased VEGF levels and activity may contribute to the abnormality of renal hemodynamic (El-Mahdy, 2016). Accumulation of ECM Thickening of glomerular basement membrane and accumulation of ECM in glomerular mesangium and tubulointerstitium are the hallmark of diabetic nephropathy. It has been reported that high glucose increased production of type IV collagen and fibronectin in mesangial cells (Geraldes, 2010). This finding could be mimicked by phorbol ester, PKC agonist, and reversed by general PKC inhibitors. Further, there are great deal of support that hyperglycemia induced increases in oxidant production via several mechanisms. In the kidney, several reports have suggested that hyperglycemia can increase PKC activities leading to activation of several isoforms of nicotinamide adenine dinucleotide phosphate (reduced form; NADPH) oxidases to produce the excessive oxidants (Zhou, 2018). The elevated levels of oxidants in combination with PKC induced activations of mitogen-activated protein kinase will lead to the overexpression of fibrotic growth factors. Many studies have suggested that transforming growth factor (TGF)-b1 play a key role in the accumulation of ECM (Lagranha, 2007). It has been reported that PKC activation can increase the production of ECM and TGF-b1 expression, and that PKC inhibitors can prevent hyperglycemia or diabetes-induced increases in ECM accumulation and TGF-b1 production in mesangial cells or renal glomeruli. Recently, we have reported that the excessive productions of TGF-b1 and connective tissue growth factor, and ECM proteins induced by diabetes were significantly reduced in PKCb-null mice as compared with their

wild-type controls (Thallas-Bonke, 2014). In addition, diabetes also induced significantly less activation of NADPH oxidase subunits, NOX 2 and 4 expression further support a regulatory role of PKC activation in the excessive production of oxidants, fibrotic factors, and ECMs observed in the renal glomeruli in diabetic state. Vascular permeability and albuminuria Increased vascular permeability is another characteristic systemic vascular abnormality in diabetic animals, suggesting endothelial cell dysfunction (Soetikno, 2011). PKC activation can directly increase the permeability of albumin and other macromolecules through barriers formed by endothelial cells, probably by phosphorylating cytoskeletal proteins forming the intracellular junctions. As well, PKC activation could also regulate vascular permeability and neovascularization via the expression of growth factors such as VEGF, which has been demonstrated as a key factor in the pathogenesis of diabetic retinopathy (Praidou, 2010). It is also not likely that the elevation of VEGF is responsible for the increases in capillary permeability observed in diabetes since several tissues such as the heart and peripheral limb vessels also have increases in vascular permeability, yet VEGF expressions are decreased in those tissues. In the kidney, however, the role of PKC activation in the genesis of albuminuria is not clearly defined (Warren, 2019). Given that the glomerular filtration barrier is a unique structure composed of three different parts including the glomerular endothelial cells, the basement membrane, and the podocytes, consideration should be given to the alteration of all of these components. Interestingly, little is known about the effects of PKC activation on the biology of glomerular endothelial cells and podocytes (Volpe, 2018). Recently, it was shown that hyperglycemia-induced downregulation of the negatively charged basement membrane heparan sulfate proteoglycan perlecan and increases in VEGF and VEGF receptor II were prevented in mice lacking PKCa, which also had a significant reduction in albuminuria It is likely that PKC activation has an important role in the thickening of basement membranes as the use of PKCb selective isoforms inhibitor, ruboxistaurin (RBX) was able to prevent both mesangial expansion and basement membrane thickening in diabetic db/db mice and hypertensive diabetic rats (Choi, 2019).

2.10 Herbalism

Herbalism is a traditional medicine practice that relied on the use of plants and plant extracts. Herbalism is also referred to as medical herbalism, herbology, and phytotherapy. Animal products and certain animal parts are also used in herbal medicine (Trogrlić, 2018). Many plants produce substances that are useful to the maintenance of health in humans and other animals. These include aromatic substances, most of which are phenols or their oxygen-substituted derivatives such as tannins (Vickers, 2001). In many cases, these substances (particularly the alkaloids) serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. Many of the herbs and spices used by humans to season food yield useful medicinal compounds.

2.10.1 Anthropology of herbalism

People all over the world have used thousands of local plants for treatment of diseases. Anthropologists have proposed that animals possess the potentials to seek out bitter parts of plants in response to diseases. This behavior arose because bitterness is an indicator of secondary metabolites. The risk of taken herbal medicine is minimal in both human and animals subject to experiment in times of sickness (Halberstein, 2005). People of different communities would have acquired a set knowledge through their instinct, trial and error on the use of herbal medicine. As this knowledge expanded over time, the role of the herbalist became noticed. This process may likely have occurred in different manners within a wide diversity of cultures (Butler, 2011).

Local healers often claim to have learned by observing that sick animals change their food preferences to bitter herbs they would normally reject. Field biologists have provided supporting evidence based on observation of diverse species, such as chimpanzees, chickens, sheep and butterflies (Buenz, 2005). Lowland gorillas take 90% of their diet from the fruits of *Aframomum Melegueta*, a relative of the ginger plant, a potent antimicrobial plant. Researchers from Ohio Wesleyan University have observed that some birds select nesting material rich in antimicrobial agents which protect their young from harmful bacteria. Sick animals tend to forage plants rich in secondary metabolites, such as tannins and alkaloids. Since these phytochemicals often have antiviral, antibacterial, antifungal and

antihelminthic properties, a plausible case can be made for self-medication by animals in the wild. Some animals have digestive systems adapted to cope with certain plant toxins. For example, the koala can live on the leaves and shoots of the eucalyptus, a plant that is dangerous to most animals. A plant that is harmless to a particular animal may not be safe for humans to ingest (Wang, 2008). A reasonable conjecture is that these discoveries were traditionally collected by the medicine people of indigenous tribes, who then passed on safety information and cautions. The use of herbs and spices in cuisine developed in part as a response to the threat of food-born pathogens. Studies show that in tropical climes where pathogens are the most abundant recipes are the most highly spiced (Heckler, 2008). Further, the spices with the most potent antimicrobial activity tend to be selected. In all cultures vegetables are spiced less than meat, presumably because they are more resistant to spoilage.

2.10.2 Herbs in history

In the written record, the study of herbs dates back over 5,000 years to the Sumerians, who described well-established medicinal uses for such plants as laurel, caraway, and thyme (Heckler, 2007). The Egyptians of 1000 B.C. are known to have used garlic, opium, castor oil, coriander, mint, indigo, and other herbs for medicine and the Old Testament also mentions herb use and cultivation, including mandrake, vetch, caraway, wheat, barley, and rye. The first Chinese herb book (or herbal), dating from about 2700 B.C., lists 365 medicinal plants and their uses - including ma Huang, the shrub that introduced the drug ephedrine to modern medicine.

The ancient Greeks and Romans made medicinal use of plants. Greek and Roman medicinal practices, as preserved in the writings of Hippocrates and - especially - Galen, provided the patterns for later western medicine. Hippocrates advocated the use of a few simple herbal drugs - along with fresh air, rest, and proper diet. Galen, on the other had, recommended large doses of drug mixtures - including plant, animal, and mineral ingredients (Wachtel-Galor, 2011). The Greek physician compiled the first European treatise on the properties and uses of medicinal plants, De Materia Medica. In the first century AD, Dioscorides wrote a compendium of more that 500 plants that remained an authoritative reference into the 17th century. Similarly important for herbalists and botanists of later centuries was the Greek

book that founded the science of botany, Theophrastus' Historia Plantarum, written in the fourth century B.C.Thyme from Project Gutenberg EBook of Culinary Herbs: Their Cultivation Harvesting Curing and Uses, by M. G. Kains (Petrovska, 2012). The uses of plants for medicine and other purposes changed little during the Middle Ages. The early Christian church discouraged the formal practice of medicine, preferring faith healing; but many Greek and Roman writings on medicine, as on other subjects, were preserved by diligent hand copying of manuscripts in monasteries. The monasteries thus tended to become local centers of medical knowledge, and their herb gardens provided the raw materials for simple treatment of common disorders (Šantić, 2017). At the same time, folk medicine in the home and village continues uninterrupted, supporting numerous wandering and settled herbalists. Among these were the "wise-women," who prescribed herbal remedies often along with spells and enchantments. It was not until the later Middle Ages that women who were knowledgeable in herb lore became the targets of the witch hysteria. One of the most famous women in the herbal tradition was Hildegard of Bingen. A twelfth century Benedictine nun, she wrote a medical text called Causes and Cures. Medical schools began to return in the eleventh century, teaching Galen's system. At the time, the Arabic world was more advanced in science than Europe (Riddle, 2002). As a trading culture, the Arabs had access to plant material from distant places such as China and India. Herbals, medical texts and translations of the classics of antiquity filtered in from east to west. Alongside the university system, folk medicine continued to thrive. Plants were burdened with a mass of both pagan and Christian superstition that often was more important than their actual properties. The continuing importance of herbs for the centuries following the Middle Ages is indicated by the hundreds of herbals published after the invention of printing in the fifteenth century (Swart, 2019). Marjoram from Project Gutenberg EBook of Culinary Herbs: Their Cultivation Harvesting Curing and Uses, by M. G. Kains The fifteenth, sixteenth, and seventeenth centuries were the great age of herbals, many of them available for the first time in English and other languages rather than Latin or Greek. The first herbal to be published in English was the anonymous Grete Herball of 1526. The two best-known herbals in English were The Herball or General History of Plants (1597) by John Gerard and The English Physician Enlarged (1653) by Nicholas Culpeper. Gerard's text was basically a pirated translation of a book by the Belgian herbalist Dodoens and his

illustrations came from a German botanical work. The original edition contained many errors due to faulty matching of the two parts (Ameri, 2015). Culpeper's blend of traditional medicine with astrology, magic, and folklore was ridiculed by the physicians of his day yet his book - like Gerard's and other herbals - enjoyed phenomenal popularity. The Age of Exploration and the Columbian Exchange introduced new medicinal plants to Europe. The Badianus Manuscript was an illustrated Aztec herbal translated into Latin in the16th century. The second millennium, however, also saw the beginning of a slow erosion of the pre-eminent position held by plants as sources of therapeutic effects (Soelberg, 2016). This began with the introduction of the physician, the introduction of active chemical drugs (like arsenic, copper sulfate, iron, mercury, and sulfur), followed by the rapid development of chemistry and the other physical sciences, led increasingly to the dominance of chemotherapy - chemical medicine - as the orthodox system of the twentieth century.

2.10.3 Role of herbal medicine in modern human society

The use of herbs to treat disease is almost universal among non-industrialized societies. A number of traditions came to dominate the practice of herbal medicine at the end of the twentieth century: The herbal medicine system, based on Greek and Roman sources. The siddha and Ayurvedic medicine systems from India Chinese herbal medicine (Chinese herbology) Unani-Tibb medicine Shamanic Herbalism Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including opium, aspirin, digitalis, and quinine (Yuan, 2016). The World Health Organization (WHO) estimates that 80 percent of the world's population presently uses herbal medicine for some aspect of primary health care. Herbal medicine is a major component in all traditional medicine systems, and a common element in Ayurvedic, homeopathic, naturopathic, traditional Chinese medicine, and Native American medicine (Ngo, 2013). The use of, and search for, drugs and dietary supplements derived from plants have accelerated in recent years. Pharmacologists, microbiologists, botanists, and natural-products chemists are combing the Earth for phytochemicals and leads that could be developed for treatment of various diseases. In fact, according to the World Health Organization, approximately 25% of modern drugs used in the United States have been derived from plants. Three quarters of plants that provide active ingredients for prescription drugs came to the attention of

researchers because of their use in traditional medicine. Among the 120 active compounds currently isolated from the higher plants and widely used in modern medicine today, 80 percent show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived (Thomford, 2018). More than two thirds of the world's plant species - at least 35,000 of which are estimated to have medicinal value - come from the developing countries. At least 7,000 medical compounds in the modern pharmacopoeia are derived from plants.

2.10.4 Biological background of herbalism

All plants produce chemical compounds as part of their normal metabolic activities. These include primary metabolites, such as sugars and fats, found in all plants, and secondary metabolites found in a smaller range of plants, some useful ones found only in a particular genus or species (Ekor 2014). Pigments harvest light, protect the organism from radiation and display colors to attract pollinators. Many common weeds have medicinal properties. The functions of secondary metabolites are varied. For example, some secondary metabolites are toxins used to deter predation, and others are pheromones used to attract insects for pollination. Phytoalexins protect against bacterial and fungal attacks. Allelochemicals inhibit rival plants that are competing for soil and light. Plants upregulate and downregulate their biochemical paths in response to the local mix of herbivores, pollinators and microorganisms (Seca, 2014). The chemical profile of a single plant may vary over time as it reacts to changing conditions. It is the secondary metabolites and pigments that can have therapeutic actions in humans which can be refined to produced drugs

Plants synthesize a bewildering variety of phytochemicals but most are derivatives of a few biochemical motifs. Alkaloids contain a ring with nitrogen. Many alkaloids have dramatic effects on the central nervous system. Caffeine is an alkaloid that provides a mild lift but the alkaloids in datura cause severe intoxication and even death (Wink, 2018). Phenolics contain phenol rings. The anthocyanins that give grapes their purple color, the isoflavones, the phytoestrogens from soy and the tannins that give tea its astringency are phenolics. Turpenoids are built up from terpene building blocks. Each terpene consists of two paired isoprenes. The names monoterpenes, sesquiterpenes, diterpenes and triterpenes are based

on the number of isoprene units. The fragrance of rose and lavender is due to monoterpenes. The carotenoids produce the reds, yellows and oranges of pumpkin, corn and tomatoes. Glycosides consist of a glucose moiety attached to an aglycone. The aglycone is a molecule that is bioactive in its free form but inert until the glycoside bond is broken by water or enzymes (Dhama, 2018). This mechanism allows the plant to defer the availability of the molecule to an appropriate time, similar to a safety lock on a gun. An example is the cyanoglycosides in cherry pits that release toxins only when bitten by a herbivore. The word drug itself comes from the Swedish word "druug", which means 'dried plant'. Some examples are inulin from the roots of dahlias, quinine from the cinchona, morphine and codeine from the poppy, and digoxin from the foxglove. The active ingredient in willow bark, once prescribed by Hippocrates, is salicin, or salicylic acid (Sucher, 2018). The discovery of salicylic acid, also known as "acetylsalicylic acid", would eventually lead to the development of "aspirin" when it was isolated from a plant known as meadowsweet. The word aspirin comes from an abbreviation of meadowsweet's Latin genus Spiraea, with an additional "A" at the beginning to acknowledge acetylation, and "in" was added at the end for easier pronunciation. "Aspirin" was originally a brand name, and is still a protected trademark in some countries. This medication was patented by Bayer AG.

2.10.5 Philosophy of herbal medicine

Since herbalism is such a diverse field few generalizations apply universally. Nevertheless a rough consensus can be inferred. Most herbalists concede that pharmaceuticals are more effective in emergency situations where time is of the essence. An example would be where a patient had elevated blood pressure that posed imminent danger (Yuan, 2016). However they claim that over the long term herbs can help the patient resist disease and in addition provide nutritional and immunological support that pharmaceuticals lack. They view their goal as prevention as well as cure. Herbalists tend to use extracts from parts of plants, such as the roots or leaves but not isolate particular phytochemicals. Pharmaceutical medicine prefers single ingredients on the grounds that dosage can be more easily quantified. Herbalists reject the notion of a single active ingredient (Ginsburg, 2011). They argue that the different phytochemicals present in many herbs will interact to enhance the therapeutic effects of the herb and dilute toxicity.

Furthermore, they argue that a single ingredient may contribute to multiple effects. Herbalists deny that herbal synergism can be duplicated with synthetic chemicals. They argue that phytochemical interactions and trace components may alter the drug response in ways that cannot currently be replicated with a combination of a few putative active ingredients (Mukherjee, 2017). Pharmaceutical researchers recognize the concept of Drug synergism but note that clinical trials may be used to investigate the efficacy of a particular herbal preparation, provided the formulation of that herb is consistent.

In specific cases the claims of synergy and multi-functionality have been supported by science. The open question is how widely both can be generalized. Herbalists would argue that cases of synergy can be widely generalized, on the basis of their interpretation of evolutionary history, not necessarily shared by the pharmaceutical community (Wells, 2011). Plants are subject to similar selection pressures as humans and therefore they must develop resistance to threats such as radiation, reactive oxygen species and microbial attack in order to survive. Optimal chemical defenses have been selected for and have thus developed over millions of years. Human diseases are multifactorial and may be treated by consuming the chemical defenses that they believe to be present in herbs. Bacteria, inflammation, nutrition and ROS (reactive oxygen species) may all play a role in arterial disease. Herbalists claim a single herb may simultaneously address several of these factors. Likewise a factor such as ROS may underlie more than one condition (Marzvanyan, 2018). In short herbalists view their field as the study of a web of relationships rather than a quest for single cause and a single cure for a single condition. In selecting herbal treatments herbalists may use forms of information that are not applicable to pharmacists. Because herbs can moonlight as vegetables, teas or spices they have a huge consumer base and large-scale epidemiological studies become feasible. Ethnobotanical studies are another source of information. For example, when indigenous peoples from geographically dispersed areas use closely related herbs for the same purpose that is taken as supporting evidence for its efficacy (Kim, 2014). Herbalists contend that historical medical records and herbals are underutilized resources. They favor the use of convergent information in assessing the medical value of plants. An example would be when in-vitro activity is consistent with traditional use. Certain strains of herbalism rely on sources that would be widely considered

unreliable and would not be accepted in a scientifically oriented herbal journal. These include astrology, the Bible, intuition, dreams, “plant spirits”, etc

2.10.6 Popularity of herbal medicine

A survey released in May 2004 by the National Center for Complementary and Alternative Medicine focused on who used complementary and alternative medicines (CAM), what was used, and why it was used. The survey was limited to adults, aged 18 years and over during 2002, living in the United States. According to this survey, herbal therapy, or use of natural products other than vitamins and minerals, was the most commonly used CAM therapy (18.9%) when all use of prayer was excluded (Welz, 2018). Herbal remedies are very common in Europe. In Germany, herbal medications are dispensed by apothecaries (e.g., Apotheke). Prescription drugs are sold alongside essential oils, herbal extracts, or herbal teas. Herbal remedies are seen by some as a treatment to be preferred to chemical medications which have been industrially produced (McIntyre, 2016). In the United Kingdom, the training of medical herbalists is done by state funded Universities. For example, Bachelor of Science degrees in herbal medicine are offered at Universities such as University of East London, Middlesex University, University of Central Lancashire, University of Westminster, University of Lincoln and Napier University in Edinburgh at the present

2.10.7 Types of herbal medicine systems

Use of medicinal plants can be as informal as, for example, culinary use or consumption of an herbal tea or supplement, although the sale of some herbs considered dangerous is often restricted to the public. Sometimes such herbs are provided to professional herbalists by specialist companies (Fürst, 2015). Many herbalists, both professional and amateur, often grow or "wildcraft" their own herbs. Some researchers trained in both western and traditional Chinese medicine have attempted to deconstruct ancient medical texts in the light of modern science. One idea is that the yin-yang balance, at least with regard to herbs, corresponds to the pro-oxidant and anti-oxidant balance (Xue, 2018). This interpretation is supported by several investigations of the ORAC ratings of various yin and yang herbs.

Eclectic medicine came out of the vitalist tradition, similar to physiomedicalism and bridged the European and Native American traditions. Cherokee medicine tends to divide herbs into foods, medicines and toxins and to use seven plants in the treatment of disease, which is defined with both spiritual and physiological aspects, according to Cherokee herbalist David Winston (Falzon, 2017). In India, Ayurvedic medicine has quite complex formulas with 30 or more ingredients, including a sizable number of ingredients that have undergone "alchemical processing", chosen to balance "Vata", "Pitta" or "Kapha." In addition there are more modern theories of herbal combination like William LeSassier's triune formula which combined Pythagorean imagery with Chinese medicine ideas and resulted in 9 herb formulas which supplemented, drained or neutrally nourished the main organ systems affected and three associated systems. His system has been taught to thousands of influential American herbalists through his own apprenticeship programs during his lifetime, the William LeSassier Archive and the David Winston Center for Herbal Studies (Ernst, 2005). Many traditional African remedies have performed well in initial laboratory tests to ensure they are not toxic and in tests on animals. Gawo, a herb used in traditional treatments, has been tested in rats by researchers from Nigeria's University of Jos and the National Institute for Pharmaceutical Research and Development. According to research in the African Journal of Biotechnology, Gawo passed tests for toxicity and reduced induced fevers, diarrhea and inflammation

2.10.8 Routes of administration of herbal remedies

There are many forms in which herbs can be administered, these include: Tinctures (alcoholic extracts of herbs such as echinacea extract. Usually obtained by combining 100% pure ethanol (or a mixture of 100% ethanol with water) with the herb (Boadu, 2017). A completed tincture has a ethanol percentage of at least 40-60% (sometimes up to 90%). Herbal wine and elixirs; these are alcoholic extract of herbs; usually with an ethanol percentage of 12-38% Herbal wine is a maceration of herbs in wine, while an elixir is a maceration of herbs in spirits (eg vodka, grappa) Tisanes (hot-water extracts of herb, such as chamomile) Decoctions (long-term boiled extract of usually roots or bark) Macerates (cold infusion of plants with high mucilage-content as sage, thyme (Semenya, 2012). Plants are chopped and added to cold water. They are then left to stand for 7 to 12 hours (depending

on herb used). For most macerates 10 hours is used. Vinegars (prepared at the same way as tinctures).

i. **Topicals**: Essential oils- application of essential oil extracts, usually diluted in a carrier oil (many essential oils can burn the skin or are simply too high dose used straight-diluting in olive oil or another food grade oil can allow these to be used safely as a topical).Salves, oils, creams and lotions- Most topical applications are oil extractions of herbs. Taking a food grade oil and soaking herbs in it for anywhere from weeks to months allows certain phytochemicals to be extracted into the oil (Leppert, 2018). This oil can then be made into salves, creams, lotions, or simply used as an oil for topical application. Many massage oils, antibacterial salves and wound healing compounds are made this way.

ii. **Poultices and compresses**: One can also make a poultice or compress using whole herb (or the appropriate part of the plant) usually crushed or dried and re hydrated with a small amount of water and then applied directly in a bandage, cloth. Whole-herb consumption. This can occur in eather dried form (herbal powder) or fresh (juice, fresh leaves and other plant parts). - Just as Hippocrates said "Let food be thy medicine", it has become clear that eating vegetables also easily fits within this category of getting health trough consumables (besides medicinal herbs) (Wodah, 2012). All of the vitamins, minerals and antioxidants are phytochemicals that we are accessing through our diet. There are clearly some whole herbs that we consume that are more powerful than others. Shiitake mushrooms boost the immune system and taste great so they are fabulous in soups or other food preparations for the cold and flu season. Alfalfa is also condisidered a health food and can be used as a regular vegetable in diet (Agyare, 2009). Guarana and Gotu kola may be used to speed up metabolism (and thus physical performance). Ginseng-species may also provide physical (and mental) benefits. Garlic lowers cholesterol, improves blood flow, fights bacteria, viruses and yeast.

ii. **Syrups**: extracts of herbs made with syrups or honey. 65 parts of sugar are mixed with 35 parts of water and herb. The whole is then boiled and macerated for 3 weeks.

iii. **Extracts:** include liquid extracts, dry extracts and nebulisates. Liquid extracts are liquids with a lower ethanol percentage than tinctures. They can (and are usually) made by

vacuumly distilling tinctures. Dry extracts are extracts of plant material which are evaporated into a dry mass. They can then be further refined to a capsule or tablet. A nebulisate is a dry extract which has been created by freeze-drying (Gumisiriza, 2019).

iv. Inhalation: as in aromatherapy can be used as a mood changing treatment to fight a sinus infection or cough, or to cleanse the skin on a deeper level (steam not direct inhalation here) (Tandon, 2017).

2.10.9 Risks associated with herbal remedies

Proper double-blind clinical trials are needed to determine the safety and efficacy of each plant before they can be recommended for medical use. In addition, many consumers believe that herbal medicines are safe because they are natural. Herbal medicines may interact with synthetic drugs causing toxicity to the patient, herbal products may have contamination that is a safety consideration, and herbal medicines, without proven efficacy, may be used to replace medicines that have a proven efficacy (Ekor 2014). Standardization of purity and dosage is not mandated in the United States, but even products made to the same specification may differ as a result of biochemical variations within a species of plant. Plants have chemical defense mechanisms against predators that can have adverse or lethal effects on humans (Zhou, 2013). Examples of highly toxic herbs include poison hemlock and nightshade. They are not marketed to the public as herbs, because the risks are well known, partly due to a long and colorful history in Europe, associated with "sorcery", "magic" and intrigue. Although not frequent, adverse reactions have been reported for herbs in widespread use. On occasion serious untoward outcomes have been linked to herb consumption. A case of major potassium depletion has been attributed to chronic licorice ingestion Black cohosh has been implicated in a case of liver failure (Rodrigues, 2013). Few studies are available on the safety of herbs for pregnant women. Herb drug interactions are a concern. In consultation with a physician, usage of herbal remedies should be clarified, as some herbal remedies have the potential to cause adverse drug interactions when used in combination with various prescription and over-the-counter pharmaceuticals (Rousseaux, 2003). Dangerously low blood pressure may result from the combination of an herbal remedy that lowers blood pressure together with prescription medicine that has the same

effect. Some herbs may amplify the effects of anticoagulants. Certain herbs as well as common fruit interfere with cytochrome P450, an enzyme critical to drug metabolism.

2.11 *Parquetina nigrescens* (Afzel.)

Origin and geographic distribution of *Parquetina nigrescens*

Parquetina nigrescens occurs a large part of Africa, from Senegal east to Sudan and from Senegal south through Central and East Africa to Zambia, Angola and eastern Zimbabwe.

Fig 2.2. *Parquetina nigrescens* leaves (Afzel.) Bullock (Odetola *et al*., 2006)

Genus: *Parquetina*

Family: *Apocynaceae*

Subfamily: *Periplocoideae*

Place of publication: Kew Bull. 15:205. 1961

2.11.1 Uses of *Parquetina nigrescens*

Parquetina nigrescens is widely used in traditional medicine. Usually small amounts are used, as the plant is very toxic, especially the latex. Many fatal accidents have been recorded. A plant or leaf decoction is taken as an enema to treat serious kidney problems, severe constipation and to induce abortionb (Kayode, 2017). Sometimes fresh crushed leaves are taken as an emetic to treat severe constipation. A plant or leaf decoction or infusion, sometimes with parts of other plant species, is drunk to treat measles, intestinal worms, diarrhoea, dysentery, diabetes, menstrual disorders and venereal diseases (Ozaslan. 2011) In very small quantities, it is given to children to treat respiratory diseases. A leaf decoction with honey added is drunk to treat fatigue, jaundice, stomach ulcers and anemia, as a tonic. It is also taken to treat hypotension and to ease child birth (Kola-Mustapha, 2019). The body is washed with a leaf decoction to treat general fatigue. The leaves are a common ingredient in medications to treat insanity.

The latex and leaf sap cause a burning sensation on the skin and are externally applied to tumours, abscesses, sores and burns; they also blacken scars and are applied to thorns in the skin to extract them. Leaves are applied as a dressing for wounds; crushed leaves are applied to skin diseases and head lice (Owoyele, 2009). A leaf maceration is applied to the legs of children with rachitis, to the head to treat headache and to the side to treat pain in the side. Pulverized bark is applied to skin incisions to treat rheumatism. Leaf sap, without latex, is used as eye drops to treat conjunctivitis and jaundice (Ochigbo, 2017). The latex is considered to cause blindness.

Pulverized root bark, rubbed on the body, is considered a potent aphrodisiac. Crushed roots with *Capsicum* peppers are used as an enema to treat venereal diseases. Crushed roots mixed with shea butter (*Vitellaria paradoxa* C.F.Gaertn.) is rubbed on hands and legs as a snake repellent. Roots paste is also applied to snakebites and a root decoction is drunk for the same purpose. Root paste is also applied to scarification on the joints to treat convulsions in children (Oyagbemi, 2018). The liquid of boiled roots with lemon juice is taken to treat nicotine poisoning; it is a tobacco revulsive.

The latex is very toxic and widely used as an ingredient of arrow poison, especially in Central Africa, to hunt bush meat. The latex is used alone, or mixed with other plants,

depending on the traditions of the poison maker and availability of these plants. It is used in a similar way as *Strophanthus* and *Acokanthera* arrow poisons. Sometimes the whole crushed plant is thrown in streams to stupefy fish. The inner bark fibre is flexible, white and strong, and is widely used to make fishing nets and fishing lines of excellent quality. The stems are also used as rope (Mahmud, 2020). The latex is white, hardening to black and has been exploited in DR Congo in the past to yield black rubber. The quality varies from excellent to poor, depending on the plant, and has been used as an adulterant for better quality rubber. The flowers are quite showy and have ornamental potential.

2.11.2 Properties of *Parquetina nigrescens*

Parquetina nigrescens is rich in cardiac glycosides (cardenolides). These glycosides, collectively called strophanthins, are most abundant in the latex and are responsible for the activity in arrow poison. The composition of the cardenolides varies depending on the provenance of the plants. One twig sample contained as main compound strophanthidin and low amounts of nigrescigenin. Strophantidol, strophanthidin-β-D-glucoside and several unidentified glycosides occurred in trace amounts (Akinrinmade, 2019). Another twig sample contained moderate amounts of 16-dehydrostrophanthidin and strophanthidin, and a range of glycosides, including strophadogenin and convallatoxin, in trace amounts. From the roots strophanthidin, cymarin and an unidentified strophanthidin glycoside were isolated, as well as the sterols β-sitosterol, campesterol and stigmasterol, and the triterpenes α- and β-amyrine. From the leaves and stem bark no cardenolides were isolated (Agarwal, 2016). From the leaves the triterpene ursolic acid as well as the flavonoids apigenin and isorhoifolin (apigenin-7-O-rutinoside) and vitamins A and C were isolated.

A hydromethanolic leaf extract exerted a stimulating and spasmogenic action on the uterine smooth muscle of pregnant rats in vitro. A leaf extract induced the contraction force and rate dose-dependently in an isolated guinea-pig heart in vitro

Diabetic rats treated orally with an aqueous leaf extract showed significant reduction of the blood glucose to levels comparable to that of the non-diabetic control and those treated with

a standard drug. In normoglycaemic rats, hypoglycaemic action of the leaf extracts was observed to be dose dependent, with prolonged hypoglycaemia at higher doses.

An aqueous extract significantly reduced gastric acid secretion, reduced significantly ethanol-induced gastric ulceration and caused elevation in gastric mucus secretion in rats. Hexane, chloroform and methanolic leaf extracts showed significant anti-oxidant activity in vitro. Rats given methanol, hexane and chloroform extracts of the leaves prior to ethanol-induced ulcer in rats showed a significant gastro-protective and antiulcer effect (Wani, 2011). An aqueous leaf showed significant analgesic, anti-inflammatory and antipyretic effects in rats.

The aqueous fraction of an ethanolic root extract improved the morphology of sickled cells in vitro, and also inhibited and reversed the sickling process in a moderate way. An aqueous leaf extract orally administered to anaemic rats showed a significant and dose-dependent increase of blood parameters. A study was carried out to determine the effect of a herbal preparation, containing *Parquetina nigrescens*, *Sorghum bicolor* and *Harungana madagascariensis*, on blood parameters in anaemic rabbits, caused by an infection with *Trypanosoma brucei brucei* (Han, 2016). The anaemic rabbits not treated with the herbal preparation died before the end of the study, while for the treated anaemic rabbits the blood parameters were restored to normal levels. An aqueous leaf extract showed moderate antimicrobial activity against a range of pathogenic bacteria. The ethanol extract was effective only against *Pseudomonas aeruginosa* and *Salmonella typhi*. Crude hot and cold water and ethanolic extracts of the leaves showed moderate antibacterial activity against *Salmonella typhi (*Azeez, 2010). An aqueous leaf extract showed moderate anthelmintic activity against parasitic (*Onchocerca ochengi*) and free-living (*Caenorhabditis elegans*) nematodes

2.11.3 Other botanical information on *Parquetina*

Parquetina was formerly included in *Periploca*, but morphological and DNA sequence analyses support the separation of 2 species in a different genus, despite their considerable

difference in flower morphology. A conspicuous characteristic of both species is that they turn black when dry.

i. *Parquetina calophylla*

The other species, *Parquetina calophylla* (Baill.) Venter (synonyms: *Ompalogonus calophyllus* Baill., *Periploca calophylla* (Baill.) Roberty), occurs from Côte d'Ivoire east to Tanzania. During some time it has been considered synonymous with *Parquetina nigrescens*, and the medicinal uses described by various sources may refer to the wrong species (Huang, 2019). In Côte d'Ivoire and Burkina Faso leaves and bark, mixed with parts of other medicinal plants, are macerated in water and heated in the sun. After filtering the liquid is taken to treat liver complaints. In Nigeria the dried, crushed leaves in water are taken to clean the liver in case of jaundice. The latex is used as arrow poison.

2.11.4 Prospect of using *Parquetina nigrescens*

Although the latex of *Parquetina nigrescens* is very toxic, the plant has many medicinal uses. Mainly the leaves are used, which are considered less toxic, and these are most often used in pharmacological trials, to evaluate its use to treat diabetes, sickle cell anaemia and stomach ulcers (Kayode, 2017) Although the results are promising in rats, more research is needed to evaluate its potential and safety in humans.

CHAPTER THREE

3.0 Materials and Methods

3.1 Collection of plant materials, extraction and GC-MS studies of methanol extract of *Parquetina nigresens* (MEPN)

The *Parquetina nigrescens* leaves used for the study were obtained from the outskirt of the University of Ibadan. Plants materials were identified at Botany Department, University of Ibadan with vouchers specimen number 22475. The leaves of the harvested plant were air-dried and then ground to powder using Thomas miller machine. 1 kg of the powder was extracted using cold maceration method with 5L of methanol as solvent. The mixture were filtered and the filtrate evaporated using regulated water bath (maintained at 90^{o}C) to produce a solid dark extract. About 1g of MEPN was used for a Gas Chromatography-Mass Spectrometry Study (GC-MS) study which revealed the active compounds in MEPN.

3.2 Toxicological Study and Median Lethal Dose (LD_{50}) of MEPN

Thirty-five rats weighing between 100-150 g were used in this study and were divided into seven groups of five animals in a group. Group 1 received 0.3 mL distilled water; Groups 2-7 were orally given graded doses of MEPN at 500 mg/kg, 1000 mg/kg, 2000 mg/kg, 3000 mg/kg, 4000 mg/kg and 5000 mg/kg respectively. Animals were placed under continuous observation for 6 hours. After 24 hours, rats were sacrificed under mild anesthesia (Sodium thiopental 30 mg/kg *i.p*) to observe changes in internal structure (OECD, 2001). The median lethal dose was calculated according to the method of Karber *et al.* (1931) and modified by Erhirhie *et al*. (2018) .This method involves the administration of different doses of test substance to various groups, which has five animals each. The first group of animals is administered with the vehicle in which the test substance was dissolved or diluted in (e.g, water or normal saline). However, from the second group onward receives different doses

of the test substance. The animals in each group receives specific doses, while increment in dose progresses from group to group (starting from group 2 which receives the lowest dose). The interval mean of a number of mortality recorded in each group and dose difference across the groups are key parameters in this method. The LD_{50} is calculated using the arithmetical method of Karber, which is as follow:

$$LD_{50} = LD_{100} - \sum \left(\frac{a \times b}{n} \right)$$

Where, LD_{50} = Median lethal dose

LD_{100} = Least dose required to kill 100%

a = Dose difference

b = Mean mortality

n = Group population.

3.3 Experimental Animals

A total of one hundred Wistar rats weighing between 90-130 g were utilized in this research. The rats were purchased from the central animal house at the College of Medicine, University of Ibadan. The animals were kept in air conditioned room (temperature 22±2^{o}C) and were allowed to acclimatize for two weeks duration and water and food were given *ad-libitum.*

3.4 Experimental Design

The study was conducted in two phases;

Study 1: Fifty Wistar rats were used: twenty-five rats were used for intestinal glucose absorption study while another twenty-five rats were used for other experiments. Animals were divided into 5 groups of 5 rats per group. Group 1 served as normal control, group 2 served as diabetic untreated, groups 3, 4 and 5 were diabetic treated with 100 mg/kg MEPN, 200 mg/kg MEPN and 100 mg/kg Metformin respectively (Kola-Mustapha, 2019).

Study 2: Fifty Wistar rats were used: twenty-five rats were used for intestinal glucose absorption study while another twenty-five rats were used for other experiments. Animals were divided into 5 groups of 5 rats per group. Group 1 served as normal control, group 2 served as diabetic untreated, groups 3, 4 and 5 were diabetic treated with 250 mg/kg Phytol, 3 mg/kg Squalene and 100 mg/kg Metformin respectively (Elmazar, 2013; Anisimov, 2013; Gohil, 2019)

3.5 Method of Diabetes Induction

Diabetes was induced after a 24-hour fasting in group 2, 3, 4 and 5 by single intra-peritoneal injection of alloxan monohydrate (Sigma Aldrich, U.S.A) at a dose of 120 mg/kg as described by Djurhuus *et al*. (2000). After 72 hours of alloxan administration, only animals with fasting blood glucose level of 250 mg/kg and above were taken as diabetic and selected for these studies.

3.6 Blood Collection

After 28 days post-treatment with MEPN, phytol and squalene, rats were given mild anesthesia (Sodium thiopental 30 mg/kg *i.p*) and blood samples were gotten from the retro-orbital sinus of the animals with heparinized microcapillary tubes as described by Parasuraman et al., (2010) into clean dry test tubes and were allowed to centrifuge at 3000 rpm for 10 minutes to obtain the serum which was frozen at -20°C. The animals were sacrificed through cervical dislocation and opened up through mid-line laparotomy. The liver, kidney and pancreas were excised and fixed in 10% formalin.

3.7 Purchase of Drugs used in this Study

Squalene and Phytol were purchased from Santa Cruz Biotechnology, Germany with Catalogue numbers sc-281155A and sc-250719, respectively.

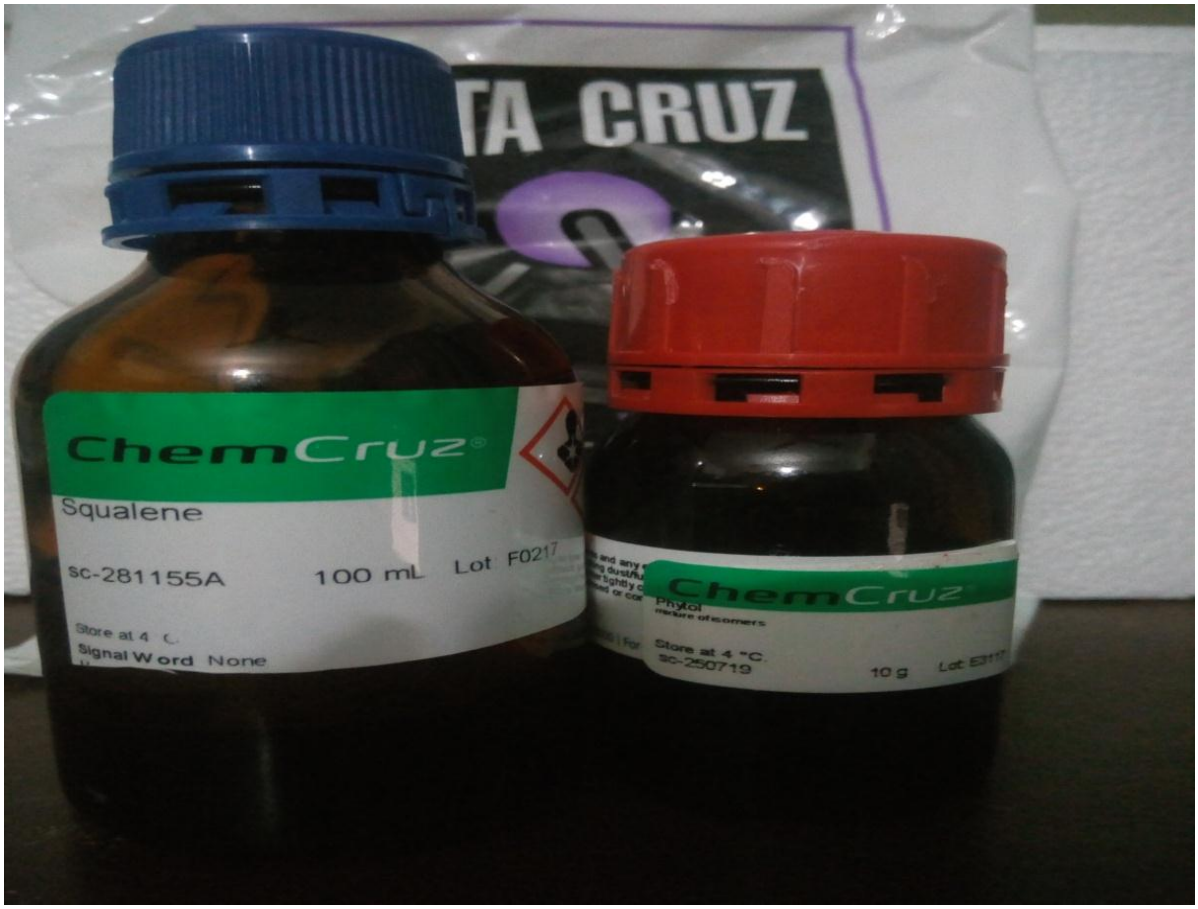

Figure 3.1. Bottles containing Phytol and Squalene

3.8 Ethical Considerations

The Wistar rats were purchased from the central animal house, Faculty of Basic Medical Sciences, University of Ibadan. Rats were housed in well aerated cages solid-bottomed polypropylene cages with wire-mesh covers for ventilation. They were fed with standard rat chow (Ladokun feed) and water *ad libitum*. The rats were allowed to acclamatize for a period of two weeks and monitored twice daily (morning and evening) and their beddings were changed daily. Physical examination was done to observe the health status of the animal. The maximum number of rats per cage was 5 in 3m x 5m cage. This research was approved by Animal Care and Use Research Ethics Committee (ACUREC), University of Ibadan, with certificate number: UI-ACUREC/18/0036

Sampling and Measurements

3.9 Determination of glucose concentration

Glucose concentration was measured using spectrophotometry procedure as described by Ambade *et al*. (1998). Glucose kits contains glucose oxidase reagents, glucose standard and a buffer. 0.3 mL of glucose reagent was mixed with the 0.1 mL buffer and placed in a tube. 0.3 mL of the glucose oxidase reagent was mixed with 0.1 mL glucose standards and placed in tubes. 3 mL of the glucose was mixed with 0.1 mL of each sample and placed in tubes. The mixture was incubated at a temperature of 40-50^{o}C and the absorbance was read at a wavelength of 510 nm in the spectrophotometer. The concentration of D-glucose in mg/dL was taken as sample absorbance over glucose standard absorbance multiplied 1000.

3.10 Measurement of intestinal glucose absorption

Glucose absorption was measured using intestinal ligation loop method as described by Chen *et al*. (2016). Each rat was opened up through a mid-line laparotomy after mild anesthesia (Sodium thiopental 30 mg/kg *ip*). Two regions of the intestine were identified for consistency. The ligament of Treiz and ileo-caecal junction were ligated using cannula, thread and syringe. This section of the small intestine was then rinsed using phosphate buffer saline to remove undigested waste that may have accumulated in this region. The middle of the small intestine was identified and ligated using thread in order to separate the duodenum from the jejunum. Using the syringe, 4 mL of kreb's bicarbonate solution was infused into each segment. The duodenum and jejunal glucose concentration was then taken at 0 min, 20 min, 40 min and 60 min. The glucose absorbed per unit length was taken to be the final glucose concentration minus the initial glucose concentration divided by length of each segment.

3.11 Determination of liver glycogen content

Liver glycogen content was determined using anthrone reagent as described by Mojibi, *et al*. (2017). The liver samples were harvested from each rat after a 24-hour fast. It was then digested in hot potassium hydroxide (KOH 30% concentrated). The liver glycogen precipitate was obtained by dissolving in alcohol. The precipitate was again dissolved in 10% trichloroacetic acid (TCA) after which it was centrifuged to sediment proteins. The

supernatant was decanted and re-precipitated with alcohol. The sedimentation was diluted with water and liver glycogen concentration measured spectrophotometrically using anthrone reagent at a wavelength of 620 nm. Glycogen concentration in mg/mL was taken as the absorbance of the sample divided by absorbance of standard multiply by volume of the extract multiply by mass of the tissue multiply by 100 multiply 0.9.

3.12 Determination of insulin concentration

Insulin ELISA kit was used for this procedure which was carried out at room temperature of 25-28^{o}C as described by Purohit *et al*. (2012). Enzyme conjugate and wash buffers were contained in the kit. The samples, insulin control solution and calibrators were also prepared. Insulin microplate wells are also contained in the kit and accommodate the samples. 25 µL of the calibrators (standards) were pipetted into appropriate wells. Thereafter, 100 mL of the enzyme conjugate was added to each well. The mixture was incubated at room temperature (25^{o}C) for about 2 hours. The mixture was thereafter discarded and about 350 µL of the wash solution was used to wash each well. The washing procedure was repeated five times to remove unreacted antigen-antibody complex. 200 µL of the substrate was then added into each well and incubated for another 15 min at room temperature. 50 µL of stop solution was added into each well. The reaction mixture was placed in a microplate reader and the optical density/absorbance was read at 450 nm. A calibrator curve was plotted. The absorbance was plotted against the standard insulin concentration. Insulin concentration of the samples were extrapolated from the calibrator curve.

3.13 Determination of catalase activity

The principle is based on the decomposition of hydrogen peroxide as described by Hadwan *et al*. (2018). Reaction standards and reaction samples were prepared. 0.05 mL Hydrogen peroxide substrate solution was placed in both the samples and the standards. 0.05 mL of assay buffer was added to the standards and to the samples. Both mixture were incubated for 5 min at 25^{o}C. 15 nM of Sodium azide as placed in each of the standard and sample. 40 µL of the mixture from the standard and sample were placed in cuvettes. The mixture was again incubated for 15 min at 25^{o}C. The standards and samples absorbance were read at 520

nm. The procedure was performed in triplicates. Catalase activity (U/mL) was calculated as change in absorbance of sample divided by change in absorbance of blank multiply by 28.85 multiplied by the dilution factor.

3.14 Determination of superoxide dismutase activity

Colorimetric kit was used to determined activity of superoxide dismutase in serum sample as described by Li *et al*. (2019). 920 µL of assay buffer from the kit was added to each cuvette. 40 µL of haematoxylin reagent was added to each cuvette to commence auto-oxidation reaction. The absorbance was read at 560 nm every 10 seconds for 5 minutes. SOD activity in U/mL was calculated by multiplying 1.25 times the percentage inhibition. Percentage is one minus the ratio of absorbance of the sample and the standards times 100%.

3.15 Determination of glutathione peroxidase activity

According to the assay procedure, a reaction mixture was prepared and assay conducted as described by Baumber *et al*. (2005). This contained the assay buffer, 40 nm NADPH solution and GSH solution. 40 µL of the reaction mix was added to samples and standard wells. These were mixed and incubated at room temperature for 15 minutes to reduce all GSSG in the samples. 10 µL of cumene hydroperoxide solution was added to the samples and the standards. The first absorbance A_1 was measured. The reaction mixture was again incubated for 5 min at 25^{o}C and absorbance measured at a wavelength 340 nm. Thereafter, the second absorbance A_2 was measured. The corrected absorbance was plotted against the standard solutions of NADP. Once the concentration of NADP was noted, GPx activity (nmol/min/mL) was calculated as concentration of NADPH divided by difference in absorbance multiply by the dilution factor.

3.16 Determination of Albumin

This assay was done using colorimeter procedure as described by Busher *et al*. (1990). 1.0 mL of the reagent was placed each in blank, sample and standard. 5 µL of the sample was added to the sample tubes while 5µL of the standard were placed in the standard tubes. The solutions were mixed and incubated for 10 min at room temperature of 25^{o}C. The absorbance of the sample, standard were read against the blank. The concentration of

albumin (g/dL) was calculated by dividing absorbance of sample by the absorbance of the standard multiply by 5.

3.17 Determination of glucose-6-phosphate dehydrogenase activity

A colorimetric kit was used for this assay as described by Zhu *et al*. (2011). A reaction mixture was prepared by adding 1.0 mL reagent to a cuvette. 0.01 mL of serum was added and mixed thoroughly and allowed to stand at room temperature (25^{o}C) for 5-10 min. Reagent 2 was added to the mixture. The cuvette was then placed into water bath and incubated for approximately 5 min. The first absorbance A_1 was read at 340 nm. The second absorbance A_2 was read after 5mins. Absorbance per min was given as A_2-A_1 divided by 5. G6PDH in (U/mL) equals change in absorbance per minute multiply by 38.3.

3.18 Determination of Sodium ion concentration

Sodium ion concentration was determined using spectrophotometry procedure as described by de Caland *et al*. (2020). Forty microlitre of the serum samples were added into sample tubes. These samples were diluted using sodium assay buffer (10 mM). 0, 2, 4, 6, 8 and 10 µL of the standard solutions were prepared and placed into sample tubes and were also diluted with 10 mM of the sodium buffer. The mixtures in the tubes were incubated for 10 min. 40 µL of substrate was added to each sample tube and later incubated at 37^{o}C for 30 min. The absorbance was measured using spectrophotometer at 405 nm wave-length. The concentration of the sample was extrapolated from the calibration curve of the standard. Sodium ion concentration was given as sodium concentration of the samples divided by sample volume added to each tube multiply by the dilution factor.

3.19 Determination of potassium ion concentration

Potassium ion concentration was determined using spectrophotometry procedure as described by de Caland *et al*. (2020). Twenty microlitre of the serum sample and 20 µL of different concentration of the standard solutions (0.8, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1) were placed in sample bottles. 200 µL of the chromogenic agent was added to each tube. The mixtures were allowed to stand for 5 min at room temperature (25^{o}C). The absorbance was taken at 450 nm wavelength using spectrophotometer. The potassium ion concentration was extrapolated from the standard calibration curves.

3.20 Determination of serum calcium ion concentration

Calcium ion concentration was determined using spectrophotometry procedure as described by de Caland *et al*. (2020). Ten microlitre of serum sample and different concentration of the standards (0, 2, 4, 6, 8, 10) were placed in tubes. The mixtures were diluted with distilled water while 90µL of the chromogenic reagent was added into each tube and shaken gently. 60 µL of the calcium assay buffer was added to each tube. The mixture was then incubated for 5-10 min at room temperature (25^{o}C). The absorbance was measured using spectrophotometer at a wavelength of 575 nm. The concentration of calcium ion was given as concentration of calcium samples minus concentration of standards divided by sample volume added to each tube.

3.21 Determination of creatinine concentration

Creatinine level was determined using spectrophotometry procedure as described by Peake, *et al*. (2006). Using serum sample for analysis, 0.1 mL of serum was placed in a tube and mixed with 0.1 mL of picric acid of about 35 mmol concentration and 0.1mL of sodium hydroxide (0.32 mol/L). 30 minutes later, the first absorbance A1 of the standard and sample were then taken at 520 nm, the second absorbance A2 was thereafter taken after 2 minutes. The concentration of serum creatinine (µmol/L) was taken as absorbance of sample divided by absorbance of the standard multiply by 177.

3.22 Determination of urea concentration

Urea level was determined using spectrophotometry procedure as described by Bojic *et al*. (2008). Reagents were prepared for this assay. Reagent 1 constituted 116 mmol/L EDTA, 6mmol/L sodium nitroprusside, 1 g/L urease. Reagent 2 constituted of 120 mmol/L and Reagent 3 constituted 27 mmol/L diluted sodium hypochloride and 0.14 N sodium hydroxide. 10 µL of each sample and standard were placed in tubes. 100 µL of reagent 1, 2.5 mL of reagent 2 and 2.5 mL of reagent 3 were added to each tube. The mixture was incubated at 37^{o}C for 15 min. The absorbance was read at 546 nm wavelength. A standard calibration curve was plotted and the concentration of serum sample were extrapolated using their absorbance.

3.23 Determination of protein kinase C alpha

All reagents, standards and samples were first prepared and the assay was performed as described by Newton *et al*. (2018). About 100 µL of each of these were placed in microplate wells. 100 µL of Reagent A was added to each well and incubated at 37^{o}C for 1 hr. The mixture was aspirated from each well. Reagent was added to each well and incubated for 30 minute at 37^{o}C. This was aspirated and wash five times using wash buffer. 90 µL of the TMB substrate solution was placed in each well and incubated for 20 min at 37^{o}C. 50 µL of the stop solution was added to each well and the absorbance were read at 450 nm. A standard calibration curve was plotted and the absorbance of the sample were used to determine the concentration of each sample from calibration curve.

3.24 Determination of diacylglycerol

Diacylglycerol ELISA kits was used and assay conducted as described by Polewski *et al*. (2015). 100 µL sample and standard were placed in microplate wells. 50 µL of the enzyme conjugate was added to each well and incubated for 1 hr at 37^{o}C. The mixture was decanted and the wells washed five times using a washing buffer. 10 µL of the substrate was added to each well and incubated at 37^{o}C for 30 minutes. 10 µL of the stop solution was added and the absorbance of the standard and sample were read at 450 nm. A calibration curve was plotted for standards while the concentration of the samples were obtained from the curve.

3.25 Determination of mitogen activated protein kinase-8

Mitogen activated protein-8 ELISA kits was used and assay conducted as described by Bardwell *et al*. (2018). Ten microlitre of serum sample was added into each well. 100 µL of the fixing solution and the quenching buffer were added into the wells and incubated for 20 min. 200 µL of a blocking buffer was added to each well and incubated for 1hr at 37^{o}C. The wells were decanted and washed five times using a washing buffer. 100 µL of the substrate was added to each well and incubated for 30 min. 50 µL of the stop solution was added to each well and read at 450 nm wavelength. A calibration curve was plotted for the standards and concentrations of the samples were thereafter extrapolated from the calibration curve.

3.26 Determination of transforming growth factor

Transforming growth factor ELISA kits was used and assay conducted as described by Khan *et al*. (2012). Fifty microlitre of the standards, sample and blank were placed in the microplate wells. 100 µL of HPR-conjugate reagent was added to each well and incubated for 60 min at 37^{o}C. The microplate well was washed four times using the wash buffer. 50 µL of chromogen A and 50 µL of chromogen B were added to each well and incubated for 15 mins at 37^{o}C. 50 µL of the stop solution was added to each well and the absorbance read at 450 nm. A calibration curve for standard was plotted and the concentration of the sample extrapolated from the calibration curve.

3.27 Histological preparation of tissues

Structural changes to the organs were determined using histological technique as described by Alturkistani *et al*. (2015). Having weighed the liver, pancreas and the kidneys, they were immersed in formalin of 10% concentration. The tissues were dehydrated using 70%, 95% and 100% alcohol at different time intervals. This alcohol was then removed from each sample using xylene and then embedded in paraffin wax for 2 hrs at 57^{o}C. The samples were section using microtome at 5 µm. Each section was picked up using slide from a water bath. Each of these slide was pre-coated with albumen adhesives. Each of the slide was then immersed in alcohol solution of hematoxylin and alcohol solution of eosin respectively. Each slide was then cleaned and mounted using cover slip and examined under the light microscope at magnification of X40, X100 and X400.

3.28 Immunohistochemical preparation of tissues

The principle is based on detecting specific antigen in tissue samples using appropriate antibody labelling (Kim, 2016). The samples were fixed in 10% formaldehyde solution for 8 hours at room temperature (25^{o}C) and the antigenic properties were still preserved. The tissues were embedded and freezed at -20 to -80^{o}C. Five microns of each tissue was cut using a cryostat and mounted on histological slide. This was allowed to dry for 30 minutes. Each slide was incubated in blocking buffer to exclude other non-specific staining. The primary antibody were thereafter applied and incubated for 6 hours at 2-8^{o}C. Each slide was

dried and mounted on the stage under the light microscope and examined at x400 magnification.

3.29 Statistical Analysis

Graphpad prism 5.0 software was used for statistical analysis and data were analyzed with one way analysis of Variance (ANOVA), and Neuman's Keul posthoc test which evaluate effect in between groups and a chance of multiple comparison between groups. Results were given as mean ± standard error of mean and p values less than 0.05 were considered to be statistically significant.

CHAPTER FOUR

4.0 Results

4.1 Phytochemical Screening of methanol extract of *Parquetina nigrescens*

Table 4.0 showed phytochemical screening of methanol extract of *Parquetina nigrscens*. The phytochemical screening showed positive results for alkaloids, cardenoloides, anthraquinones, tannins and flavonoids.

Table 4.0: Phytochemical analysis of MEPN

Phytochemicals compounds	**Methanol extract of *Parquetina nigrescens* (MEPN)**
Alkaloids	+
Cardenoloids	+
Anthraquinones	+
Saponins	-
Tannins	+
Flavonoids	+

+ = Present - = Absent

4.2 Gas chromatography mass spectrometry of methanol extract of *Parquetina nigrescens*

Table 4.1 GC-MS analysis of MEPN showed the presence of twenty-two (22) bio active compounds which include Alpha-phellandrene (5.819),Cymene(6.117), Betacurcumene (12.005), Alpha begarmotene (12.126), Beta-bisabolene (12.262), Naphthalene (12.455), Diethylphthalate (12.995), 1,12-tridecadiene (15.033), 5-ethyl-2-furaldehyde (15.283), 7-hexadecyne (15.478), Pyranos (15.576), decanoic acid (15.867) Mannos (16.275), Thiophene (16.438), Hexadecanoic acid (16.813), Octadecanoic acid (17.710), Catrienoate (17.772), Phytol (18.148), Octadecatrienoate (18.274), Octadecanoic (18.388), 9-Octadecenamide (20.222), Squalene (21.021). Table 4.1 also showed the percentage concentration of the compounds in MEPN.

Fig 4.0 showed the spectral analysis of the compounds embedded in methanol extract of *Parquetina nigrescens*.

Fig 4.1 showed the spectral analysis of phytol and squalene. The arrows indicate the molecular weights of phytol (299 g/mol) and squalene (410 g/mol)

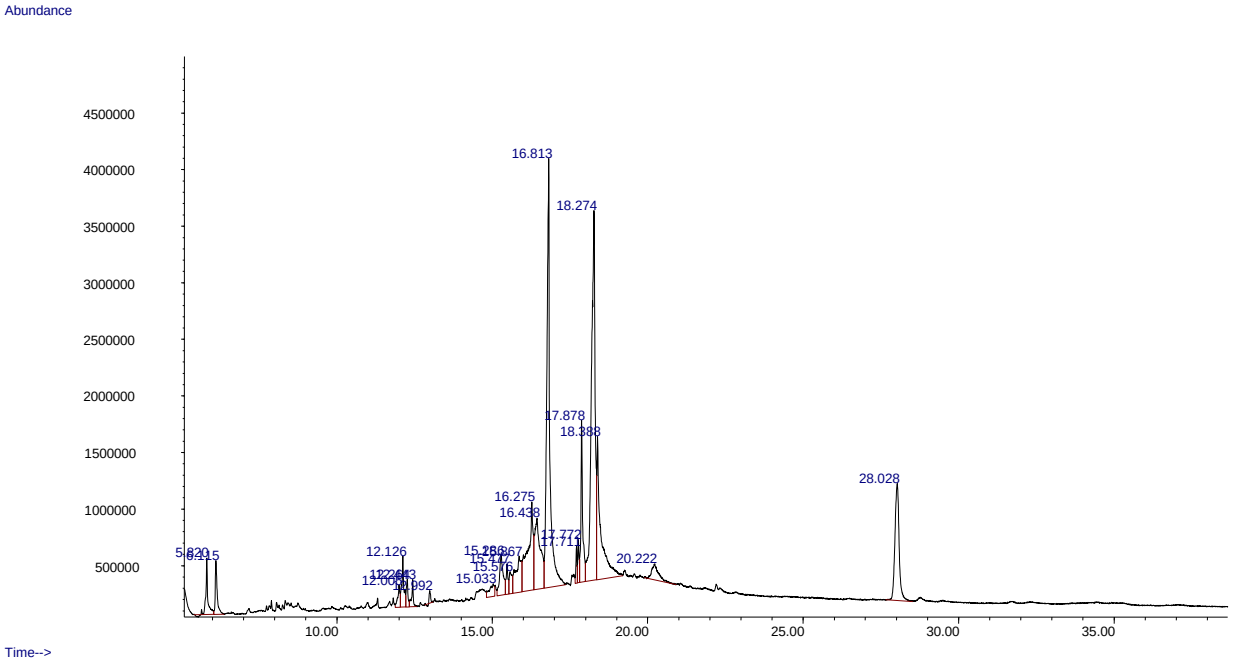

Fig 4.0 Gas chromatography-mass spectrometry analysis of MEPN

Table 4.1 Compounds in methanol extract of *Parquetina nigrescens* (MEPN)

S/n	Name	Reaction time (min)	% Concentration
1.	Alpha-phellandrene	5.819	1.726
2.	Cymene	6.117	1.720
3.	Beta-curcumene	12.005	0.742
4.	Alpha-begarmotene	12.125	1.739
5.	Beta-bisabolene	12.262	0.766
6.	Naphthalene	12.455	0.750
7.	Diethylphthalate	12.995	0.238
8.	1, 12- tridecadiene	15.032	0.981
9.	5-ethyl-2-furaldehyde	15.283	2.412
10.	7-hexadecyne	15.478	0.932
11.	Pyranos	15.575	0.991
12.	Decanoic acid	15.478	3.222
13.	Mannos	16.273	7.765
14.	Thiophene	16.439	6.814
15.	Hexadecanioc acid	16.811	21.378
16.	Octadecanoic acid	17.710	0.646
17.	Catrienoate	17.773	0.884
18.	Phytol	18.148	4.772
19.	Octadecatrienoate	18.390	22.488
20.	Octadecanoate	18.390	9.268
21.	9-Octadecenamide	20.222	1.777
22.	Squalene	21.021	7.990

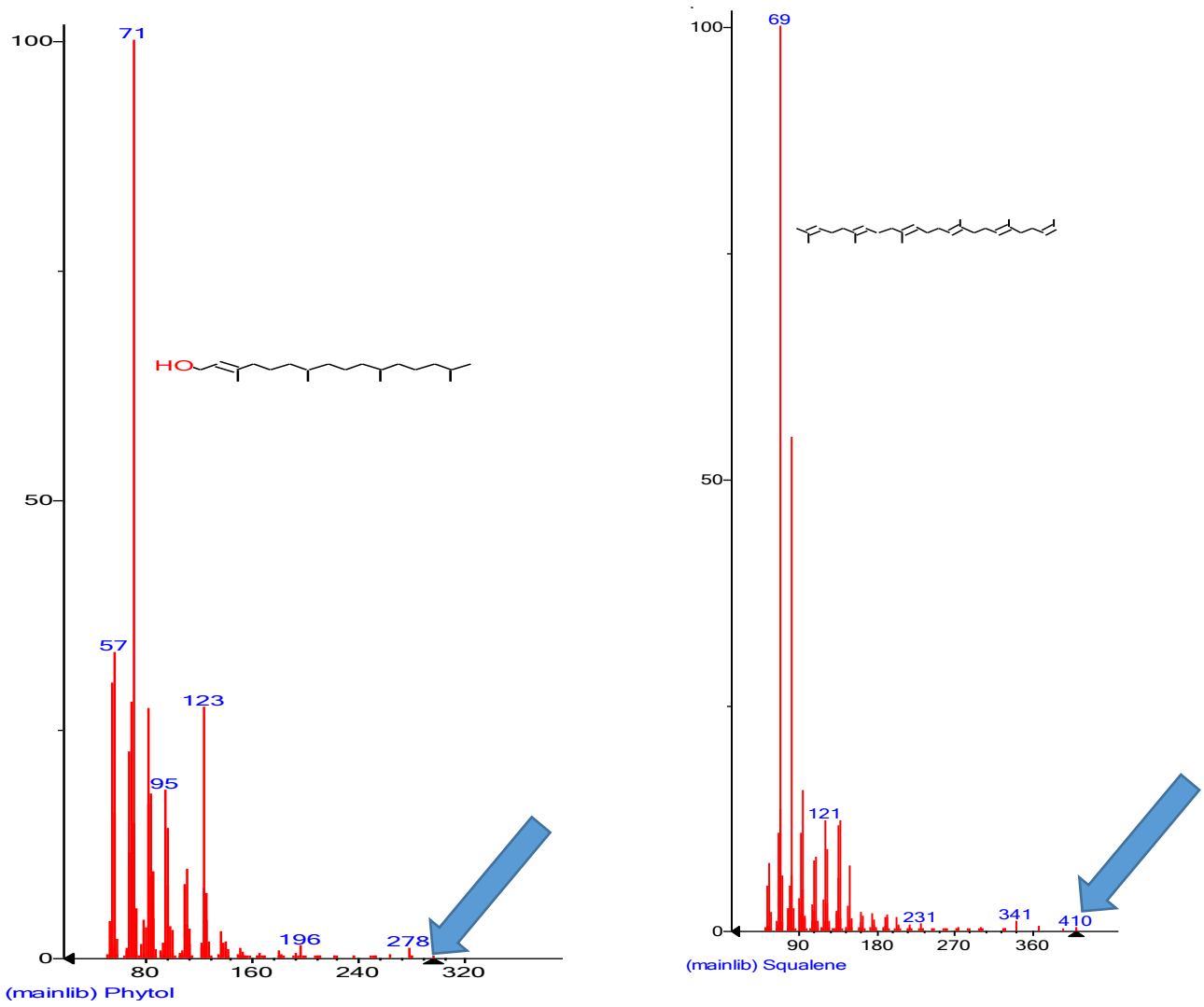

Fig 4.1 Spectral analysis of phytol and squalene.

4.3 Toxicological study of different doses of MEPN

Table 4.2 showed the effect of graded doses of MEPN in normal rats. There were no cases of mortality in the experimental rats and this means that the LD_{50} of MEPN was greater than 5000 mg/kg. However, fecal materials and urine were found more in group administered with 5000 mg/kg MEPN.

Table 4.2: Toxicological study of different doses of MEPN

S/n	Groups	Mortality x/N	Symptoms (0-6 hrs)
Group 1	0.3mL distilled water	0/5	Nil
Group 2	500 mg/kg	0/5	Nil
Group 3	1000 mg/kg	0/5	Nil
Group 4	2000 mg/kg	0/5	Nil
Group 5	3000 mg/kg	0/5	Nil
Group 6	4000 mg/kg	0/5	Nil
Group 7	5000 mg/kg	0/5	Increased defecation and urination

4.4 Body, liver and kidney weights in rats treated with methanol extract of *Parquetina nigrescens* (MEPN)

Table 4.3 showed body weights in rats treated with MEPN. Body weight increased significantly ($p<0.05$) in normal control group at weeks 2, 3 and 4 when compared to the initial value at week 0. The percentage increases are 9.92%, 14.18% and 19.15% respectively. Body weight decreased significantly ($p<0.05$) in diabetic untreated at weeks 3 and 4 when compared to the initial value at week 0. The percentage decreases are 10.48% and 14.5% respectively. There was significant increase ($p<0.05$) in body weight in diabetic group treated with 200 mg/kg MEPN at weeks 3 and 4 when compared to the initial value at week 0. The percentage increases are 11.43% and 17.14% respectively.

Table 4.4 showed the percentage differences in body weights, 72 hours of alloxan administration and 28 days post-treatment with MEPN. The percentage differences includes normal control (22.3%), diabetic untreated (14.5%), diabetes treated with 100 mg/kg MEPN (1.1%), diabetes treated with 200 mg/kg (17.2%) and diabetes treated with 100 mg/kg metformin (12.6%)

Table 4.5 showed the mean and relative weights of the liver of the control and the treated groups. Liver weight increased significantly ($p<0.05$) in normal control when compared to diabetic untreated. There was significant increase ($p<0.05$) in liver weight in diabetic treated with 100 mg/kg and 200 mg/kg MEPN when compared to diabetic untreated. Liver weight was significantly higher ($p<0.05$) in diabetic treated with 100 mg/kg metformin when compared to diabetic untreated.

Table 4.6 showed the mean and relative weights of the kidney of the control and the treated groups. Kidney weight increased significantly ($p<0.05$) in normal control when compared to diabetic untreated. There was significant increase ($p<0.05$) in kidney weight in diabetic treated with 100 mg/kg and 200 mg/kg MEPN when compared to diabetic untreated. Kidney weight was significantly higher ($p<0.05$) in diabetic treated with 100 mg/kg metformin when compared to diabetic untreated.

Table 4.3 Body weight changes in rats treated with methanol extract of *Parquetina nigrescens* (MEPN)

Experimental group	Week 0(g)	Week 1(g)	Week 2 (g)	Week 3(g)	Week 4 (g)
Normal control (0.3 ml distilled water)	121.00± 1.70	128.00 ± 1.86	135.00 ± 2.00*	141.00 ± 2.21*	148.20 ± 3.29*
Diabetic untreated (0.3 ml distilled water)	124.2 ± 2.44	119.2 ± 2.27	115.0±1.92	111.4±1.91*	106.2±2.22*
Diabetic + 100 mg/kg MEPN	106.6 ± 3.64	106.8 ± 4.12	105.2 ± 3.40	104.0 ± 3.73	105.4 ± 2.54
Diabetic + 200 mg/kg MEPN	105.6±4.27	106.8±4.64	112.4±4.40	117.2±3.99*	123.8±3.15*
Diabetic + 100 mg/kg Metformin	92.80±7.97	97.80±8.42	100.4±8.58	104.6±5.71	106.2±6.18

. Data were expressed as Mean ± SEM, * indicate values significantly different when compared with week 0 values (n = 5).

Table 4.4 Percentage Difference in the Body weights after 72 hours of Alloxan Injection and 28 days post-treatment with Methanol extract of *Parquetina nigrescens*

Experimental groups	72 hours after alloxan injection (g)	Values after 28 days of treatment (g)	% Differences	
			Increase	Decrease
Normal control	121.0±1.7	148.2±3.3	22.3	
Diabetic untreated	124.2±2.4	106.2±2.2		14.5
Diabetic + 100 mg/kg MEPN	106.6±3.6	105.4±2.5		1.1
Diabetic + 200 mg/kg MEPN	105.6±4.3	123.8±3.2	17.2	
Diabetic + 100 mg/kg Metformin	92.8±7.9	106.2±6.2	12.6	

Table 4.5 Mean and relative weights of the liver in rats treated with MEPN

Experimental groups	Mean weights of the liver (g)	Relative weights of liver (%)
Normal control	5.1±0.2	3.44
Diabetic untreated	4.0±0.01*	3.77
Diabetic + 100 mg/kg MEPN	4.8±0.3	4.55
Diabetic + 200 mg/kg MEPN	5.9±0.2	4.76
Diabetic + 100 mg/kg MEPN	5.4±0.6	5.08

Data were expressed as Mean ± SEM,* indicate values significantly different from normal contro, diabetes + 200 mg/kg, diabetes + 100 mg/kg metformin (n = 5).

Table 4.6 Mean and relative weights of the kidney in rats treated with MEPN

Experimental groups	Mean weights of the kidney (g)	Relative weights of kidney (%)
Normal control	0.5±0.0.2	0.34
Diabetic untreated	0.3±0.02*	0.28
Diabetic + 100 mg/kg MEPN	0.4±0.01	0.38
Diabetic + 200 mg/kg MEPN	0.5±0.02	0.40
Diabetic + 100 mg/kg MEPN	0.4±0.02	0.38

Data were expressed as Mean ± SEM,* indicate values significantly different from normal control, diabetes + 100 mg/kg MEPN, diabetes + 200 mg/kg, diabetes + 100 mg/kg metformin (n = 5).

4.5 Fasting blood glucose level in rats treated with methanol extract of *Parquetina nigrescens* (MEPN).

Figure 4.3 showed changes in fasting blood glucose level in the normal control, diabetic untreated, MEPN and Metformin treated rats. There was significant increase ($p<0.05$) in fasting blood glucose level at weeks 1,2, 3 and 4 in diabetic untreated group when compared to the normal control. However, there was significant decrease ($p<0.05$) in fasting blood glucose in MEPN and metformin treated groups at weeks 3 and 4 when compared to the diabetic untreated group. There was no significant difference ($p>0.05$) in fasting blood glucose in MEPN, metformin treated groups and normal control at weeks 3 and 4 post-treatment.

There was no significant difference in fasting blood glucose in diabetic untreated group at weeks 1, 2, 3 and 4 when compared to the value 72 hours after diabetes induction. However, there was significant decrease ($p<0.05$) in fasting blood glucose level in MEPN and metformin treated groups at week 2, 3, and 4 when compared to the value 72 hours after diabetes induction.

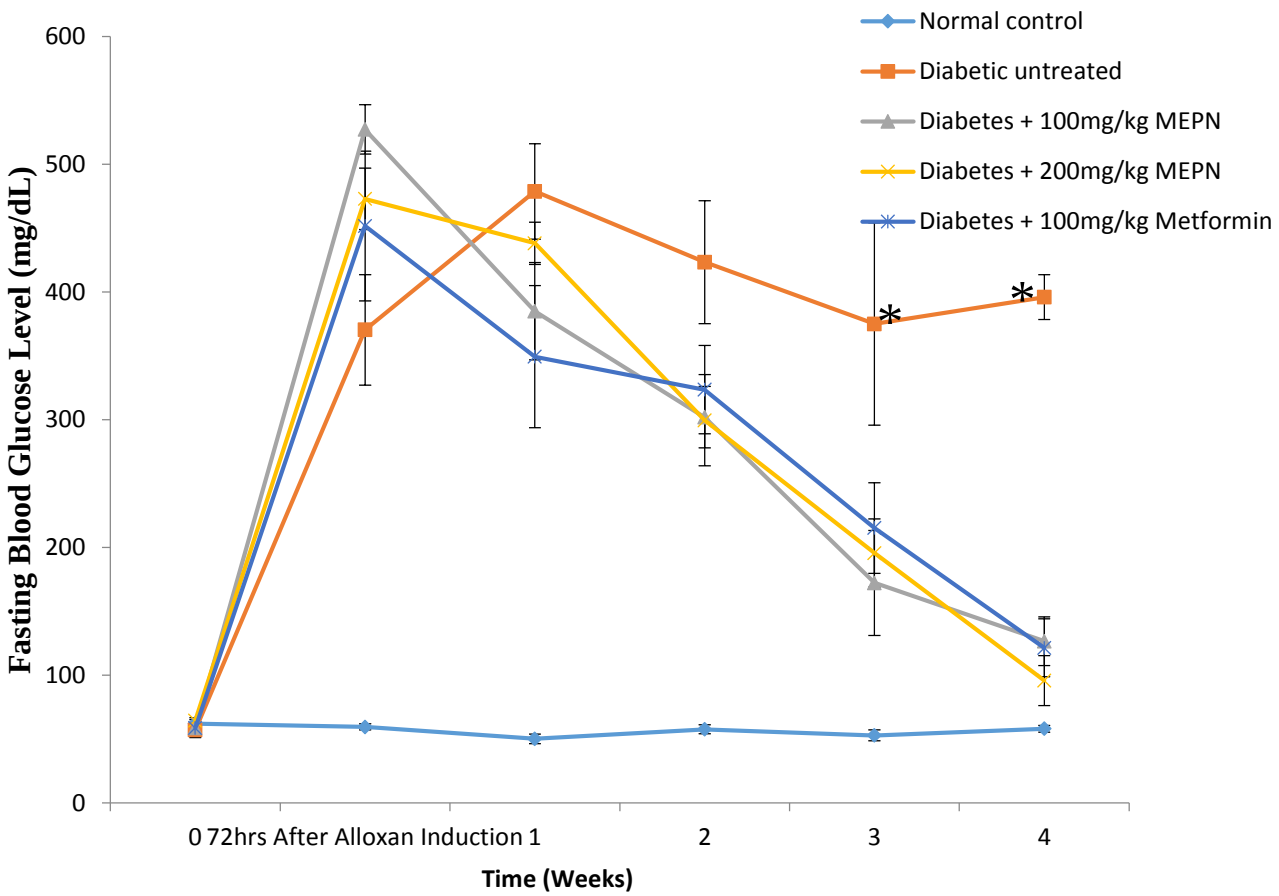

Fig 4.3 Effect of Methanol extract of *Parquetina nigrescens* (MEPN) on fasting blood glucose level in experimental rats.

Data were expressed as Mean ± SEM; $p<0.05$. * indicate values significantly different from normal control, diabetes + 100 mg/kg MEPN, diabetes + 200 mg/kg MEPN and diabetes + 100 mg/kg Metformin (n=5)

4.6 Liver glycogen content in rats treated with MEPN

Figure 4.4 showed changes in liver glycogen content in the normal control, diabetic untreated, MEPN and Metformin treated rats. There was significant decrease ($p<0.05$) in liver glycogen in diabetic untreated group when compared to normal control, diabetes + 100 mg/kg MEPN, diabetes + 200 mg/kg MEPN and diabetes + metformin treated groups. There was significant decrease ($p<0.05$) in glycogen content in diabetes + 100 mg/kg MEPN treated group when compared to the normal control. However, there was no significant difference ($p>0.05$) in liver glycogen content in normal control, diabetes + 200 mg/kg MEPN and diabetes + 100 mg/kg metformin treated groups.

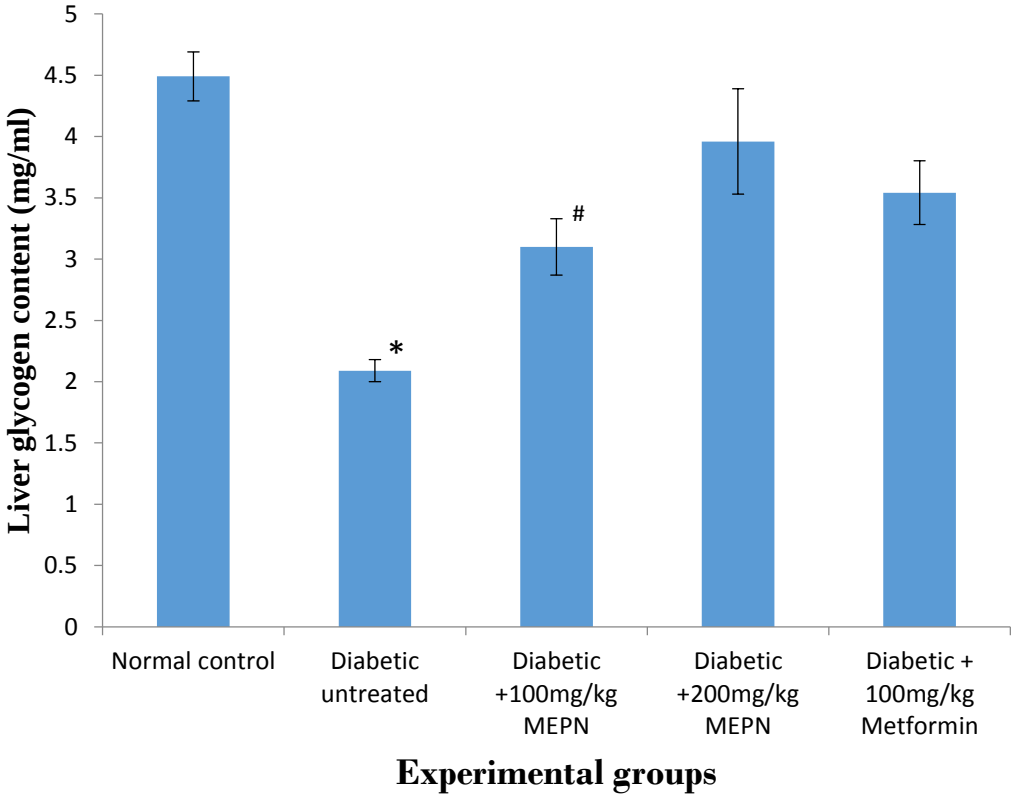

Figure 4.4 Effect of Methanol extract of *Parquetina nigrescens* (MEPN) on liver glycogen content in experimental rats.

[#] indicates value significantly different from the normal control while * indicates value significantly different from normal control, diabetes + 100 mg/kg MEPN, diabetes + 200 mg/kg MEPN and diabetes + 100 mg/kg Metformin (n=5)

4.7 Histological changes in the liver of rat treated with methanol extract of *Parquetina nigrescens* (MEPN)

Plate 4.0 showed transverse section of the liver in normal, diabetic untreated, diabetic treated with 100 mg/kg MEPN, diabetic treated with 200 mg/kg MEPN and diabetic treated with 100 mg/kg metformin respectively.

Photomicrograph of liver section stained by haematoxylin and eosin shows good architecture as seen in magnification (x400). The central venules are normal and not congested and the sinusoids appear normal without infiltration of inflammatory cells in normal control, diabetic untreated, diabetic treated with 100 mg/kg MEPN, diabetic treated with 200 mg/kg MEPN and diabetic treated with 100 mg/kg metformin. The hepatocytes however, showed severe hepatic macro steatosis and infiltration of fat within cytoplasms of the hepatocytes in all the groups.

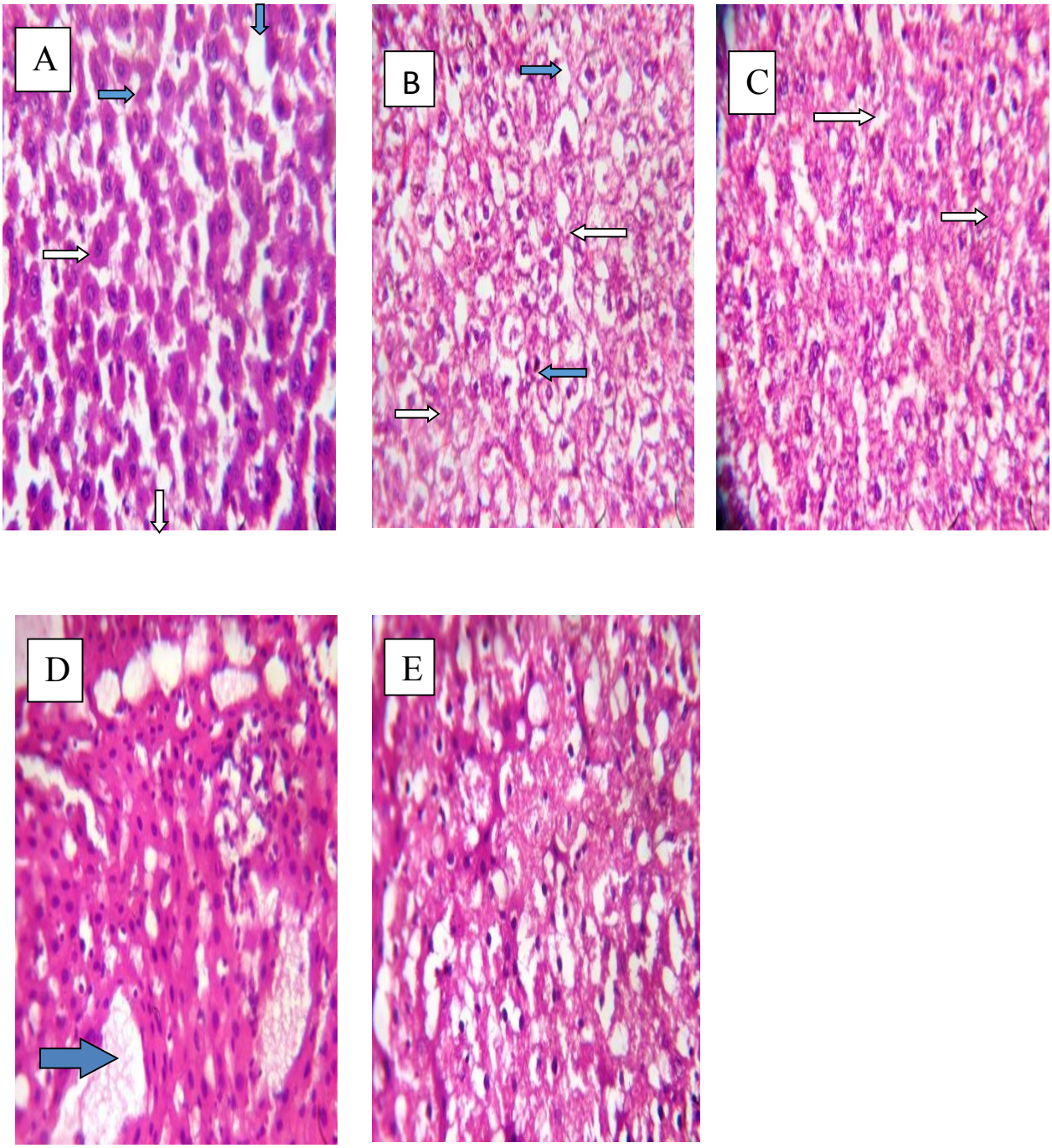

Plate 4.0 (A-E): Sections stained with H & E showing architecture of the liver in A (Control), B (Diabetic untreated), C, D & E (Diabetes treated with 100 mg/kg MEPN, 200 mg/kg MEPN and 100 mg/kg Metformin respectively). Each of the slides showed no sign of toxicity but are infiltrated with fats (Blue Arrows) and sinusoids (White Arrows) with no visible lesions. Slide D showed infiltration and deposition of fat (Blue Arrow) X 400.

4.8 Intestinal glucose absorption in rats treated with MEPN

Table 4.7 showed duodenal glucose absorption in normal control, diabetic untreated, MEPN and Metformin treated rats. There was increase in duodenal glucose absorption in diabetic untreated group when compared to normal control. However, duodenal glucose absorption significantly decreased ($p<0.05$) at 20 and 60 minutes in diabetes+MEPN treated groups when compared to the normal control and diabetic untreated groups respectively. There was significant decrease in duodenal glucose absorption at 20, 40 and 60 minutes in diabetes+metformin treated group when compared to normal control and diabetic untreated groups respectively. Duodenal glucose absorption significantly decrease ($p<0.05$) in diabetes+ metformin group when compared to diabetes+100 mg/kg MEPN and diabetes+200 mg/kg MEPN.

Table 4.8 showed jejunal glucose absorption in normal control, diabetic untreated, MEPN and Metformin treated rats. Jejunal glucose absorption significantly decrease ($p<0.05$) at 20 minutes in diabetes+MEPN treated groups when compared to the normal control and diabetic untreated group respectively. There was no significant decrease ($p>0.05$) at 40 and 60 minutes of glucose absorption in diabetes+MEPN treated groups when compared to normal control and diabetic untreated groups. There was significant decrease in glucose absorption at 20 and 40 minutes in metformin treated group when compared to normal control and diabetic untreated groups respectively. There was significant difference in glucose absorption at 40 minutes in diabetes+metformin group when compared to diabetes+100 mg/kg MEPN. Similarly, glucose absorption significantly decrease at 20 and 40 minutes in diabetes+metformin group when compared to diabetes+200 mg/kg MEPN

Table 4.7 Effect of MEPN on duodenal glucose absorption in experimental rats

Experimental group	20 min (mg/dl/cm)	40 min (mg/dl/cm)	60 min (mg/dl/cm)
Normal Control	3.33±0.36	3.84±0.26	2.90±0.43
Diabetic untreated	3.192±0.34*#	4.12±0.11#	3.37±0.29*#
Diabetic +100mg/kg MEPN	1.87±0.25a	3.84±0.18	2.04±0.32a
Diabetic + 200mg/kg MEPN	2.06±0.18a	3.82±0.24	1.91±0.22a
Diabetic + 100mg/kg Metformin	1.12±0.21b,c	1.50±0.39b,c	1.34±0.29b,c

*&C indicate values significantly different from diabetes + 100 mg/kg MEPN and diabetes + 200 mg/kg MEPN, # indicates values significantly different from diabetes + 100 mg/kg metformin, a & b indicate values significantly different from the normal control, (n=5).

Table 4.8 Effect of MEPN on jejunal glucose absorption in experimental rats

Experimental group	20 min (mg/dl/cm)	40 min (mg/dl/cm)	60 min (mg/dl/cm)
Normal Control	3.52±0.42	2.74±0.34	2.32±0.27
Diabetic untreated	4.46±0.24*	3.02±0.24	2.44±0.26
Diabetic + 100mg/kg MEPN	1.00±0.35[a]	2.60±0.36[c]	1.91±0.30
Diabetic + 200mg/kg MEPN	2.06±0.33[a,c]	3.23±0.22[c]	1.99±0.47
Diabetic + 100mg/kg Metformin	1.29±0.17[b]	1.34±0.34[b]	2.35±0.38

Data were expressed as Mean ± SEM; $p<0.05$. [a] indicate values significantly different from the normal control, * indicates values significantly different from diabeties+100 mg/kg MEPN and diabetes+200 mg/kg MEPN, [b] indicates values significantly different from the normal control and diabetic untreated, [c] indicates value significantly different from diabetes+metformin (n=5)

4.9 Glucose, insulin concentration, pancreatic beta cell function, insulin sensitivity, and insulin resistance in rats treated with MEPN

Figure 4.5 showed glucose concentration in normal, diabetic untreated, MEPN and metformin treated rats. Serum glucose significantly increase ($p<0.05$) in diabetic untreated group when compared to normal control, diabetes + MEPN treated groups and diabetes+metformin treated group.

Figure 4.6 showed insulin concentrations in normal, diabetic untreated, MEPN and metformin treated rats. Insulin concentration significantly decreased ($p<0.05$) in diabetic untreated when compared to normal control, diabetes + MEPN treated groups and diabetes+metformin treated group.

Figure 4.7 showed pancreatic beta cell function, in normal, diabetic untreated, MEPN and metformin treated rats. There was significant increase ($p<0.05$) in pancreatic beta cell function in diabetes+200 mg/kg MEPN when compared to normal control, diabetic untreated, diabetes + 100 mg/kg MEPN treated and diabetes+metformin treated groups.

Figure 4.8 showed insulin sensitivity in normal, diabetic untreated, MEPN and metformin treated rats. There was significant decrease in insulin sensitivity in diabetic untreated compared to normal control and MEPN treated groups.

Figure 4.9 showed insulin resistance in normal, diabetic untreated, MEPN and metformin treated rats. There was no significant difference in insulin resistance in between the groups

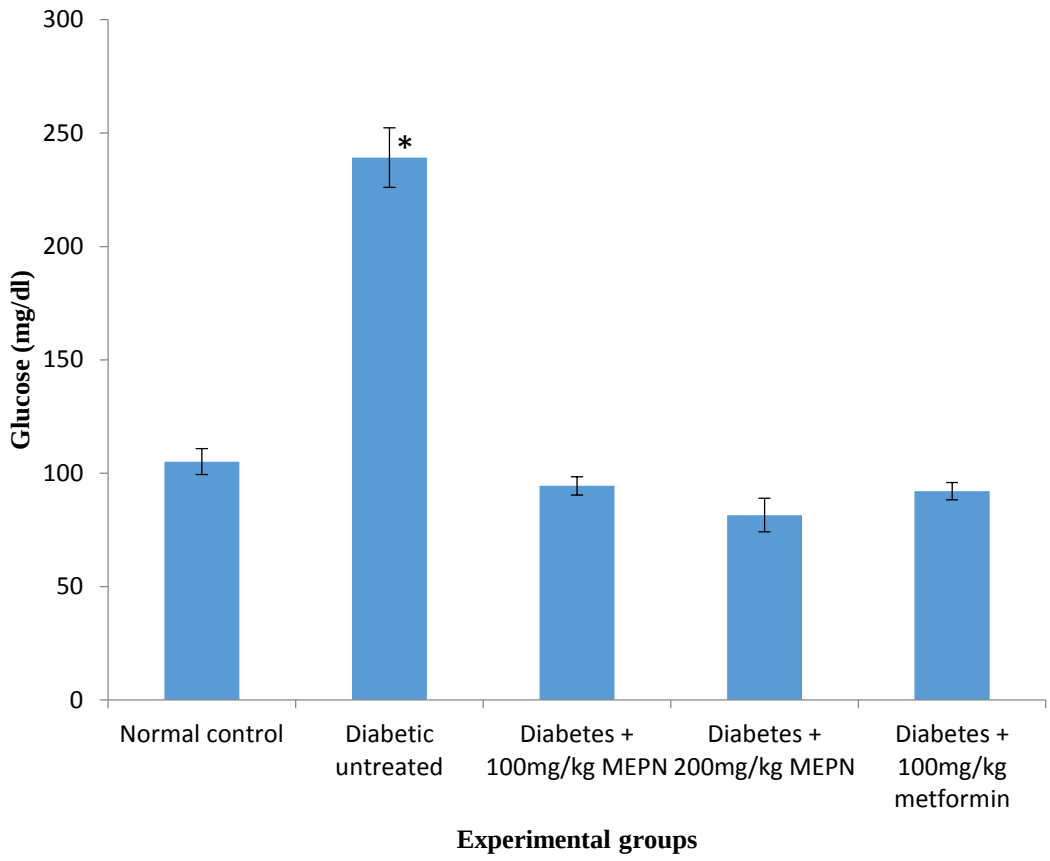

Figure 4.5 Effect of Methanol extract of *Parquetina nigrescens* (MEPN) on glucose concentration in experimental rats.

* indicate values significantly different from the normal control, diabetes+100 mg/kg MEPN, diabetes+200 mg/kg MEPN and diabetes+100 mg/kg metformin,

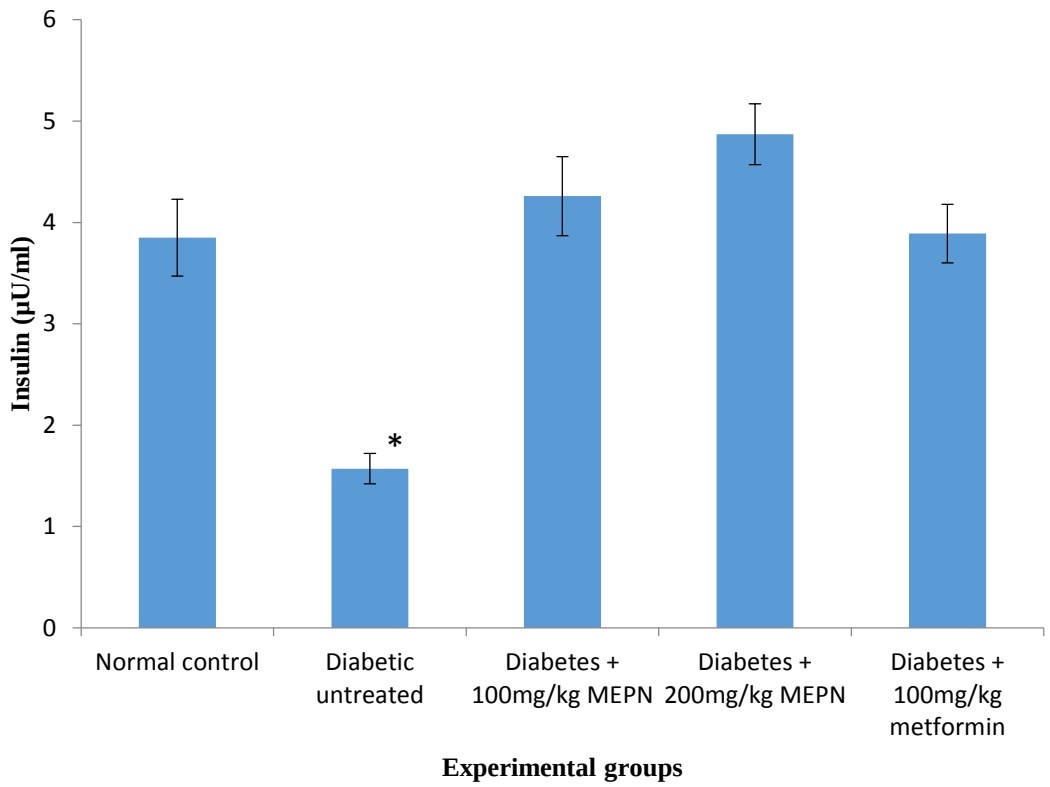

Figure 4.6 Effect of Methanol extract of *Parquetina nigrescens* (MEPN) on insulin concentration in experimental rats.

* indicate values significantly different from the normal control, diabetes+100 mg/kg MEPN, diabetes+200 mg/kg MEPN and diabetes+100 mg/kg metformin,

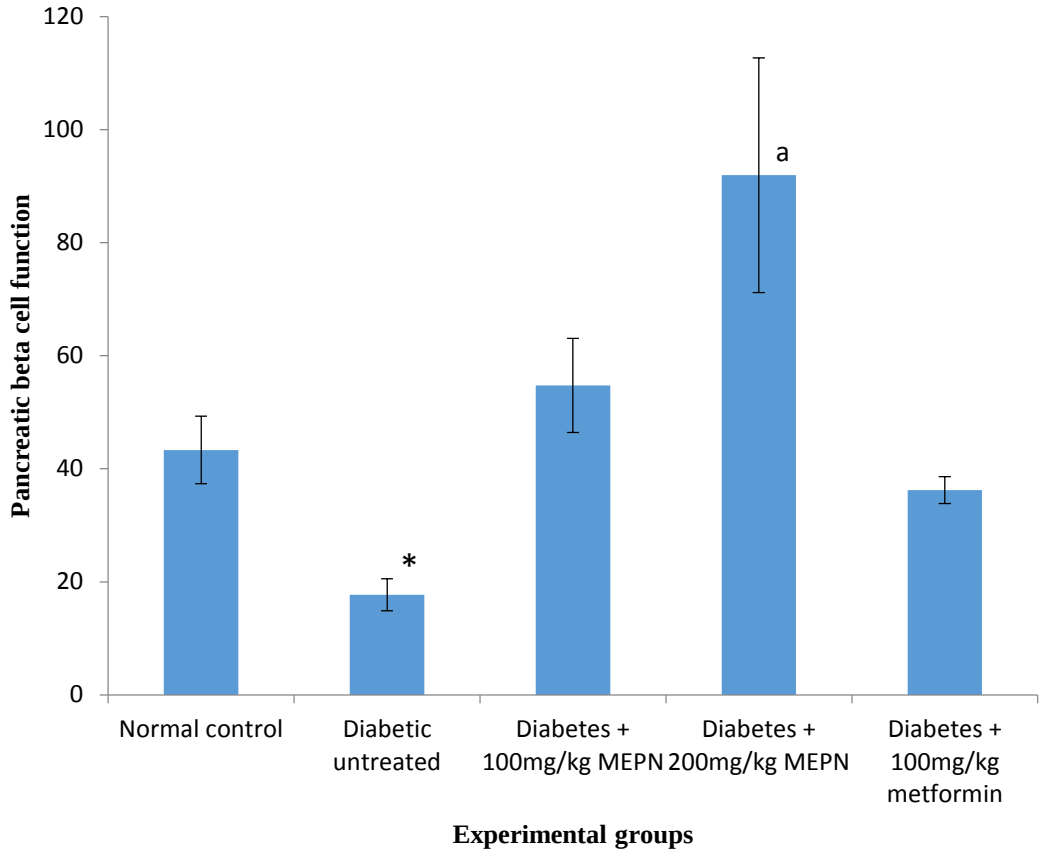

Figure 4.7 Effect of Methanol extract of *Parquetina nigrescens* (MEPN) on pancreatic beta cell function in experimental rats.

* indicates value significantly different from normal control, diabetes + 100 mg/kg MEPN, diabetes + 200 mg/kg MEPN and diabetes + Metformin; [a] indicate value significantly different from normal control, diabetes untreated, diabetes+100 mg/kg MEPN, and diabetes+100 mg/kg metformin (n=5)

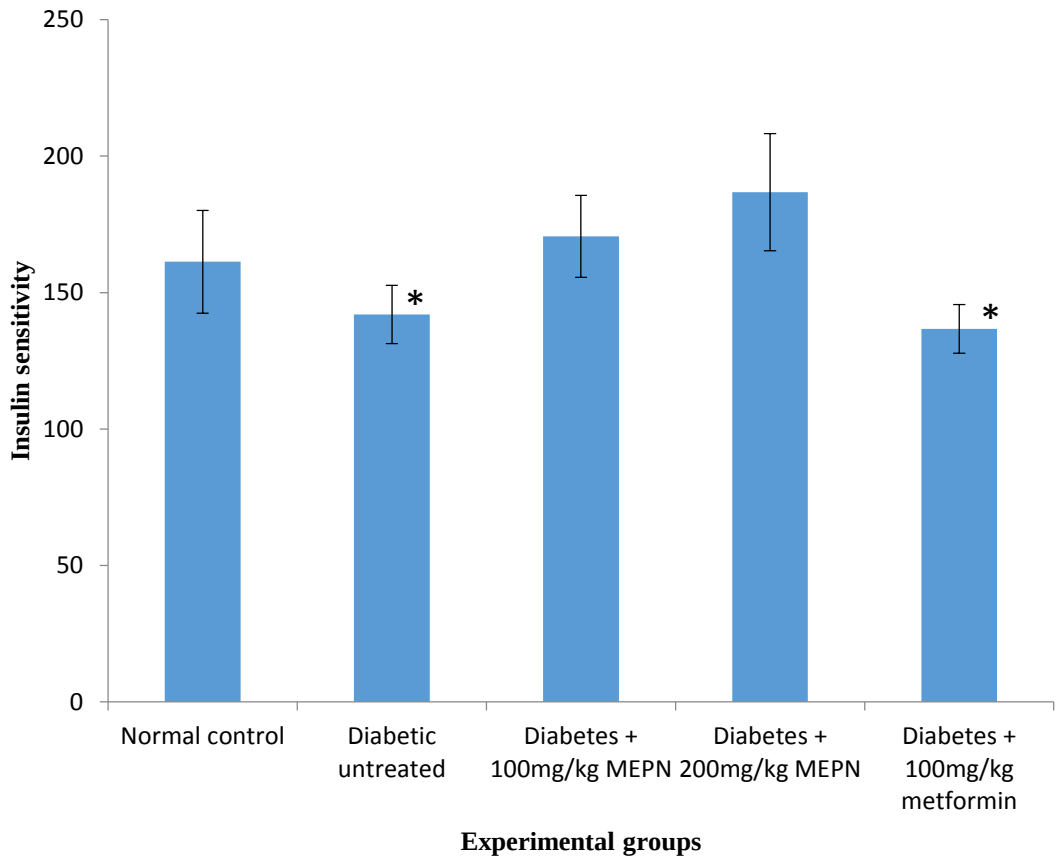

Figure 4.8 Effect of Methanol extract of *Parquetina nigrescens* (MEPN) on insulin sensitivity in experimental rats.

* indicates value significantly different from normal control, diabetes + 100 mg/kg MEPN, diabetes + 200 mg/kg MEPN (n=5)

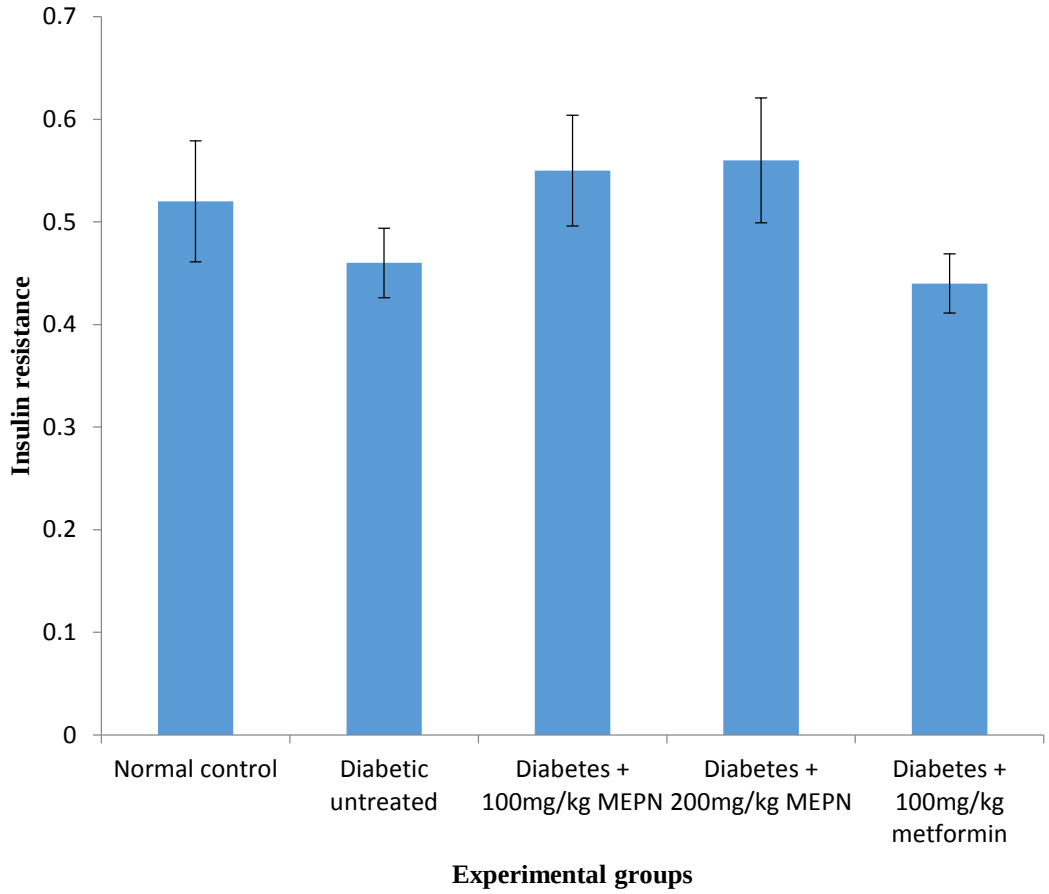

Figure 4.9 Effect of Methanol extract of *Parquetina nigrescens* (MEPN) on insulin resistance in experimental rats.

4.10 Histological changes in the pancreas of rat treated with methanol extract of *Parquetina nigrescens* (MEPN)

Plate 4.2 showed transverse section of the pancreas in normal, diabetic untreated, diabetic treated with 100 mg/kg MEPN, diabetic treated with 200 mg/kg MEPN and diabetic treated with 100 mg/kg metformin respectively.

Photomicrograph of a pancreas section stained by haematoxylin and eosin and showing normal architecture. The parenchyma of the pancreas showed normal serous acinar and zymogenic cells containing abundant granular eosinophilic cytoplasm, normal interlobular connective tissues and septa were also seen. There are large Islets of Langerhan consisting of normal round to oval collections of endocrine cells in diabetic treated with 100 mg/kg MEPN, diabetic treated with 200 mg/kg MEPN and diabetic treated with 100 mg/kg metformin. Thick walled vessels were also observed.

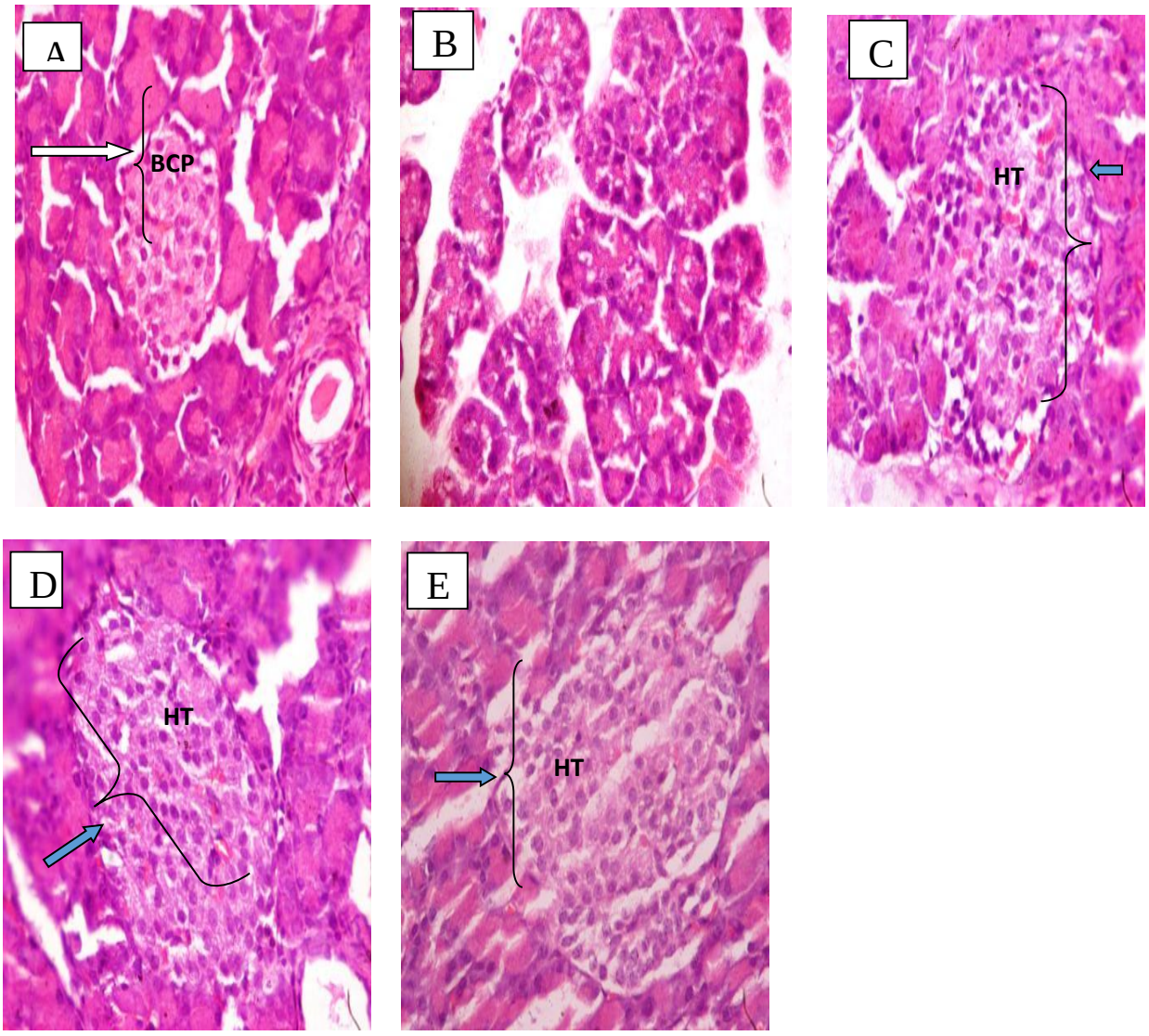

Plate 4.1 (A – E): Sections stained with H & E showing architecture of the pancreas in A (Control), B (Diabetic untreated), C, D & E (Diabetes treated with 100 mg/kg MEPN, 200 mg/kg MEPN and 100 mg/kg Metformin respectively). A showed architecture of the pancreas with normal pancreatic beta cells (white Arrow), B showed no visible beta cells, C, D & E showed enlarged pancreatic Islet cells (Hypertrophy) (Blue Arrows) X 400.

4.11 Anti-oxidative changes in rats treated with methanol extract of *Parquetina nigrescens* (MEPN)

Figure 4.10 showed changes in glucose 6 phosphate dehydrogenase activity in normal and treated rats. There was significant increase ($p<0.05$) in G6PDH activity in normal control, diabetic treated with 200 mg/kg MEPN and 100 mg/kg metformin groups when compared to diabetic untreated and diabetes treated with 100 mg/kg MEPN respectively. There was also significant increase in G6PDH activity in diabetes treated with 100 mg/kg metformin when compared to normal control and diabetes + 200 mg/kg MEPN.

Figure 4.11 showed changes in lactate dehydrogenase activity in normal and treated rats. Lactate dehydrogenase activity (LDH) was significantly lower ($p<0.05$) in diabetes treated with 100 mg/kg MEPN when compared to diabetes treated with 100 mg/kg metformin.

Figure 4.12 showed changes in superoxide dismutase activity in normal and treated rats Superoxide dismutase activity (SOD) significantly decrease ($p<0.05$) in diabetic untreated when compared to normal control, diabetes treated with 100 mg/kg MEPN, diabetes treated with 200 mg/kg MEPN and 100 mg/kg Metformin groups respectively.

Figure 4.13 showed changes in catalase activity in normal and treated rats. Catalase (CAT) activity significantly increase ($p<0.05$) in normal control, diabetes + 100 mg/kg MEPN, diabetes + 200 mg/kg MEPN, diabetes + 100 mg/kg Metformin when compared to diabetic untreated. There was significant increase in CAT activity in normal control, diabetes + 200 mg/kg MEPN, diabetes + 100 mg/kg Metformin when compared to diabetes treated with 100 mg/kg MEPN. A significant increase was also observed in CAT activity in normal control and diabetes + 200 mg/kg MEPN when compared to diabetes treated with 100 mg/kg metformin

Figure 4.14 showed changes in glutathione peroxidase activity in normal and treated rats. Glutathione peroxidase activity (GPx) significantly increased ($p<0.05$) in normal control, diabetes treated with 200 mg/kg MEPN and 100 mg/kg Metformin groups when compared to diabetic untreated and diabetes treated with 100 mg/kg MEPN respectively.

Figure 4.15 showed changes in albumin level in normal and treated rats. The level of albumin was significantly higher ($p<0.05$) in normal control, diabetes treated with 100 mg/kg, 200 mg/kg MEPN and diabetes treated with 100 mg/kg Metformin when compared to diabetic untreated.

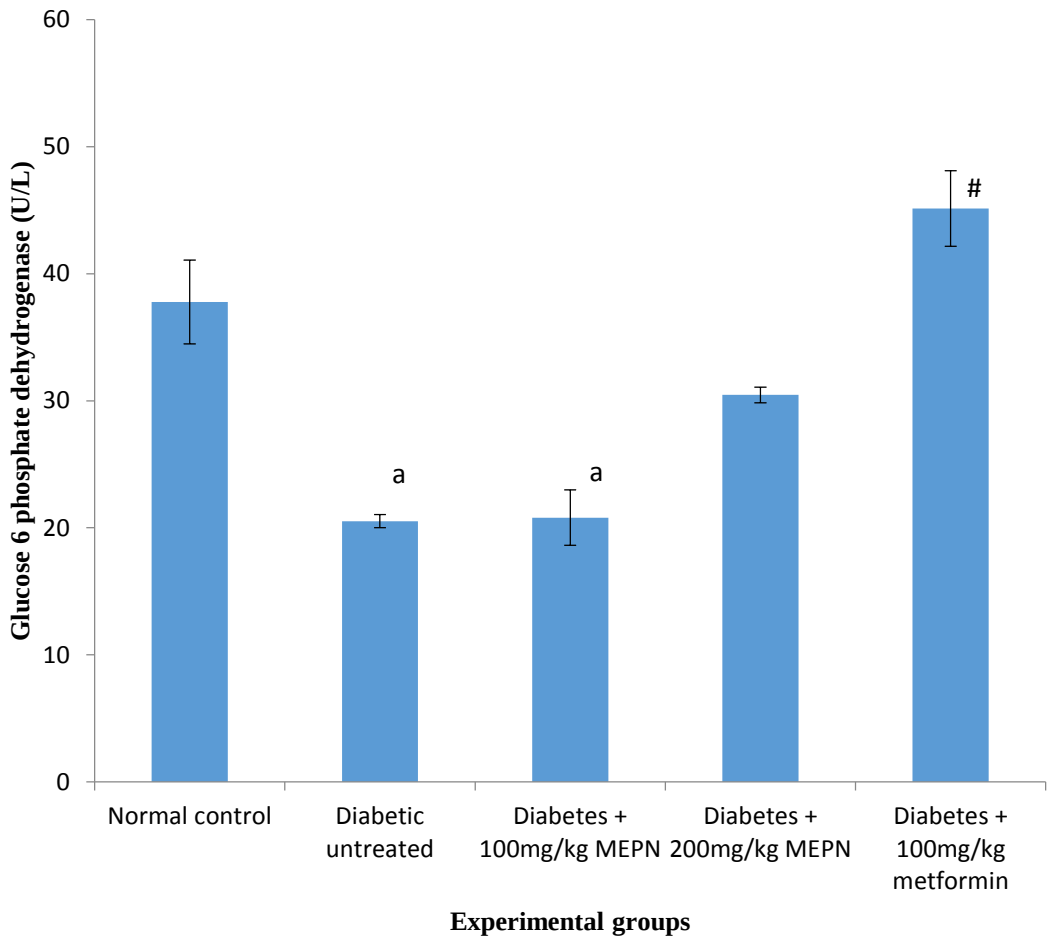

Figure 4.10 Effect of Methanol extract of *Parquetina nigrescens* (MEPN) on glucose-6-phosphate dehydrogenase activity in experimental rats.

[a] indicates value significantly different from normal control, diabetes + 200 mg/kg MEPN, diabetes + 100 mg/kg Metformin; [#] indicates value significantly different from normal control, diabetes + 200 mg/kg MEPN. (n=5)

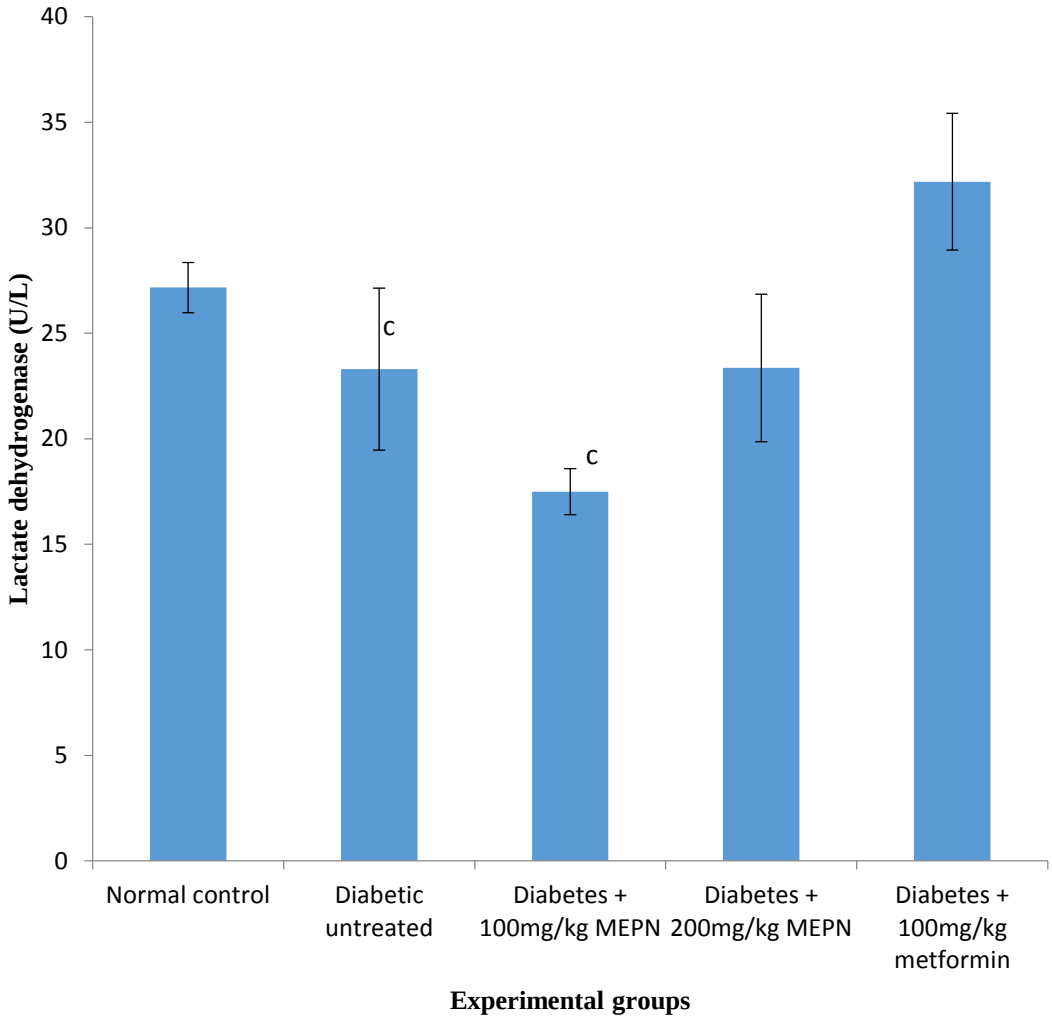

Figure 4.11 Effect of Methanol extract of *Parquetina nigrescens* (MEPN) on lactate dehydrogenase activity in experimental rats.

[c] indicates value significantly different from normal control, diabetes + 100 mg/kg metformin. (n=5)

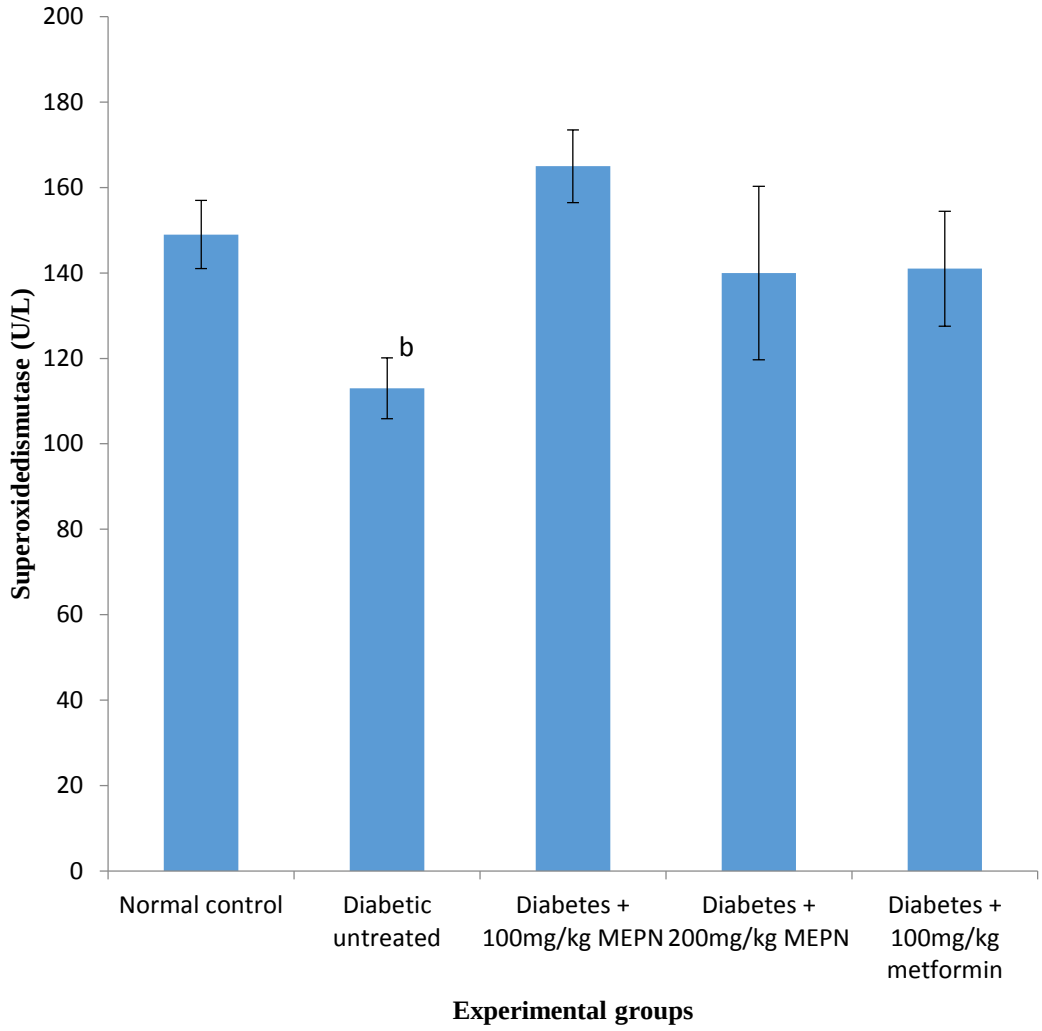

Figure 4.12 Effect of Methanol extract of *Parquetina nigrescens* (MEPN) on superoxide dismutase activity in experimental rats.

[b] indicates value significantly different from normal control, diabetes + 100 mg/kg MEPN, diabetes + 200 mg/kg MEPN, diabetes + 100 mg/kg Metformin. (n=5)

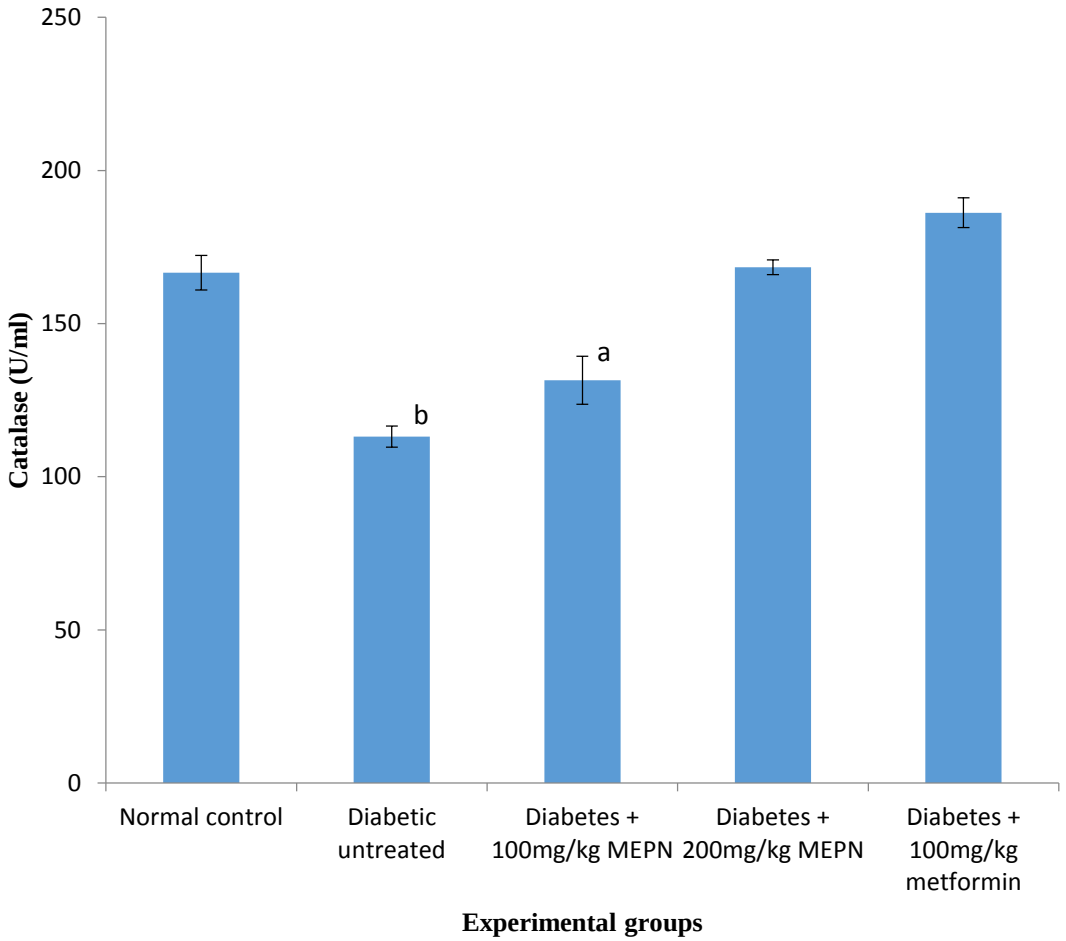

Figure 4.13 Effect of Methanol extract of *Parquetina nigrescens* (MEPN) on catalase activity in experimental rats.

[a] indicates value significantly different from normal control, diabetes + 200 mg/kg MEPN, diabetes + 100 mg/kg Metformin. [b] indicates value significantly different from normal control, diabetes + 100 mg/kg MEPN, diabetes + 200 mg/kg MEPN, diabetes + 100 mg/kg Metformin. (n=5)

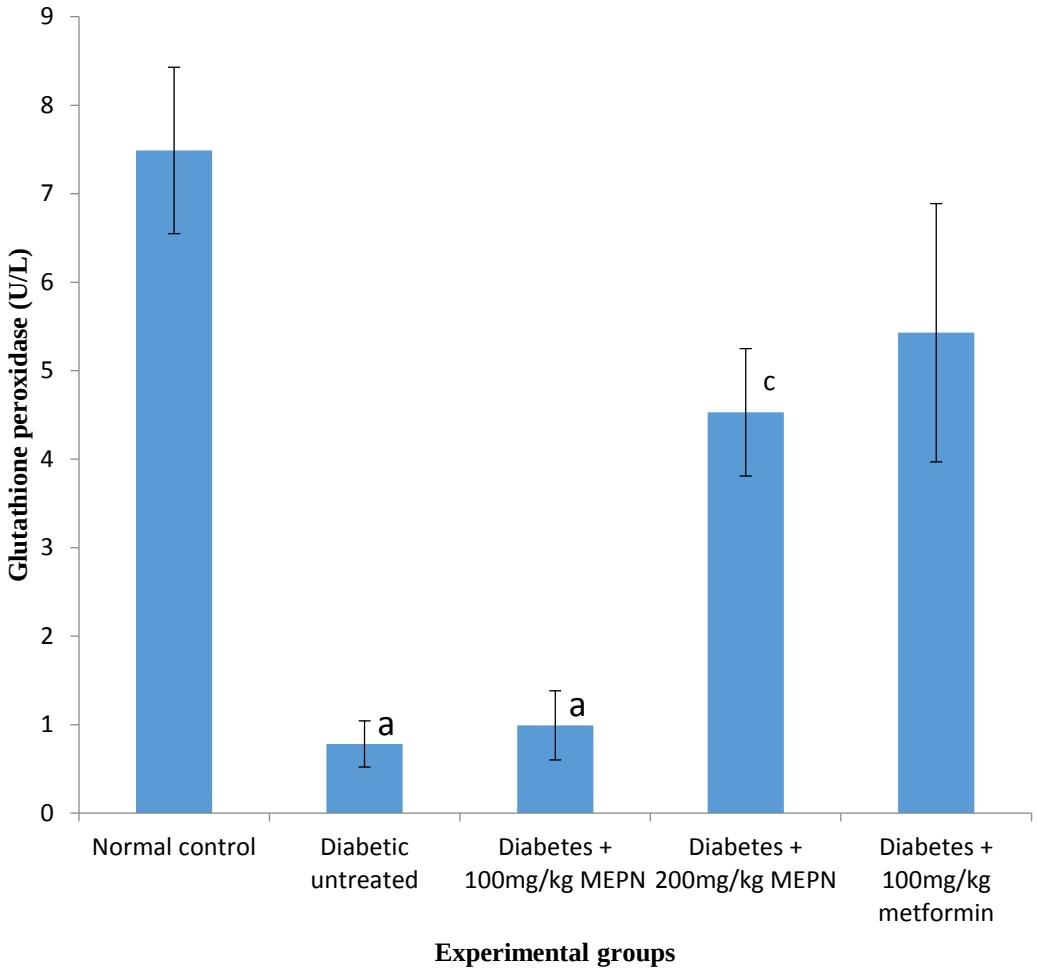

Figure 4.14 Effect of Methanol extract of *Parquetina nigrescens* (MEPN) on glutathione peroxidase activity in experimental rats.

[a] indicates value significantly different from normal control, diabetes + 200 mg/kg MEPN, diabetes + 100 mg/kg metformin. [c] indicates value significantly different from normal control (n=5)

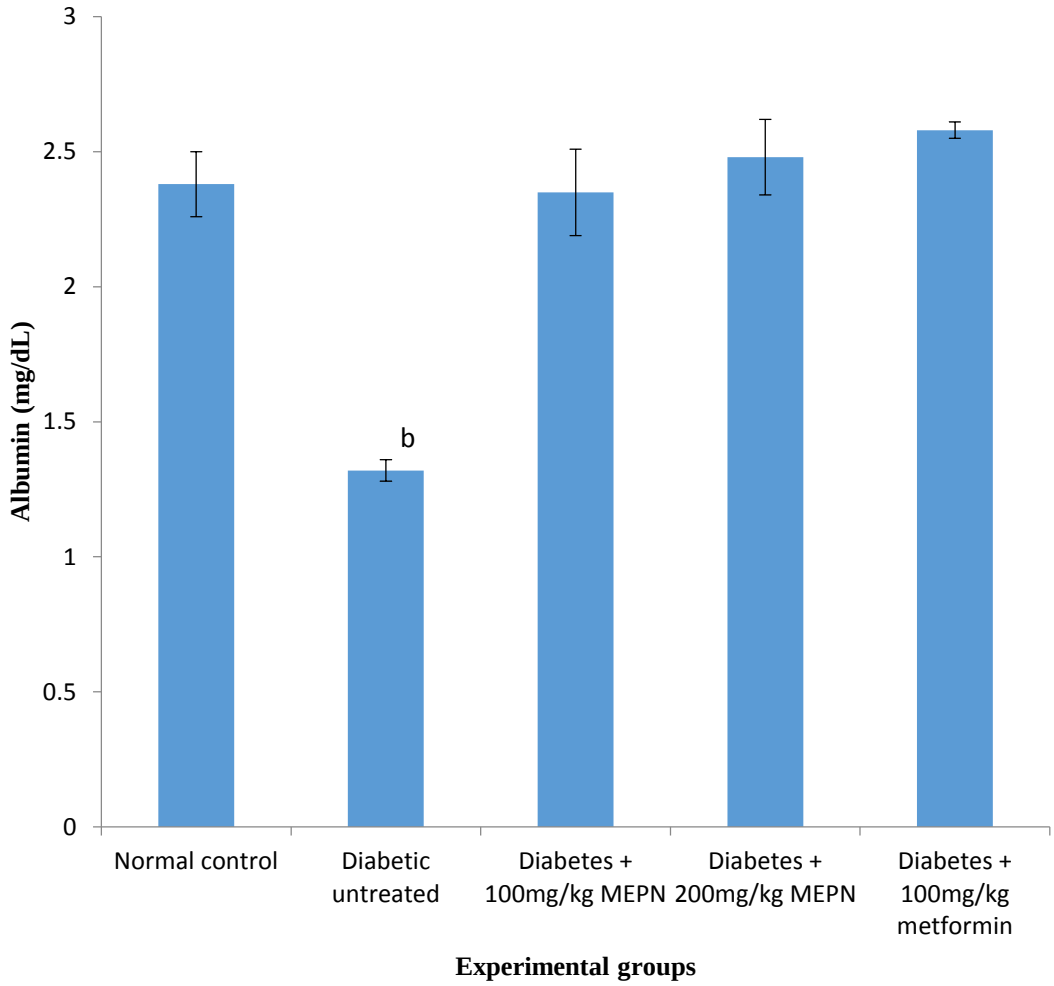

Figure 4.12 Effect of Methanol extract of *Parquetina nigrescens* (MEPN) on albumin level in experimental rats.

[b] indicates value significantly different from normal control, diabetes + 100 mg/kg MEPN, diabetes + 200 mg/kg MEPN, diabetes + 100 mg/kg Metformin (n=5).

4.12 Electrolyte changes in rats treated with methanol extract of *Parquetina nigrescens* (MEPN)

Figure 4.16 and 4.17 showed changes in sodium and potassium ions in normal control, diabetic untreated, MEPN and metformin treated rats. There was significant decrease ($p<0.05$) in Na^+ and K^+ concentrations in diabetic untreated when compared to diabetic+100 mg/kg MEPN, diabetes+200 mg/kg MEPN and 100 mg/kg metformin treated groups. There was significant difference in Na^+ concentration in diabetes+100 mg/kg MEPN when compared to normal control, diabetes+200 mg/kg MEPN and diabetes+100 mg/kg metformin. Na^+ concentration in diabetes+200 mg/kg was significantly different from diabetes+100 mg/kg MEPN and diabetes+100 mg/kg metformin.

Figure 4.18 showed changes in magnesium ions in normal control, diabetic untreated, MEPN and metformin treated rats. There was no significant difference ($p>0.05$) in Mg^{2+} in the normal control, diabetic untreated and treatment groups.

Figure 4.19 showed changes in calcium ions in normal control, diabetic untreated, MEPN and metformin treated rats. Ca^{2+}concentration significantly decrease ($p<0.05$) in diabetic untreated when compared to diabetes+200 mg/kg MEPN and diabetes+100 mg/kg metformin treated groups. There was significant difference in Ca^{2+} in diabetes+200 mg/kg MEPN when compared to normal control, diabetic untreated, diabetes+100 mg/kg MEPN and diabetes+100 mg/kg metformin.

Figure 4.20 showed changes in bicarbonate ions in normal control, diabetic untreated, MEPN and metformin treated rats. There was significant decrease in HCO_3^- in diabetic untreated when compared to diabetic treated with 100 mg/kg MEPN, diabetes treated with 200 mg/kg, diabetic treated with 100 mg/kg metformin and normal control.

Figure 4.21 showed changes in chloride ions in normal control, diabetic untreated, MEPN and metformin treated rats. There was significant decrease in Cl^- in diabetic untreated, diabetic treated with 100 mg/kg MEPN and diabetic treated with 100 mg/kg metformin when compared to normal control and diabetic treated with 200 mg/kg.

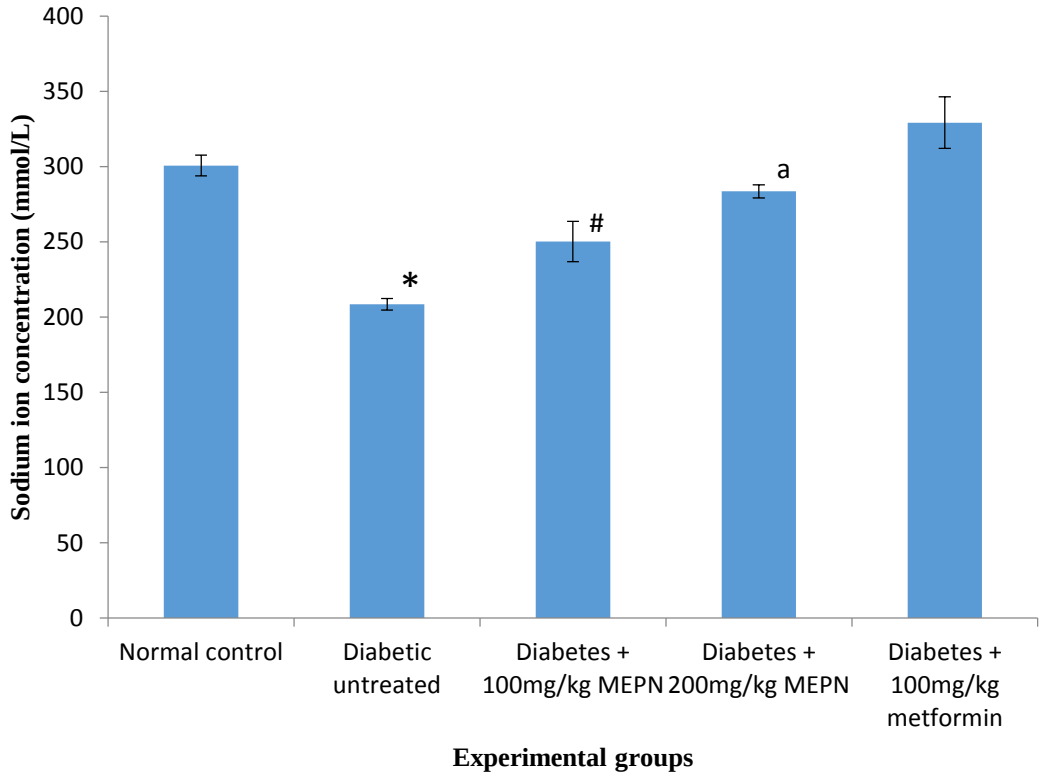

Figure 4.16 Effect of Methanol extract of *Parquetina nigrescens* (MEPN) on sodium ion concentration in experimental rats.

* indicate values significantly different from normal control, diabetes+100mg/kg MEPN, diabetes+200 mg/kg MEPN and diabetes+100 mg/kg metformin, # indicate values significantly different from normal control, diabetes+200 mg/kg MEPN and diabetes+100 mg/kg metformin, a indicate value significantly different from diabetes+100 mg/kg MEPN and diabetes+100 mg/kg metformin, (n=5).

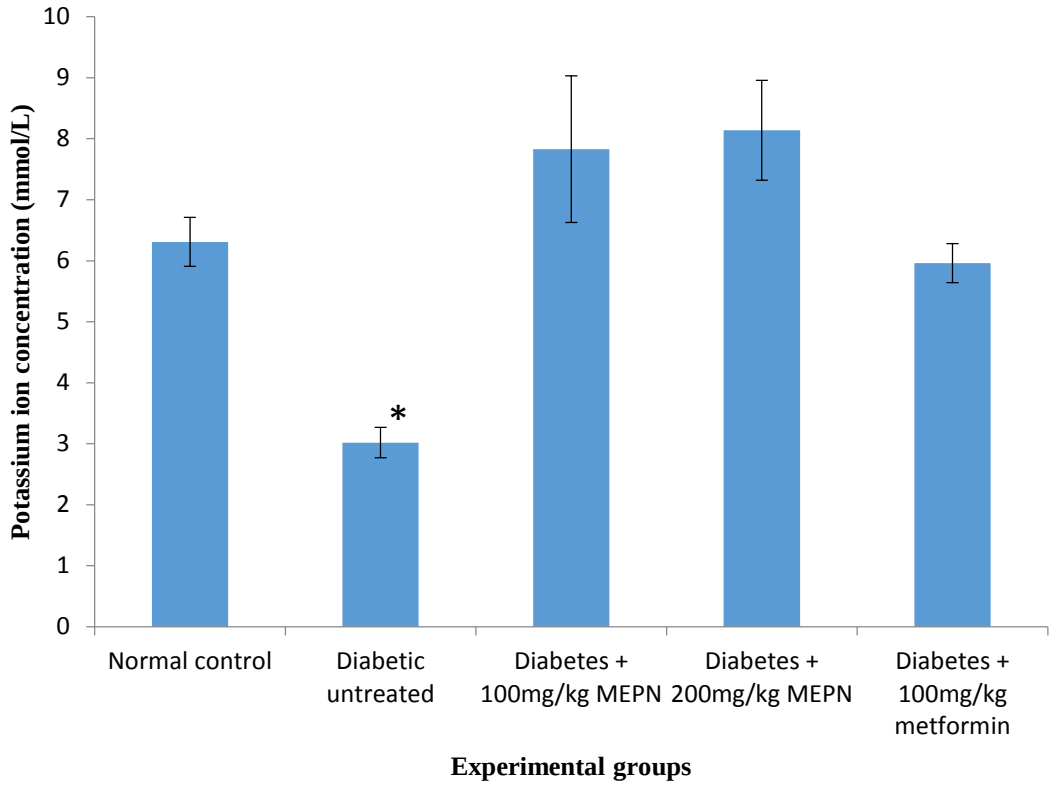

Figure 4.17 Effect of Methanol extract of *Parquetina nigrescens* (MEPN) on potassium ion concentration in experimental rats.

* indicate values significantly different from normal control, diabetes+100 mg/kg MEPN, diabetes+200 mg/kg MEPN and diabetes+100 mg/kg metformin (n=5).

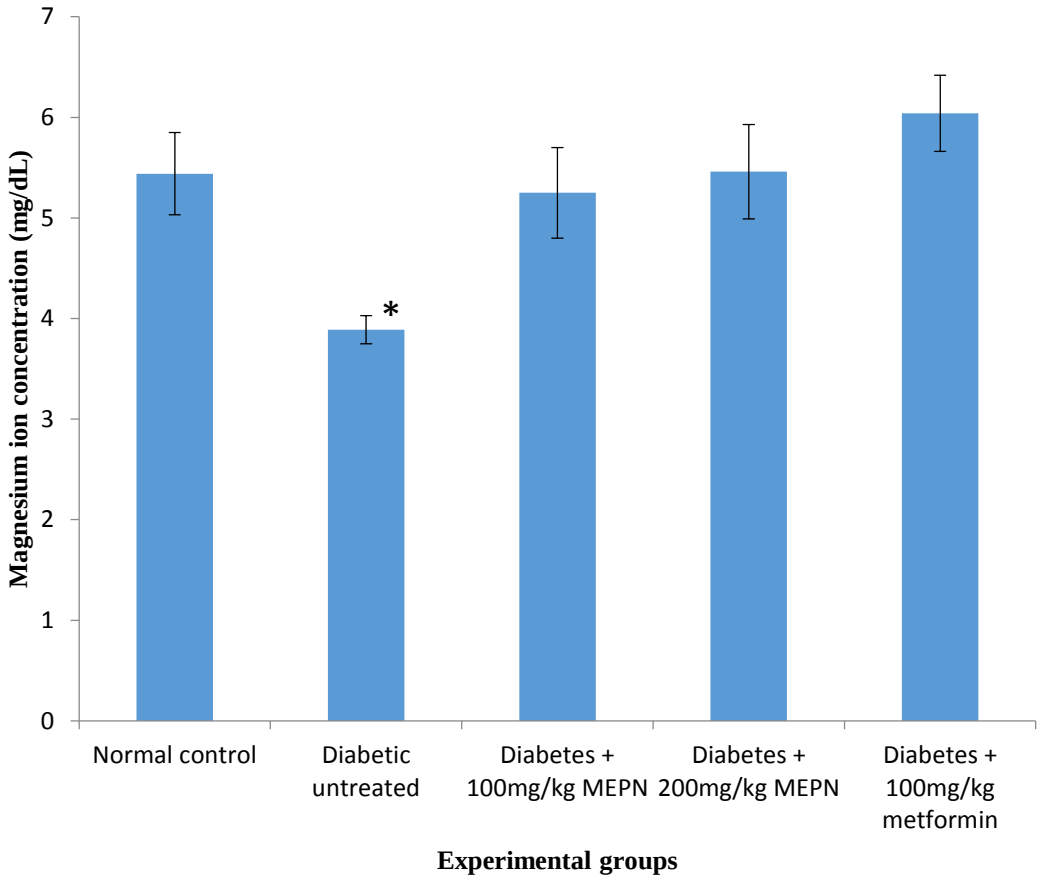

Figure 4.18 Effect of Methanol extract of *Parquetina nigrescens* (MEPN) on magnesium ion concentration in experimental rats.

* indicate values significantly different from normal control, diabetes+100 mg/kg MEPN, diabetes+200 mg/kg MEPN and diabetes+100 mg/kg metformin, (n=5).

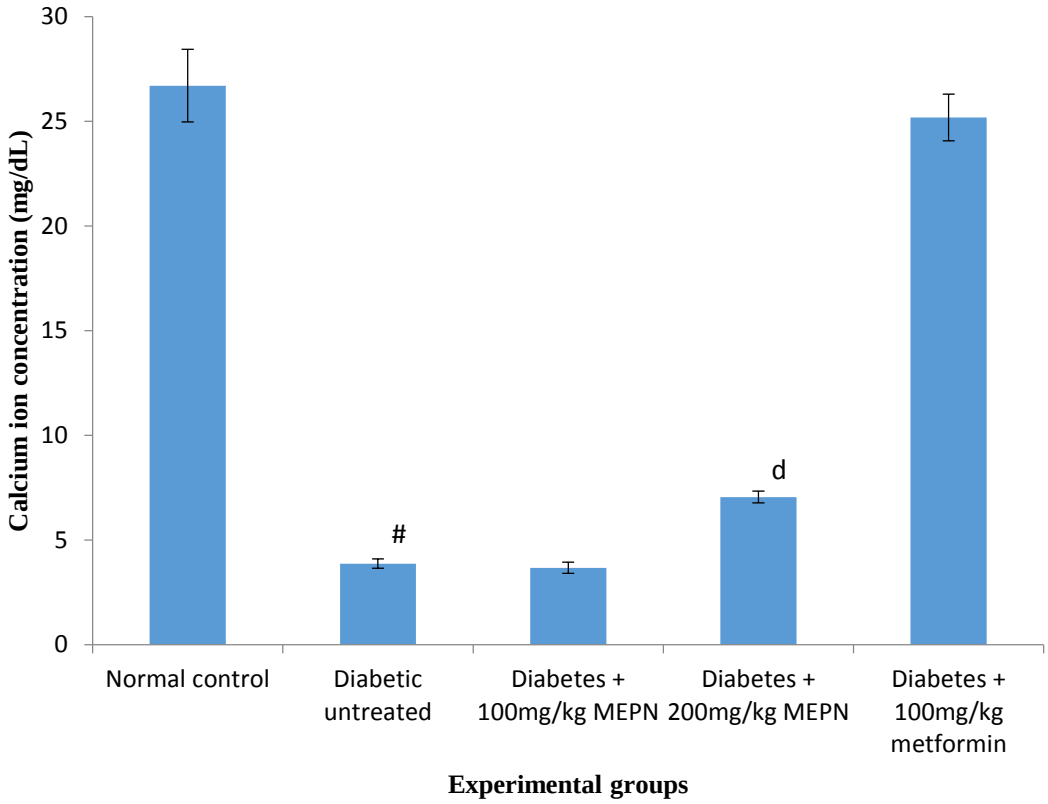

Figure 4.19 Effect of Methanol extract of *Parquetina nigrescens* (MEPN) on calcium ion concentration in experimental rats.

[#] indicate values significantly different from normal control, diabetes+200 mg/kg MEPN and diabetes+100 mg/kg metformin, [d] indicates values significantly different from normal control, diabetic untreated, diabetes+100 mg/kg MEPN and diabetes+100 mg/kg metformin. (n=5).

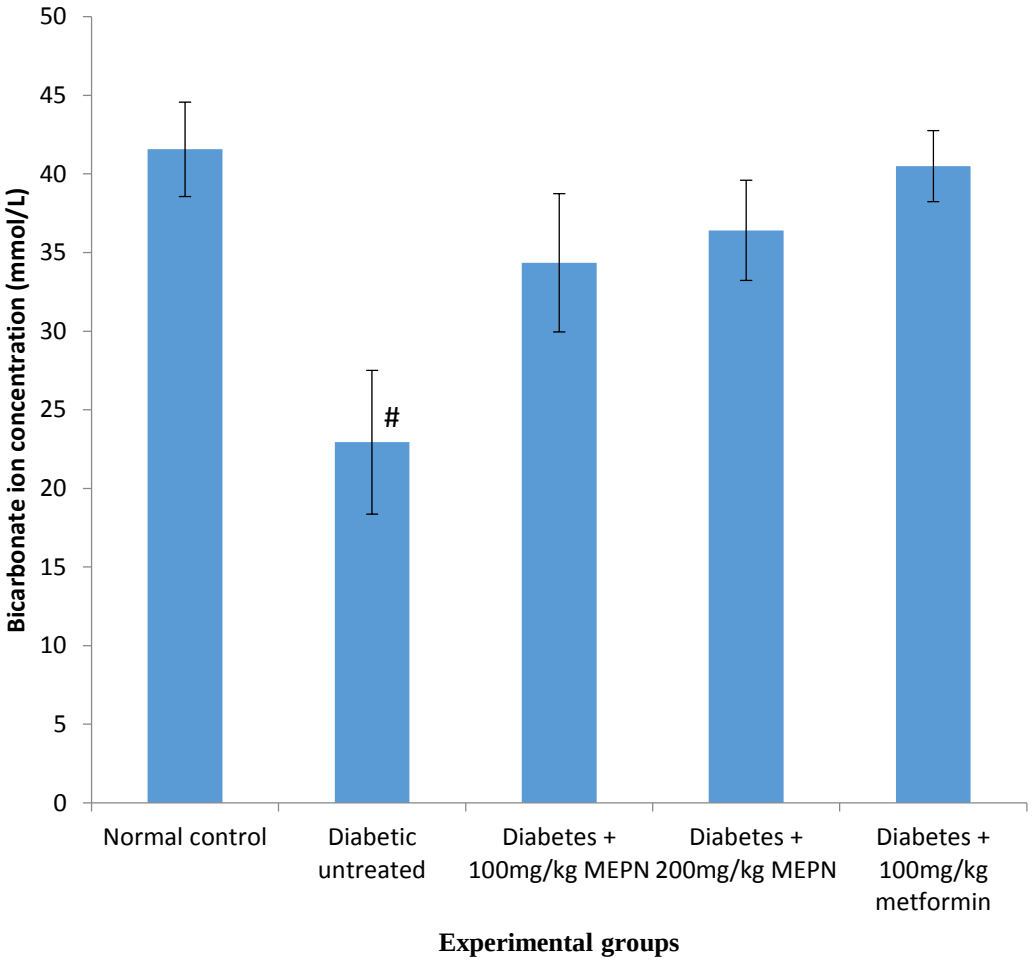

Figure 4.20 Effect of Methanol extract of *Parquetina nigrescens* (MEPN) on bicarbonate ion concentration in experimental rats.

[#] indicate values significantly different from normal control, diabetes+200 mg/kg MEPN and diabetes+100 mg/kg metformin (n=5).

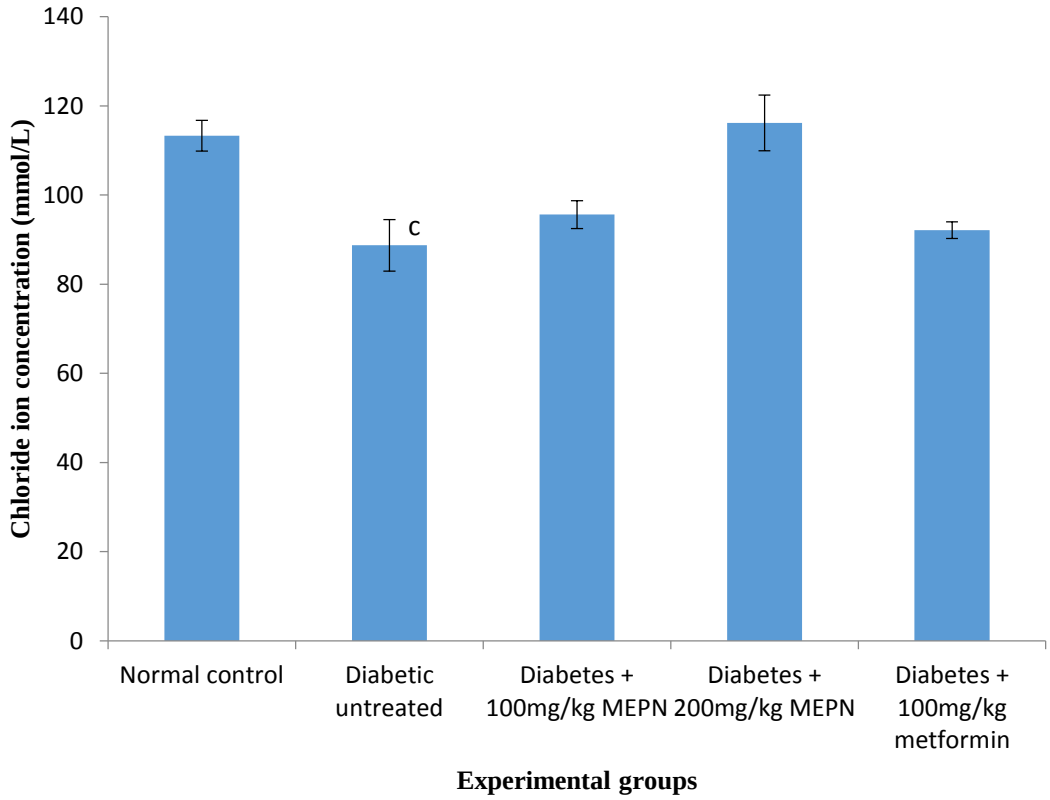

Figure 4.21 Effect of Methanol extract of *Parquetina nigrescens* (MEPN) on chloride ion concentration in experimental rats.

[c] indicate values significantly different from normal control and diabetes+200 mg/kg MEPN (n=5).

4.13 Blood Urea Nitrogen in rats treated with methanol extract of *Parquetina nigrescens* (MEPN)

Figure 4.22 showed blood urea nitrogen (BUN) in normal, diabetes treated with MEPN and metformin rats. BUN significantly decreased ($p<0.05$) in normal control, diabetes treated with 100 mg/kg and 200 mg/kg MEPN when compared to diabetic untreated. BUN significantly increased ($p<0.05$) in diabetes treated with 100 mg/kg Metformin when compared to normal control and diabetes treated with 100 and 200 mg/kg MEPN. There was no significant difference in BUN in MEPN treated groups when compared to normal control but, BUN significantly increased in metformin treated group when compared to normal control.

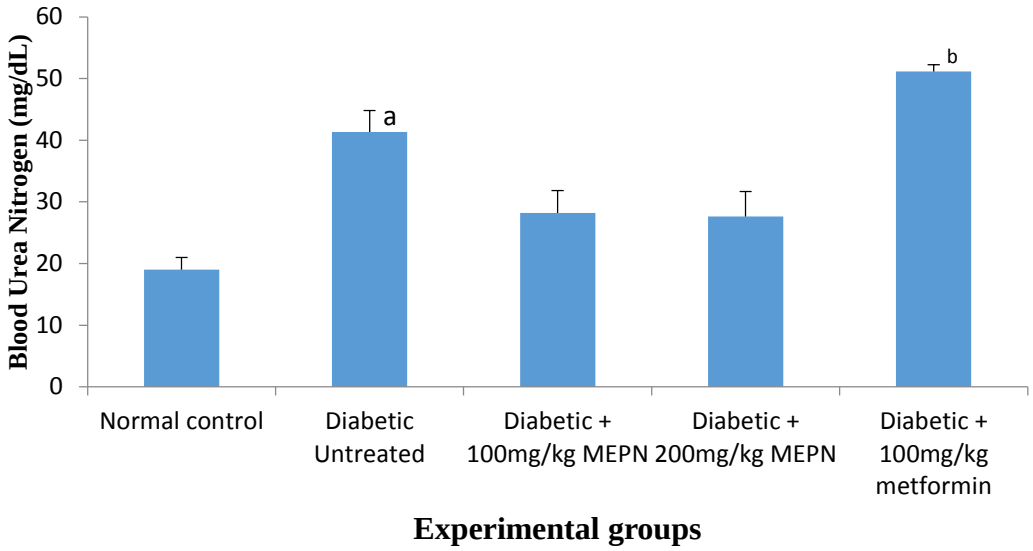

Figure 4.22 Effect of Methanol extract of *Parquetina nigrescens* (MEPN) on blood urea nitrogen in experimental rats.

[a] indicates value significantly different from the normal control, diabetes + 100 mg/kg MEPN, diabetes + 200 mg/kg MEPN, diabetes + 100 mg/kg Metformin. [b] indicates value significantly different from normal control, diabetes + 100 mg/kg MEPN, diabetes + 200 mg/kg MEPN (n=5).

4.14 Creatinine level in rats treated with methanol extract of *Parquetina nigrescens* (MEPN)

Figure 4.23 showed creatinine level in normal, MEPN and Metformin treated groups. There was significant increase ($p<0.05$) in creatinine level in diabetic untreated group when compared to normal control. Similarly, creatinine level significantly increased in 100 mg/kg MEPN treated group when compared to normal control. However, there was significant decrease ($p<0.05$) in creatinine level in 200 mg/kg MEPN treated group when compared to diabetes untreated and diabetes treated with 100 mg/kg MEPN.

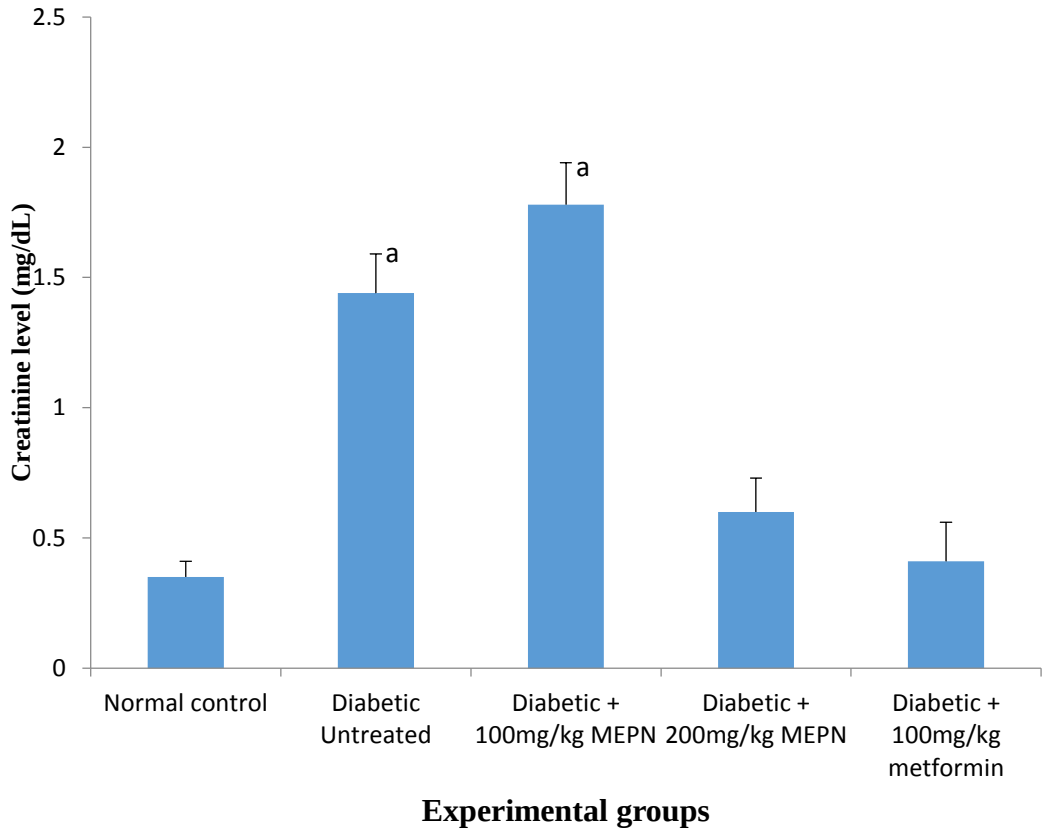

Figure 4.23 Effect of Methanol extract of *Parquetina nigrescens* (MEPN) on creatinine level in experimental rats.

[a] indicate values significantly different from the normal control, diabetes+200 mg/kg MEPN, diabetes+100 mg/kg metformin (n=5)

4.15 Histological changes in the kidney of rat treated with methanol extract of *Parquetina nigrescens* (MEPN)

Plate 4.2 showed transverse section of the kidney in normal, diabetic untreated, diabetic treated with 100 mg/kg MEPN, diabetic treated with 200 mg/kg MEPN and diabetic treated with 100 mg/kg metformin respectively.

Photomicrographs of kidney sections stained by Haematoxylin and Eosin showed normal architecture as seen with a high magnification x400, the renal cortex showed normal glomeruli with normal mesengial cells and capsular spaces in the normal control and diabetic treated 200 mg/kg MEPN. Moreso, no visible pathological lesions were seen in the groups mentioned above.

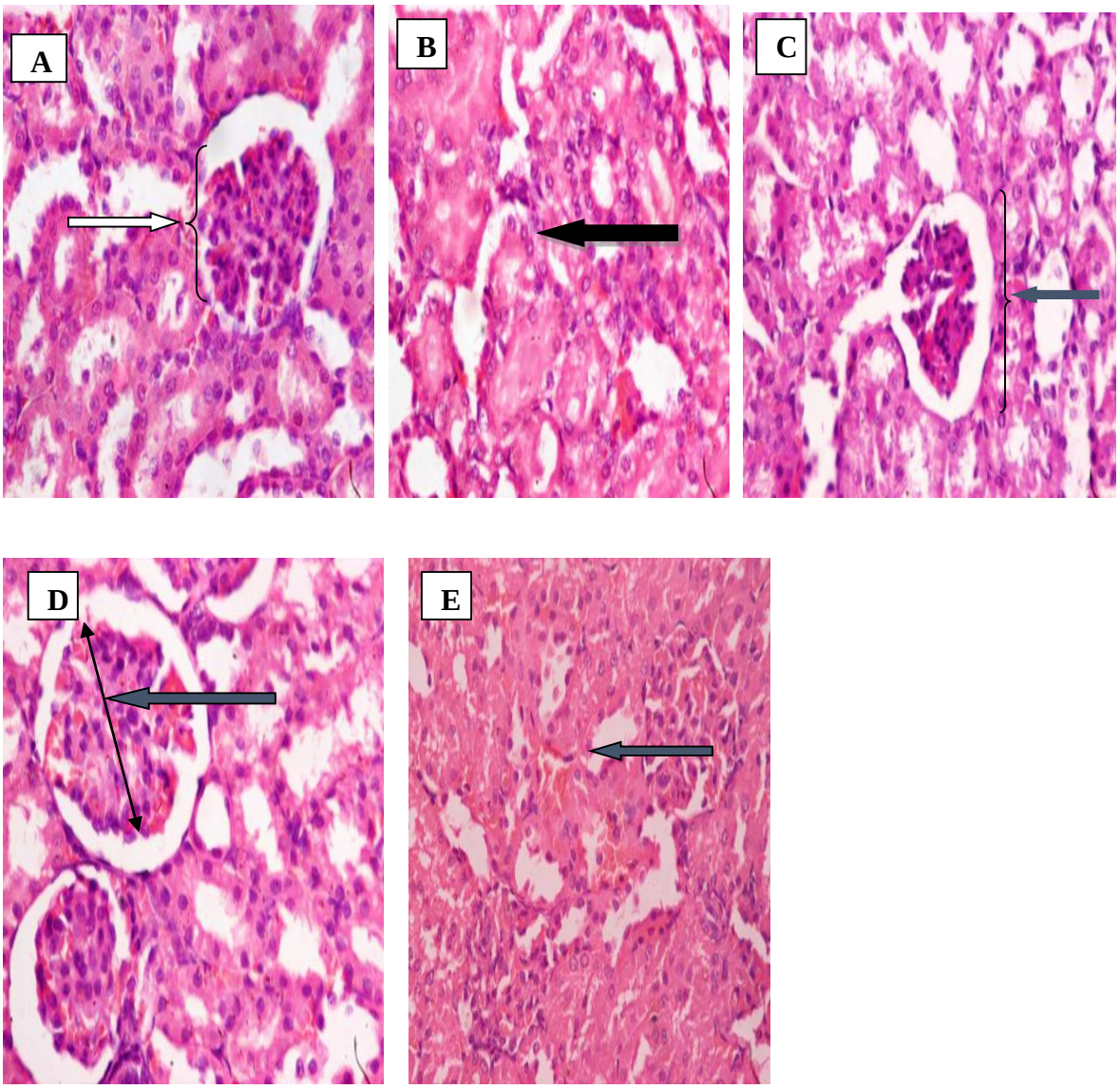

Plate 4.2 (A – E): Shows sections stained with H & E showing architecture of the Kidney in A (Control), B (Diabetic untreated), C (Diabetes + 100 mg/kg MEPN), D (Diabetes + 200 mg/kg MEPN), E (Diabetes + 100 mg/kg Metformin). Plate 4.2A showed architecture of the kidney with normal glomerulus and capsular space (white Arrow), Plate 4.2B showed architecture of the kidney with degenerated glomerulus and Bowman's capsule with inflammatory cells (Black Arrows). Plate 4.2C& E showed architecture of the kidneys with distorted glomerulus. Plate 4.2D showed architecture of the kidney with glomerulus and capsular space comparable with the normal control but with enlarged capsular space (Plate 4.2A) (Green Arrow) X 400.

4.16 Body weight changes in rats treated with Phytol and Squalene

Table 4.9 showed the body weights of the rats in the control and the treated groups. Body weight increased significantly ($p<0.05$) in normal control group at weeks 2, 3 and 4 when compared to the initial value at week 0. The percentage increases are 20.86%, 29.62% and 33.24% respectively. Body weight decreased significantly ($p<0.05$) in diabetic untreated at weeks 2, 3 and 4 when compared to the initial value at week 0. The percentage decreases are 10.44%, 17.45% and 28.89% respectively. There was significant increase ($p<0.05$) in body weight in diabetic group treated with 250 mg/kg Phytol at weeks 2, 3 and 4 when compared to the initial value at week 0. The percentage increases are 11.94% and 20.25% and 22.92% respectively. There was also significant increase ($p<0.05$) in body weight in diabetic group treated with 3 mg/kg Squalene at weeks 2, 3 and 4 when compared to the initial value at week 0. The percentage increases are 15.11% and 18.30% and 21.26% respectively.

Table 4.10 showed the percentage differences in body weights, 72 hours of alloxan administration and 28 days post treatment with MEPN. The percentage differences includes normal control (33.2%), diabetic untreated (28.9%), diabetes treated with 250 mg/kg Phytol (22.92%), diabetes treated with 3 mg/kg Squalene (17.2%) and diabetes treated with 100 mg/kg metformin (8.7%).

Table 4.11 showed the mean and relative weights of the liver of the control and the treated groups. Liver weight increased significantly ($p<0.05$) in normal control when compared to diabetic untreated. There was significant increase ($p<0.05$) in liver weight in diabetic treated with 250 mg/kg Phytol and 3 mg/kg Squalene when compared to diabetic untreated. Liver weight was significantly higher ($p<0.05$) in diabetic treated with 100 mg/kg metformin when compared to diabetic untreated.

Table 4.12 showed the mean and relative weights of the kidney of the control and the treated groups. Kidney weight increased significantly ($p<0.05$) in normal control when compared to diabetic untreated. There was significant increase ($p<0.05$) in kidney weight in diabetic treated with 250 mg/kg Phytol and 3 mg/kg Squalene when compared to diabetic untreated. Kidney weight was significantly higher ($p<0.05$) in diabetic treated with 100 mg/kg metformin when compared to diabetic untreated.

4.9 Body weight changes in rats treated with Phytol and Squalene

Experimental groups	Week 0	Week 1	Week 2	Week 3	Week 4
Normal control (0.3ml distilled water)	96.00± 1.92	114.00 ± 2.19	121.30 ± 3.21*	136.40 ± 2.19*	143.80 ± 3.71*
Diabetic untreated (0.3ml distilled water	128.4 ± 1.99	121.80 ± 1.77	115.00±2.91*	106.00±3.42*	91.3±4.10*
Diabetic + 250 mg/kg Phytol	115.0± 2.53	124.80± 2.59	130.60 ± 4.69*	144.20 ± 3.09*	149.20 ± 3.51*
Diabetic + 3 mg/kg Squalene	115.2±2.83	123.60±4.63	135.70±4.41*	141.00±2.07*	146.3±3.01*
Diabetic + 100 mg/kg Metformin	109.2±5.50	111.60±4.17	115.00±3.27	116.60±3.52	119.6±6.11

* indicate values significantly different from week 0 values (n = 5).

4.10 Percentage Difference in the Body weights after 72 hours of Alloxan Injection and 28 days post-treatment with Phytol and Squalene

Experimental groups	72 hours after alloxan injection	Values after 28 days of treatment	% Differences	
			Increase	Decrease
Normal control	96.0±1.9	143.8.30±3.7	33.24	
Diabetic untreated	91.3±1.9	128.4±4.1		28.89
Diabetic + 250 mg/kg Phytol	115.0±2.5	149.20±3.5		22.92
Diabetic + 3 mg/kg Squalene	115.2±2.8	146.3±3.0	17.2	
Diabetic + 100 mg/kg Metformin	109.2±5.5	119.6±6.1	8.7	

Table 4.11 Mean and relative weights of the liver in rats treated with Phytol and Squalene

Experimental groups	Mean weights of the liver (g)	Relative weights of liver (%)
Normal control	5.07±0.18	3.53
Diabetic untreated	3.07±0.38*	3.36
Diabetic + 250 mg/kg Phytol	4.42±0.15	2.96
Diabetic + 3 mg/kg Squalene	4.41±0.33	3.01
Diabetic + 100 mg/kg metformin	5.45±0.56	4.56

* indicate values significantly different from normal control, diabetes + 250 mg/kg Phytol, diabetes + 3 mg/kg Squalene, diabetes + 100 mg/kg metformin (n = 5).

Table 4.12 Mean and relative weights of the kidney in rats treated with Phytol and Squalene

Experimental groups	Mean weights of the kidney (g)	Relative weights of kidney (%)
Normal control	0.48±0.03	0.33
Diabetic untreated	0.24±0.01*	0.26
Diabetic + 250 mg/kg Phytol	0.46±0.02	0.31
Diabetic + 3 mg/kg Squalene	0.46±0.02	0.32
Diabetic + 100 mg/kg metformin	0.46±0.03	0.38

Data were expressed as Mean ± Standard Error of mean, $p<0.05$. * indicate values significantly different from normal control, diabetes + 250 mg/kg Phytol, diabetes + 3 mg/kg Squalene, diabetes + 100 mg/kg metformin (n = 5).

4.17 Fasting blood glucose level in rats treated with Phytol and Squalene

Figure 4.24 showed changes in fasting blood glucose level in the normal control, diabetic untreated, Phytol, Squalene and Metformin treated rats. There was significant increase ($p<0.05$) in fasting blood glucose level at weeks 1,2, 3 and 4 in diabetic untreated group when compared to the normal control. Moreso, fasting blood glucose significantly decreased ($p<0.05$) in Phytol, Squalene and metformin treated groups at weeks 3 and 4 when compared to the diabetic untreated group. There was no significant difference ($p>0.05$) in fasting blood glucose in Phytol, Squalene and metformin treated groups and normal control at the various time intervals.

There was no significant difference ($p>0.05$) in fasting blood glucose in diabetic untreated group at weeks 1, 2, 3 and 4 when compared to the initial value 72 hours after diabetes induction. However, there was significant decrease ($p<0.05$) in fasting blood glucose level in phytol, squalene and metformin treated groups at week 2, 3, and 4 when compared to the initial value 72 hours after diabetes induction.

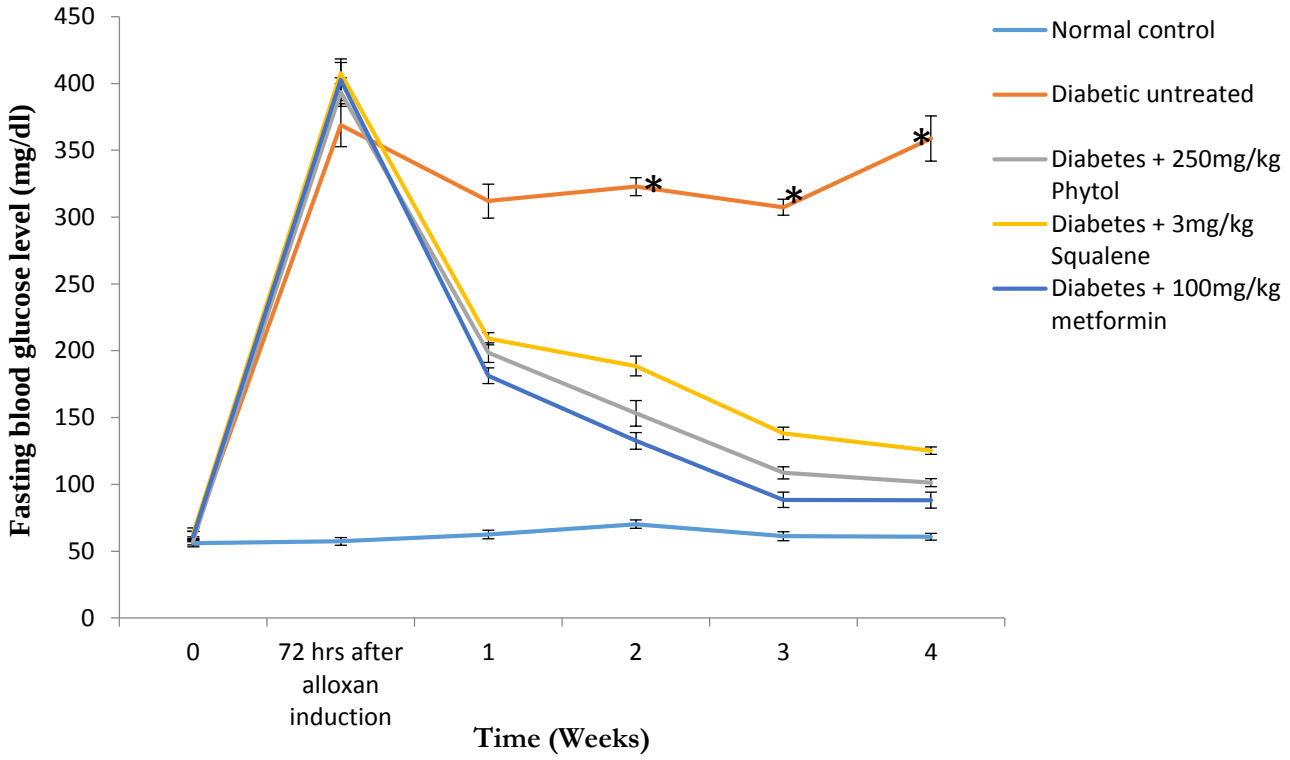

Figure 4.24 Effect of Phytol and Squalene on fasting blood glucose level in experimental rats.

* indicate values significantly different from normal control, diabetes + 250 mg/kg Phytol, diabetes + 3 mg/kg Squalene and diabetes + 100 mg/kg Metformin (n=5)

4.18 Liver glycogen content in rats treated with Phytol and Squalene

Figure 4.25 showed changes in liver glycogen content in the normal control, diabetic untreated, Phytol, Squalene and Metformin treated rats. There was significant decrease ($p<0.05$) in liver glycogen in diabetic untreated group when compared to normal control, diabetes + 250 mg/kg Phytol, diabetes + 3 mg/kg Squalene and diabetes + metformin treated groups. There was no significant difference ($p>0.05$) in glycogen content in diabetes + 3 mg/kg Squalene treated group when compared to the normal control. There was no significant difference ($p>0.05$) in liver glycogen content in normal control, diabetes + 250 mg/kg Phytol and diabetes + 100 mg/kg metformin treated groups.

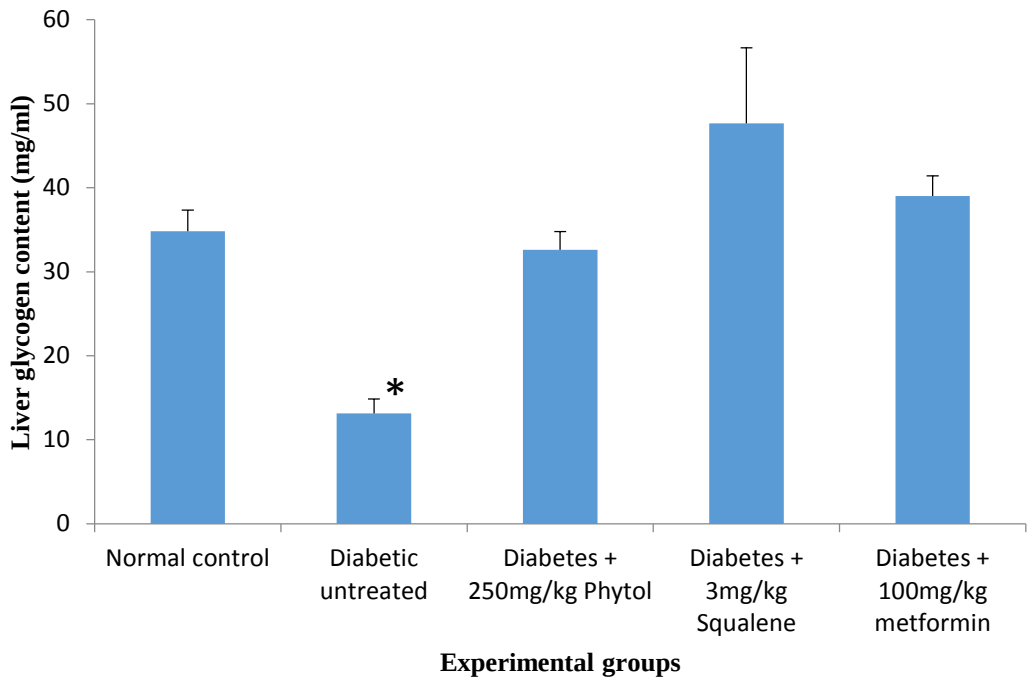

Figure 4.25 Effect of Phytol and Squalene on liver glycogen content in experimental rats.

* indicate values significantly different from normal control, diabetes + 250 mg/kg Phytol, diabetes + 3 mg/kg Squalene and diabetes + 100 mg/kg Metformin (n=5)

4.19 Histological changes in the liver of rat treated with Phytol and Squalene

Plate 4.3 showed transverse section of the liver in normal, diabetic untreated, diabetic treated with 250 mg/kg Phytol, diabetic treated with 3 mg/kg Squalene and diabetic treated with 100 mg/kg metformin respectively.

Photomicrograph of liver section stained by haematoxylin and eosin showed good architecture as seen in magnification (x400). The central venules are normal and not congested and the sinusoids appear normal without infiltration of inflammatory cells in normal control, diabetic treated with 250 mg/kg Phytol, diabetic treated with 3 mg/kg Squalene and diabetic treated with 100 mg/kg metformin. The hepatocytes however, showed severe hepatic macro steatosis and infiltration of inflammatory cells in diabetic untreated group.

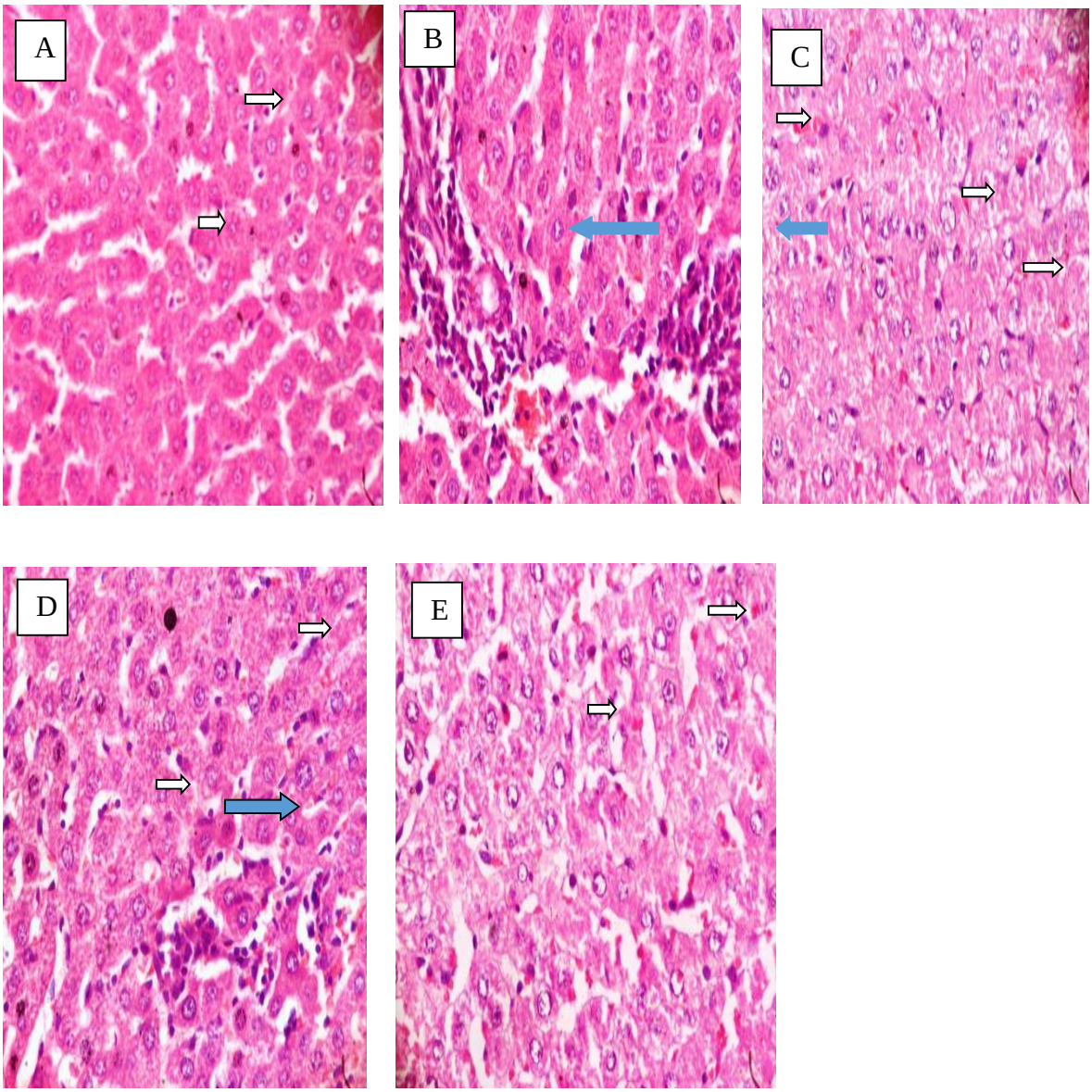

Plate 4.4 (A-E): Photomicrograph of the liver in normal, diabetic untreated and treated groups (x 400). The above slides are sections stained with H&E. Each slide represents A (Control), B (Diabetic untreated), C (250 mg/kg Phytol), D (3 mg/kg Squalene) & E (100 mg/kg Metformin). Each of the slides has normal sinusoids (White Arrows) with no visible lesions. Slide B & D however showed mild necrosis (Blue Arrows).

4.20 Intestinal glucose absorption in rats treated with Phytol and Squalene

Table 4.13 showed duodenal glucose absorption in normal control, diabetic untreated, Phytol, Squalene and Metformin treated rats. There was significant increase ($p<0.05$) in duodenal glucose absorption in diabetic untreated group when compared to normal control. There was no significant difference ($p>0.05$) in duodenal glucose absorption at 20, 40 and 60 minutes in diabetes+ 250 mg/kg Phytol and 3mg/kg diabetes + Squalene treated groups when compared to the diabetic untreated groups. There was significant increase ($p<0.05$) in duodenal glucose absorption in diabetes+ 250 mg/kg Phytol and diabetes + 3 mg/kg Squalene treated groups at 20, 40 and 60 minutes when compared to normal control. Duodenal glucose absorption significantly decrease ($p<0.05$) in diabetes+ metformin group when compared to diabetic untreated, diabetes+250 mg/kg phytol and diabetes+3 mg/kg Squalene at 40 and 60 min.

Table 4.14 showed jejunal glucose absorption in normal control, diabetic untreated, Phytol, Squalene and Metformin treated rats. Jejunal glucose absorption significantly increased ($p<0.05$) at 60 minutes in diabetic untreated groups when compared to the normal control and diabetes + metformin treated group. There was no significant difference ($p>0.05$) at 20 and 40 minutes of jejunal glucose absorption in diabetes+ 250 mg/kg Phytol and diabetes + 3 mg/kg Squalene treated groups when compared to normal control and diabetic untreated groups. There was significant decrease in glucose absorption at 40 and 60 minutes in metformin treated group when compared to normal control and diabetic untreated groups respectively. There was significant decrease in jejunal glucose absorption at 20 and 40minutes in diabetes+metformin group when compared to diabetes+250 mg/kg Phytol and diabetes+3 mg/kg Squalene.

Table 4.13 Effect of Phytol and Squalene on duodenal glucose absorption in experimental rats

Experimental group	20 min (mg/dl/cm)	40 min (mg/dl/cm)	60 min (mg/dl/cm)
Normal Control	4.21±0.88	3.84±1.05	3.11±0.95
Diabetic untreated	5.96.±0.47*	5.92±0.79*	6.13±1.05*
Diabetic +250 mg/kg Phytol	5.13±1.67	5.62±0.73	5.31±0.95
Diabetic + 3 mg/kg Squalene	5.77±1.54	5.82±0.88	5.93±0.94
Diabetic + 100 mg/kg Metformin	4.85±0.86	3.29±0.65	3.04±0.52

*indicate values significantly different from normal control and diabetes + 100 mg/kg Metformin (n=5).

Table 4.14: Effect of Phytol and Squalene on jejunal glucose absorption in experimental rats

Experimental group	20 min (mg/dl/cm)	40 min (mg/dl/cm)	60 min (mg/dl/cm)
Normal Control	3.22±0.82	3.65±0.96	3.07±0.77
Diabetic untreated	4.46±0.85*	4.19±0.67 *	4.84±0.93#
Diabetic + 250 mg/kg Phytol	4.10±0.71	4.53±0.92	4.90±0.98
Diabetic + 3 mg/kg Squalene	4.09±0.61	3.85±0.86	4.69±1.11
Diabetic + 100 mg/kg Metformin	3.32.±0.67	2.21±0.52 [a]	2.49±0.80 [a]

* indicate values significantly different from diabeties+100 mg/kg Metformin, [#] indicates value significantly different from the normal control and diabetes+ 100 mg/kg metformin, [a] indicate values significantly different from diabeties+250 mg/kg Phytol, diabetes +3 mg/kg Squalene (n=5)

4.21 Glucose concentration, insulin concentration, pancreatic beta cell function, insulin sensitivity and insulin resistance in rats treated with Phytol and Squalene

Figure 4.26 showed glucose concentration in normal, diabetic untreated, Phytol, Squalene and metformin treated rats. Serum glucose significantly increase ($p<0.05$) in diabetic untreated group when compared to normal control, diabetes + Phytol treated, diabetes + Squalene treated and diabetes+metformin treated group.

Figure 4.27 showed insulin concentration in normal, diabetic untreated, Phytol, Squalene and metformin treated rats. Insulin concentration significantly decrease ($p<0.05$) in diabetic untreated when compared to normal control, diabetes+3 mg/kg squalene, diabetes + 250 mg/kg Phytol treated and diabetes+metformin treated group.

Figure 4.28 showed pancreatic beta cell function in normal, diabetic untreated, Phytol, Squalene and metformin treated rats. There was significant decrease ($p<0.05$) in pancreatic beta cell function in diabetic untreated when compared to normal control, diabetes + 250 mg/kg Phytol, diabetes + 3 mg/kg Squalene and diabetes+metformin treated groups.

Figure 4.29 showed insulin sensitivity in normal, diabetic untreated, Phytol, Squalene and metformin treated rats. There was significant decrease ($p<0.05$) in insulin sensitivity in diabetic untreated when compared to normal control and the treated groups.

Figure 4.30 showed insulin resistance in normal, diabetic untreated, Phytol, Squalene and metformin treated rats. Insulin resistance significantly decrease ($p<0.05$) in diabetic + 3 mg/kg Squalene group when compared to normal control, diabetic untreated and the treated groups.

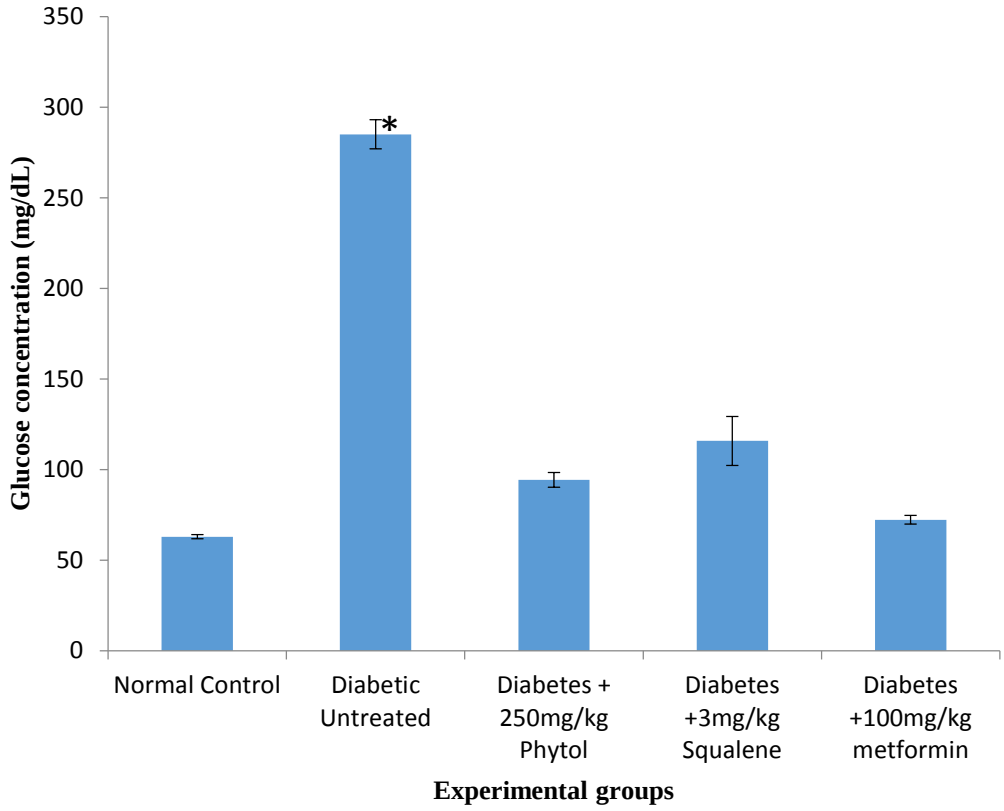

Figure 4.26 Effect of Phytol and Squalene on glucose concentration in experimental rats.

* indicate values significantly different from the normal control, diabetes+250 mg/kg Phytol, diabetes+3 mg/kg Squalene and diabetes+100mg/kg metformin, (n=5).

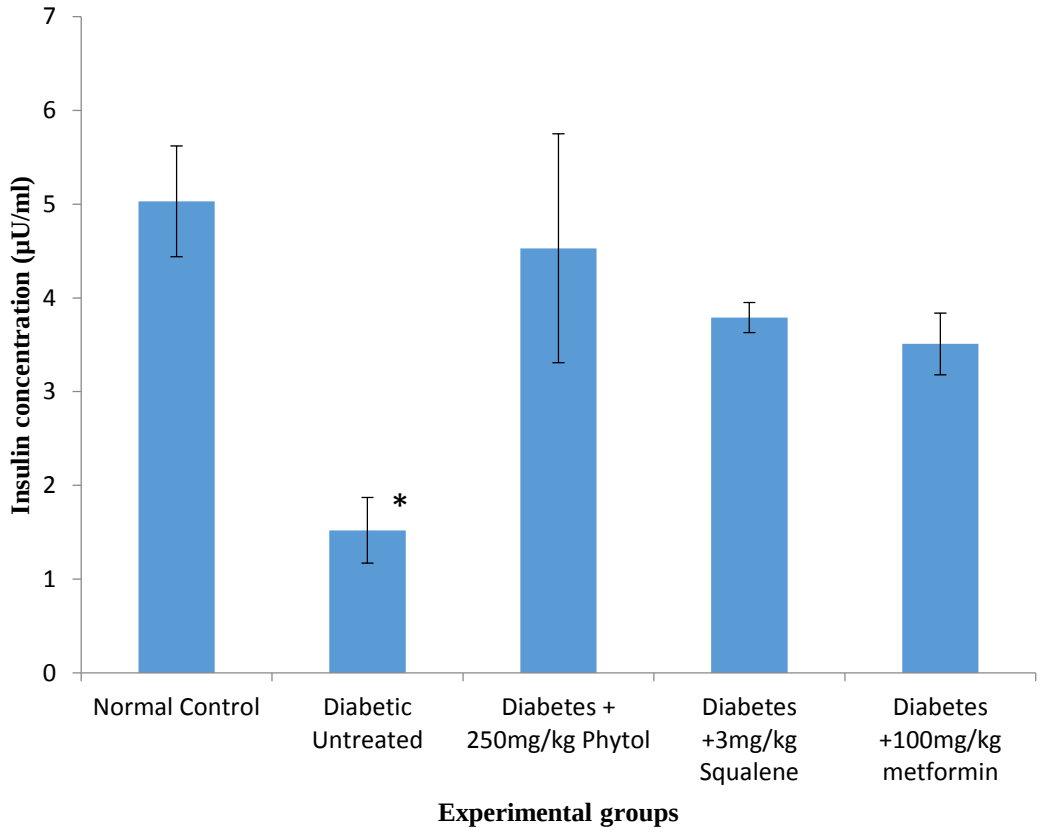

Figure 4.27 Effect of Phytol and Squalene on insulin concentration in experimental rats.

* indicate values significantly different from the normal control, diabetes+250 mg/kg Phytol, diabetes+3 mg/kg Squalene and diabetes+100 mg/kg metformin (n=5).

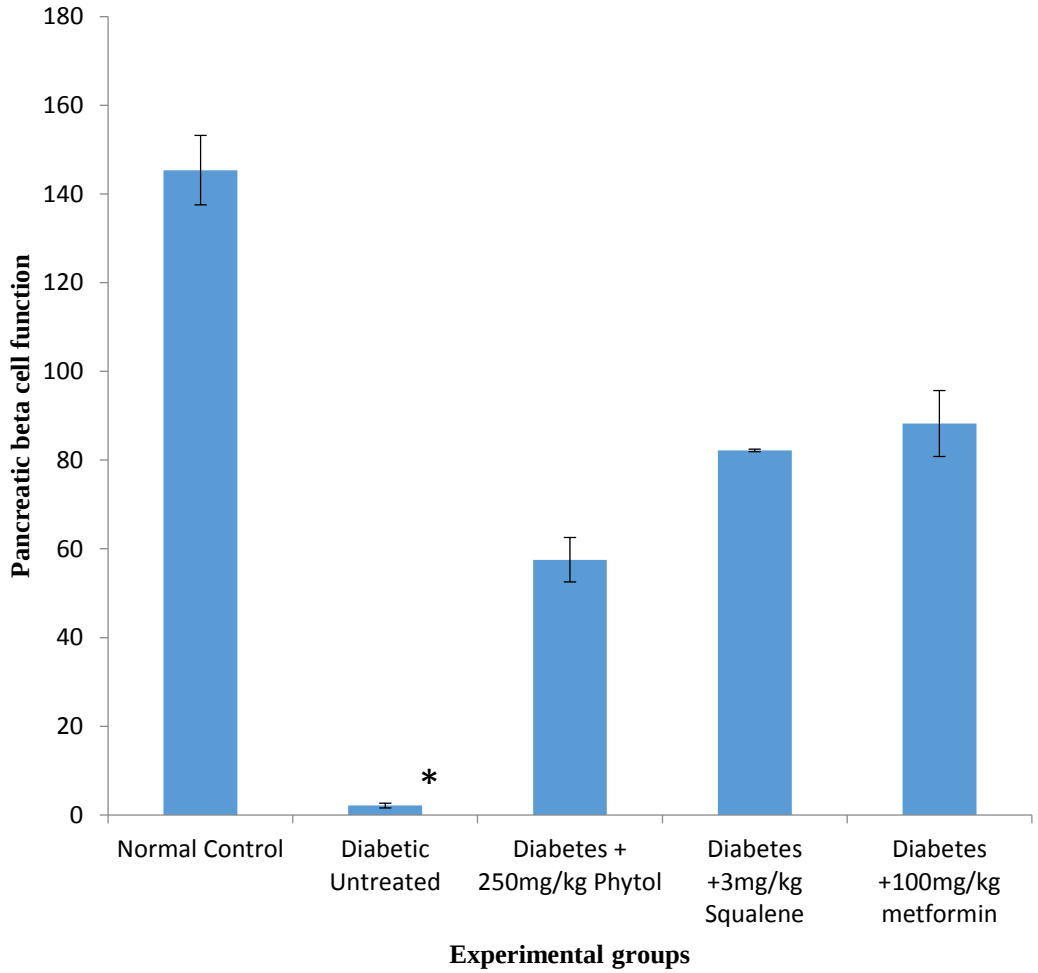

Figure 4.28 Effect of Phytol and Squalene on pancreatic beta cell function in experimental rats.

* indicate values significantly different from the normal control, diabetes+250 mg/kg Phytol, diabetes+3 mg/kg Squalene and diabetes+100 mg/kg metformin, (n=5).

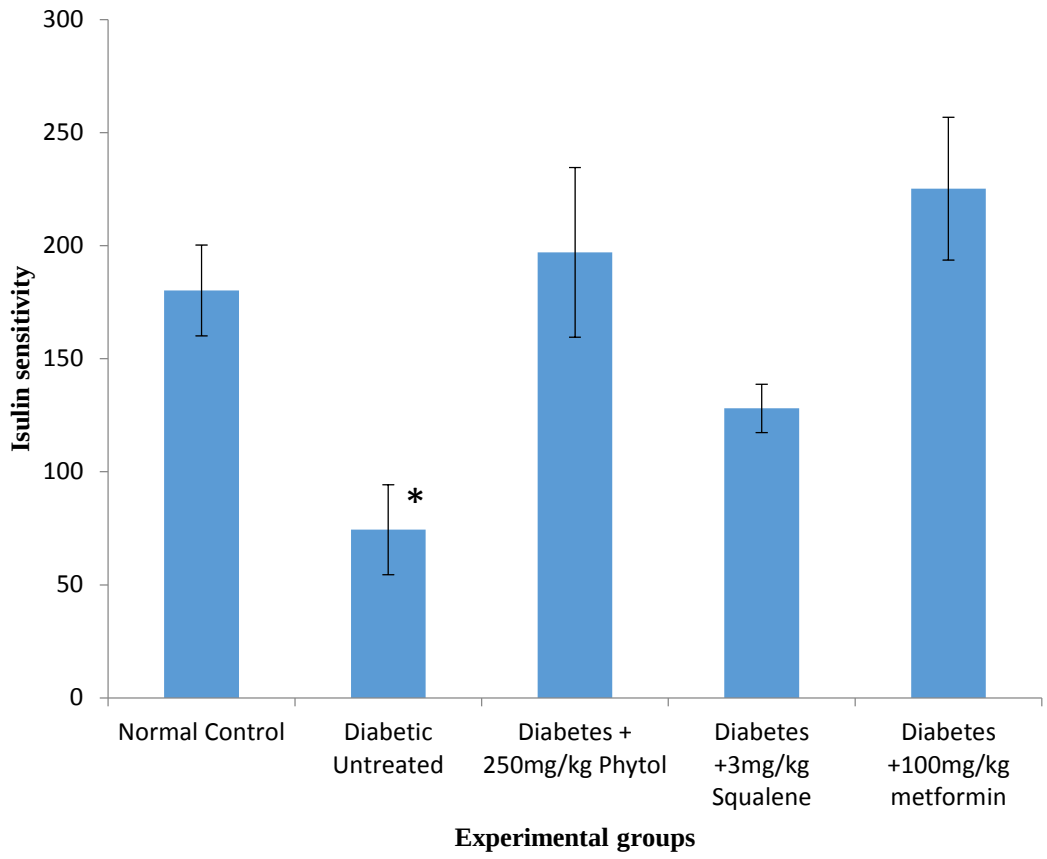

Figure 4.29 Effect of Phytol and Squalene on insulin sensitivity in experimental rats.

* indicate values significantly different from the normal control, diabetes+250 mg/kg Phytol, diabetes+3 mg/kg Squalene and diabetes+100 mg/kg metformin (n=5).

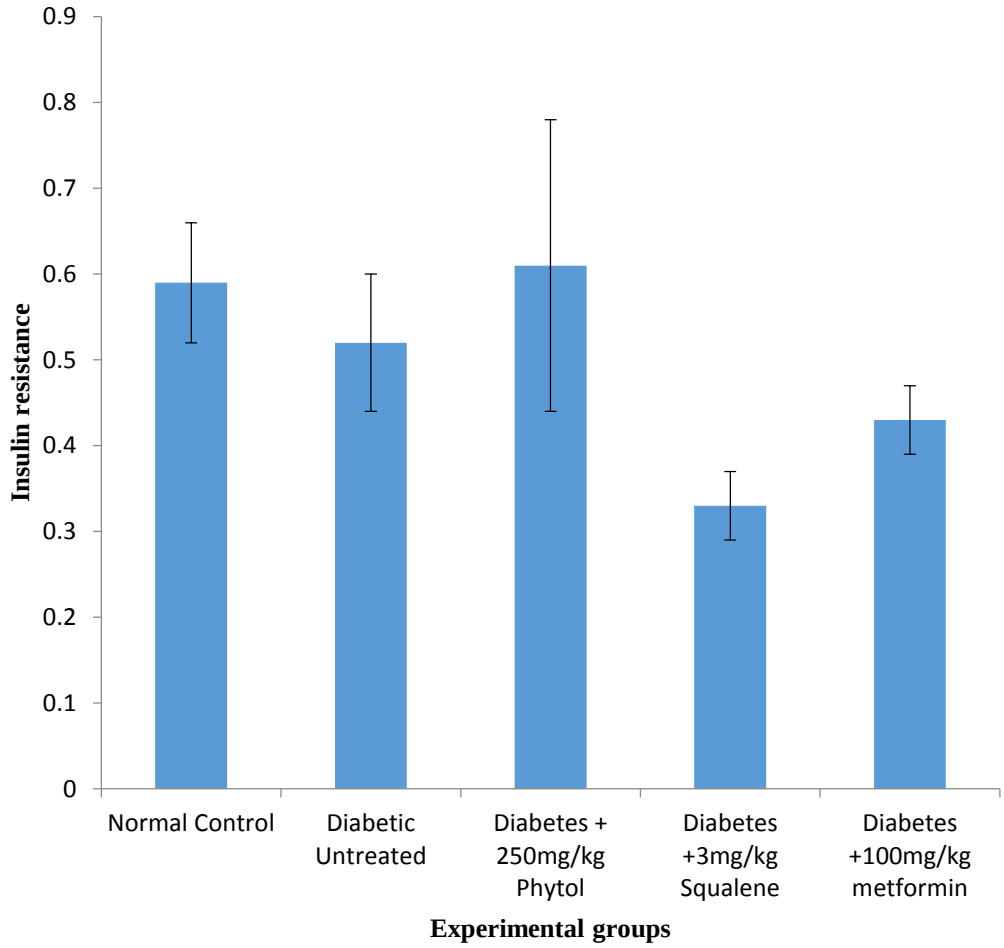

Figure 4.30 Effect of Phytol and Squalene on insulin resistance in experimental rats.

* indicate values significantly different from normal control, diabetic untreated, diabetes + 250 mg/kg Phytol, and diabetes + 100 mg/kg Metformin (n=5)

4.22 Histological changes in the pancreas in rats treated with Phytol and Squalene

Plate 4.4 showed transverse section of the pancreas in normal, diabetic untreated, diabetic treated with 250 mg/kg Phytol, diabetic treated with 3 mg/kg Squalene and diabetic treated with 100 mg/kg metformin respectively.

Photomicrograph of a pancreas section stained by haematoxylin and eosin and showed normal architecture. The parenchyma of the pancreas showed normal serous acinar and zymogenic cells containing abundant granular eosinophilic cytoplasm, normal interlobular connective tissues and septa were also seen. There are large islets of Langerhan consisting of normal round to oval collections of endocrine cells in diabetic treated with 250 mg/kg Phytol, diabetic treated with 3 mg/kg Squalene and diabetic treated with 100 mg/kg metformin. Thick walled vessels were also observed in the groups.

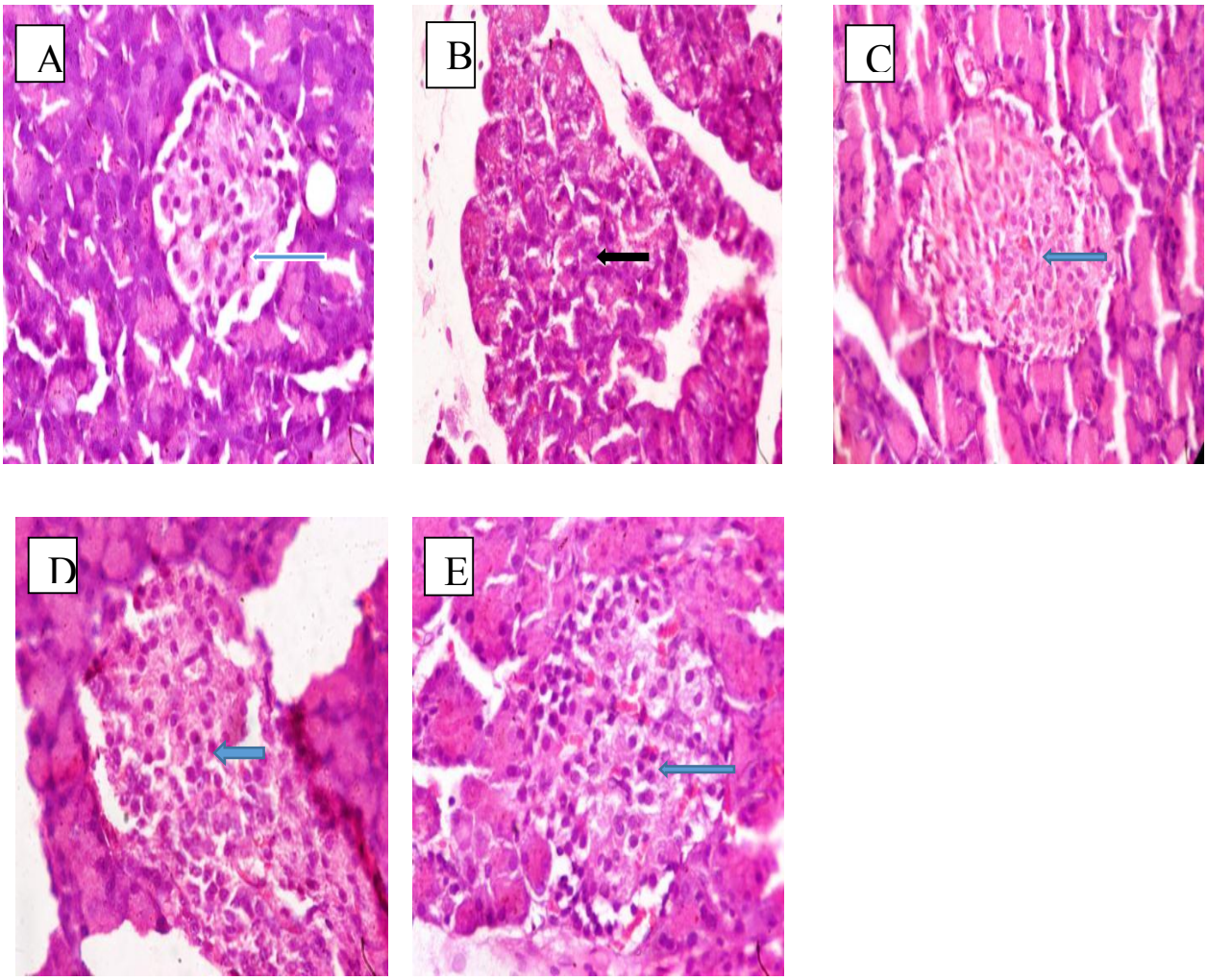

Plate 4.4 (A – E): Sections stained with H & E showing architecture of the pancreas in A (Control), B (Diabetic untreated), C, D & E (Diabetes treated with 250 mg/kg Phytol, 3 mg/kg Squalene and 100 mg/kg Metformin respectively). A showed architecture of the pancreas with normal pancreatic beta cells (White Arrow), B showed no visible pancreatic beta cells (Black Arrow), C, D & E showed hypertrophy and distorted pancreatic beta cells (Blue Arrows) X 400.

4.23 Antioxidative changes in rats treated with Phytol and Squalene

Figure 4.31 showed glucose 6 phosphate dehydrogenase activity in normal control, diabetic untreated, Phytol, Squalene, and Metformin treated rats. There was significant increase ($p<0.05$) in G6PDH activities in normal control, diabetic + 250 mg/kg Phytol, diabetic + 3 mg/kg Squalene and 100 mg/kg metformin groups when compared to diabetic untreated. There was no significant difference in G6PDH activities in diabetic + 250 mg/kg Phytol, diabetic + 3 mg/kg Squalene and 100 mg/kg metformin when compared to the normal control.

Figure 4.32 showed lactate dehydrogenase activity in normal control, diabetic untreated, Phytol, Squalene, and Metformin treated rats. There was significant increase ($p<0.05$) in LDH activities in normal control, diabetic + 250 mg/kg Phytol, diabetic + 3 mg/kg Squalene and 100 mg/kg metformin groups when compared to diabetic untreated. There was no significant difference in LDH activities in diabetic + 250 mg/kg Phytol, diabetic + 3 mg/kg Squalene and 100 mg/kg metformin when compared to the normal control.

Figure 4.33 showed superoxide dismutase activity in normal control, diabetic untreated, Phytol, Squalene, and Metformin treated rats. There was significant difference in SOD activity in normal control, diabetic + 250 mg/kg Phytol, diabetic + 3 mg/kg Squalene and 100 mg/kg metformin groups when compared to diabetic untreated. There was no significant difference in SOD activity in diabetic + 250 mg/kg Phytol, diabetic + 3 mg/kg Squalene and 100 mg/kg metformin when compared to normal control.

Figure 4.34 showed catalase activity in normal control, diabetic untreated, Phytol, Squalene, and Metformin treated rats. There was significant increase ($p<0.05$) in catalase activity in normal control, diabetic + 250 mg/kg Phytol, diabetic + 3 mg/kg Squalene and 100 mg/kg metformin groups when compared to diabetic untreated. However, there was no significant difference in CAT activity in diabetic + 250 mg/kg Phytol, diabetic + 3 mg/kg Squalene and 100 mg/kg metformin when compared to the normal control.

Figure 4.35 showed glutathione peroxidase activity in normal control, diabetic untreated, Phytol, Squalene, and Metformin treated rats. Glutathione peroxidase (GPx) significantly decreased ($p<0.05$) in diabetic untreated and diabetic + 3mg/kg Squalene

when compared to normal control, diabetic + 250 mg/kg Phytol and 100 mg/kg metformin groups respectively.

Figure 4.36 showed albumin level in normal control, diabetic untreated, Phytol, Squalene, and Metformin treated rats. There was significant decrease ($p<0.05$) in ALB level in diabetic + 250 mg/kg Phytol, diabetic + 3 mg/kg Squalene and 100 mg/kg metformin groups when compared to normal control. However, there was no significant difference in ALB level in diabetic + 250 mg/kg Phytol, diabetic + 3 mg/kg Squalene and 100 mg/kg metformin when compared to the diabetic untreated.

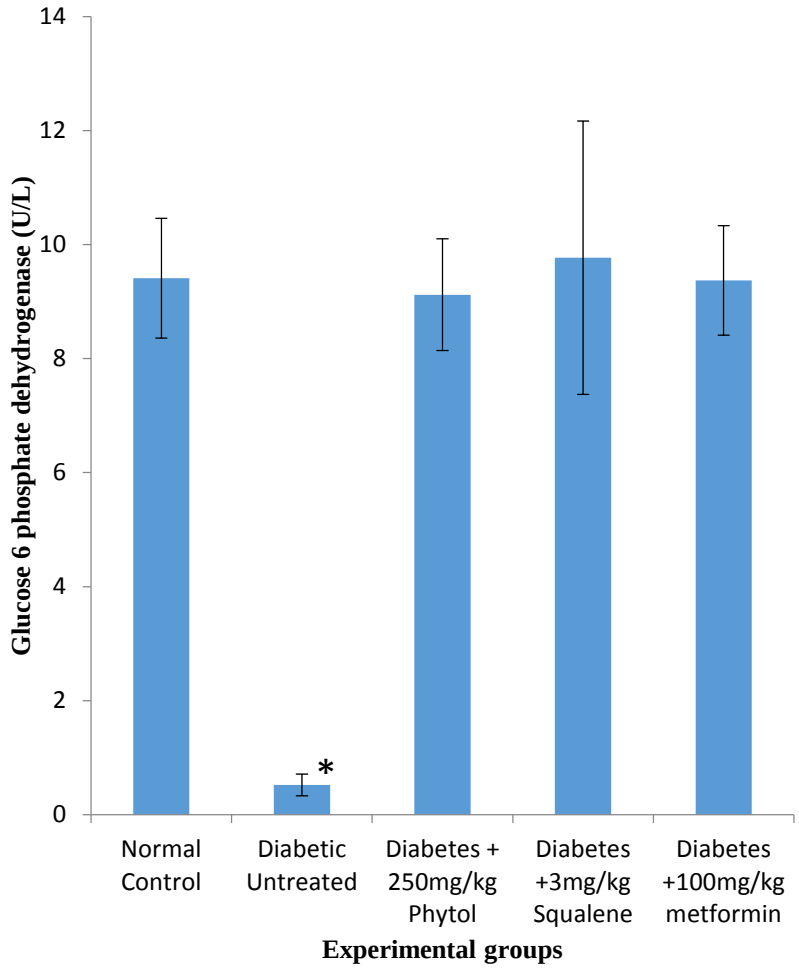

Figure 4.31 Effect of Phytol and Squalene on glucose 6 phosphate dehydrogenase activity in experimental rats.

* indicates value significantly different from normal control, diabetes + 250 mg/kg Phytol, diabetes + 3 mg/kg Squalene and diabetes + 100 mg/kg Metformin (n=5)

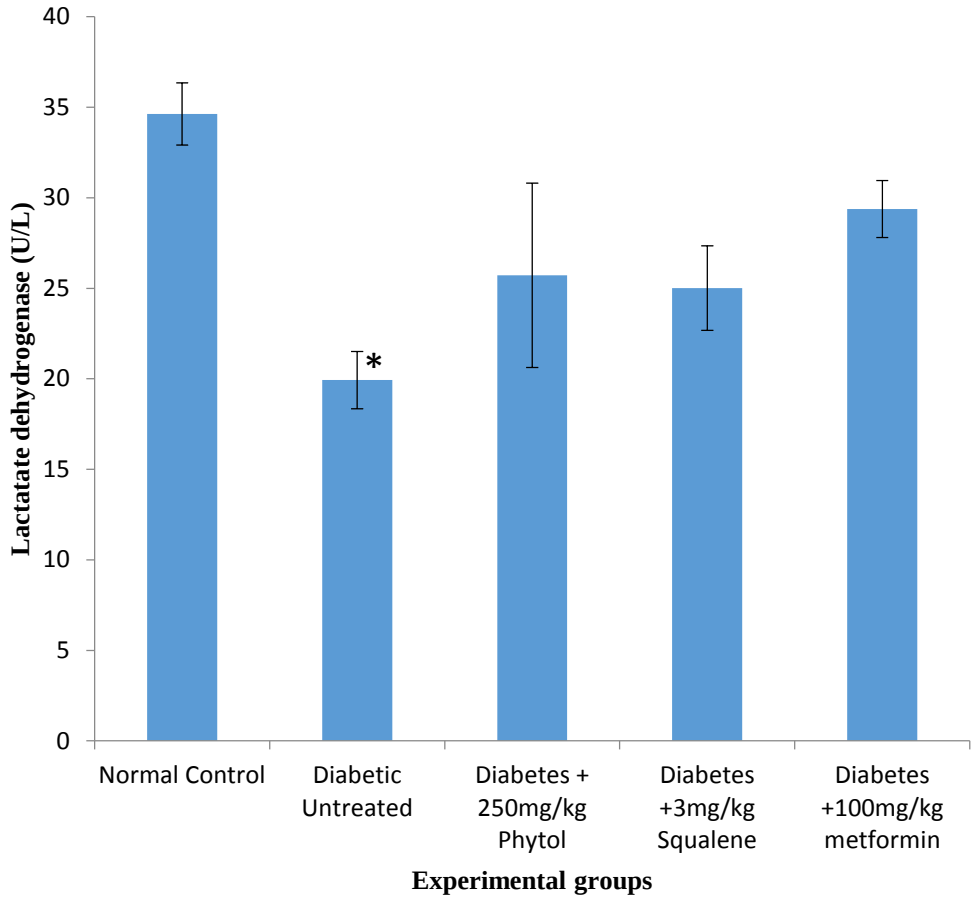

Figure 4.32 Effect of Phytol and Squalene on lactate dehydrogenase activity in experimental rats.

[a] indicates value significantly different from normal control, diabetes + 250 mg/kg Phytol, diabetes + 3 mg/kg Squalene and diabetes + 100 mg/kg Metformin. (n=5)

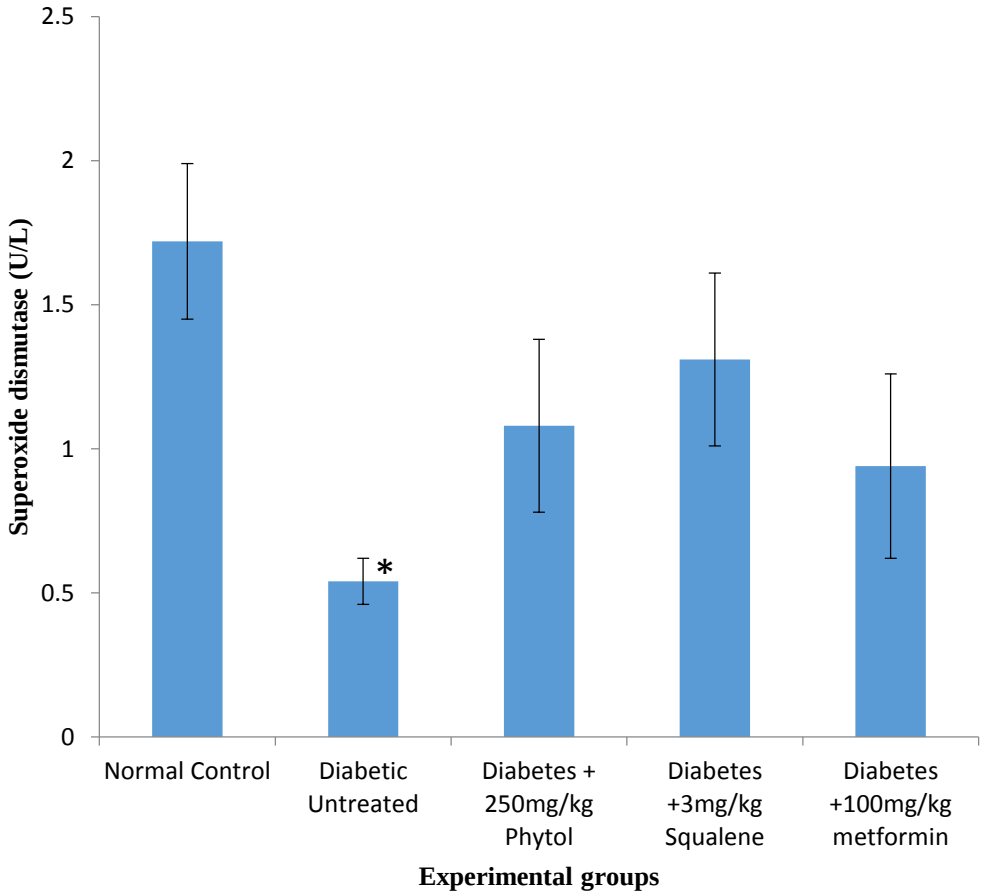

Figure 4.33 Effect of Phytol and Squalene on superoxide dismutase activity in experimental rats.

* indicate values significantly different from normal control, diabetes + 250 mg/kg Phytol, diabetes + 3 mg/kg Squalene and diabetes + 100 mg/kg Metformin (n=5).

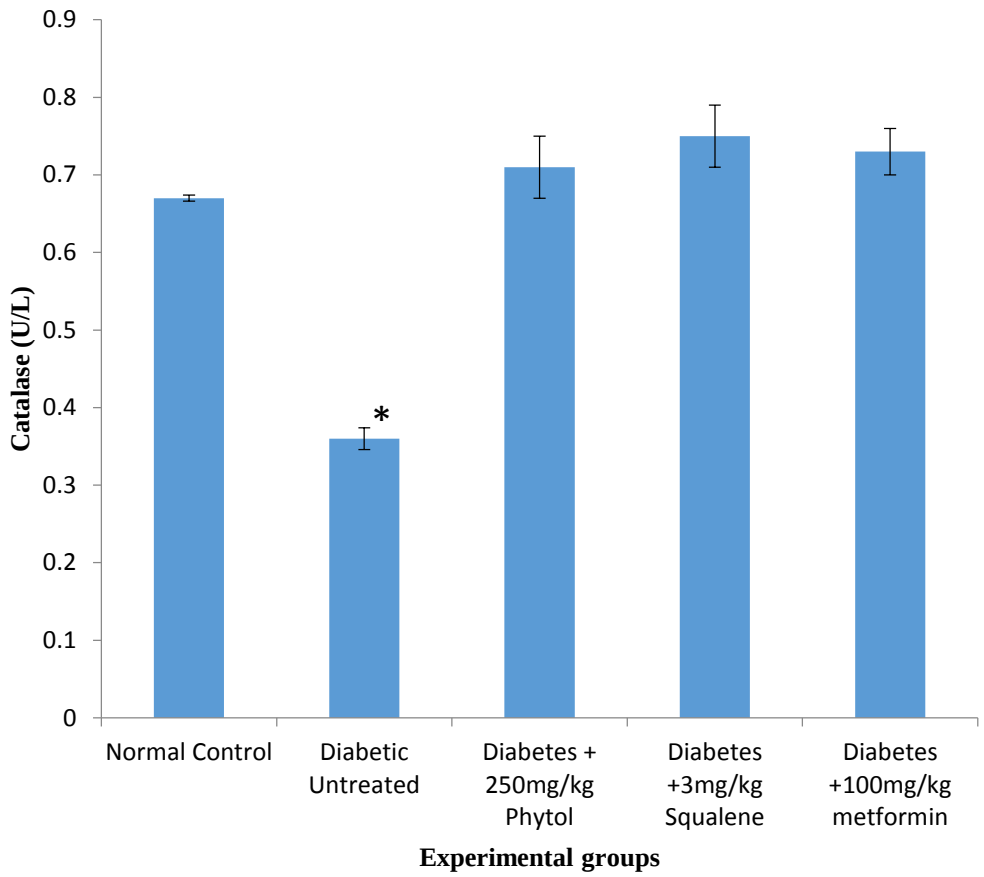

Figure 4.34 Effect of Phytol and Squalene on catalase activity in experimental rats.

* indicates value significantly different from normal control, diabetes + 250 mg/kg Phytol, diabetes + 3 mg/kg Squalene and diabetes + 100 mg/kg Metformin (n=5).

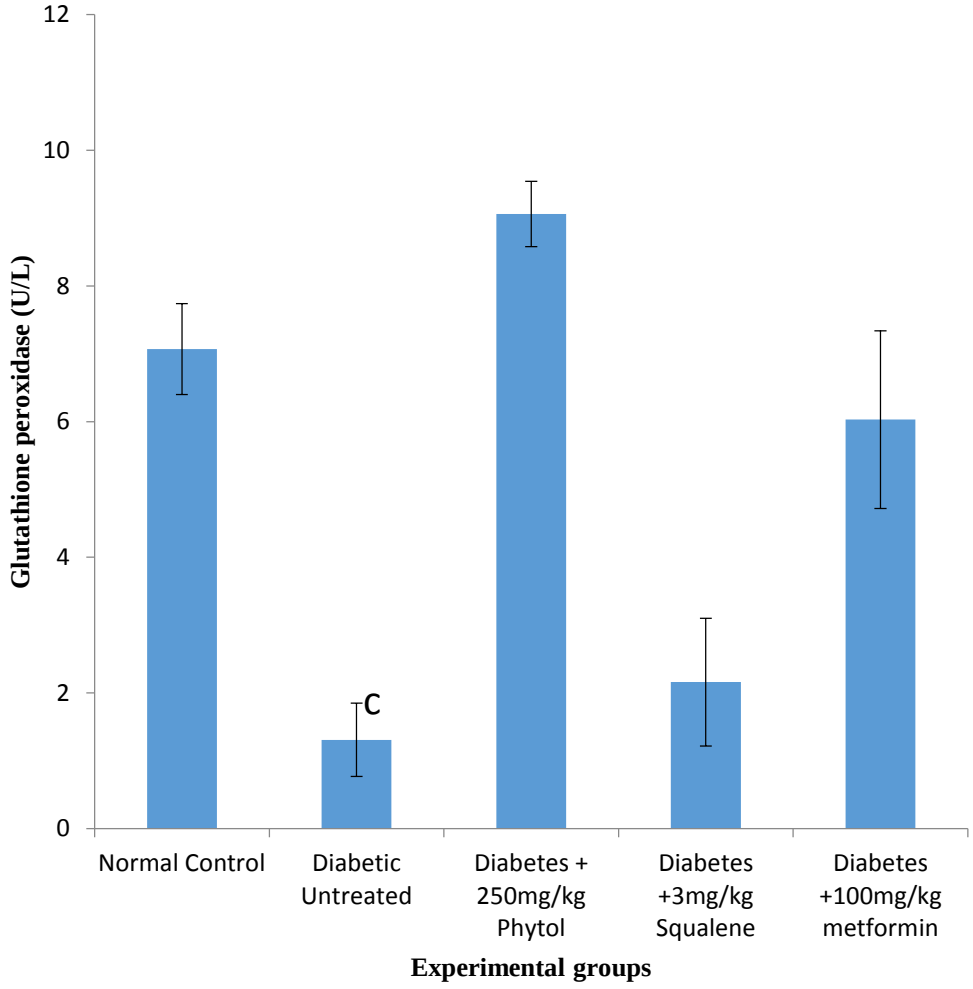

Figure 4.35 Effect of Phytol and Squalene on glutathione peroxidase in experimental rats.

[C] indicates value significantly different from normal control, diabetes + 250 mg/kg Phytol, diabetes + 100 mg/kg metformin (n=5).

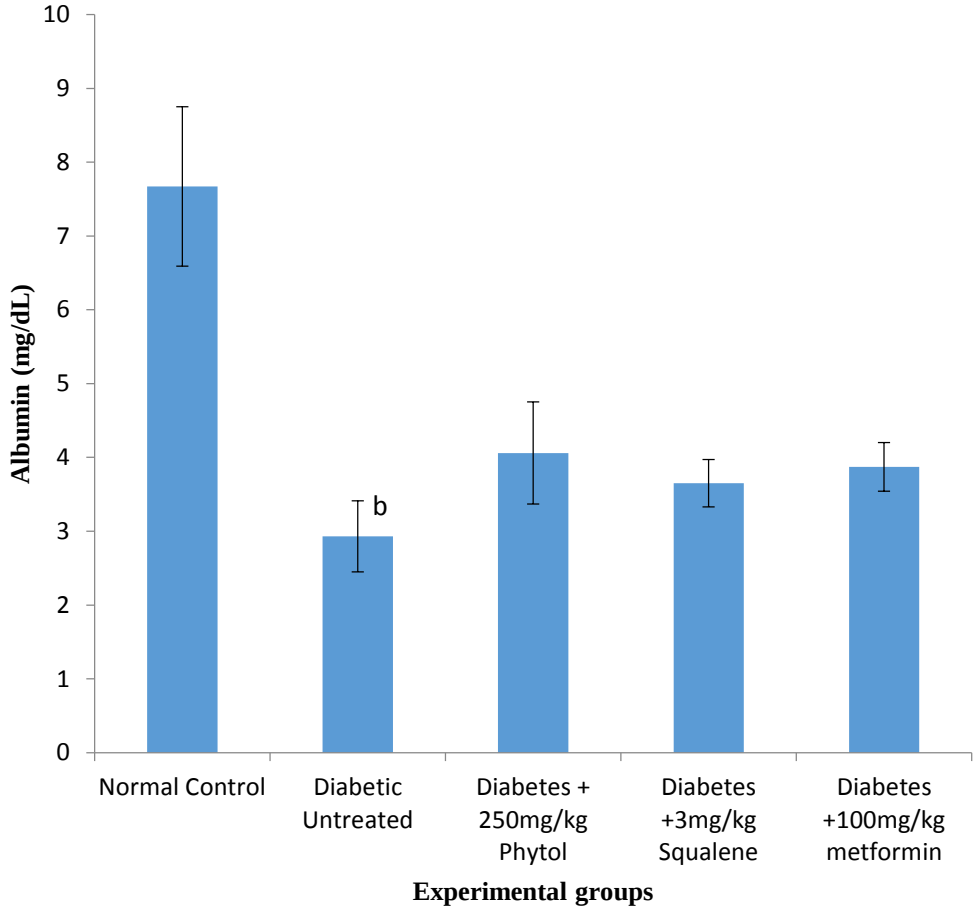

Figure 4.36 Effect of Phytol and Squalene on albumin level in experimental rats.

[b] indicates value significantly different from normal control (n=5).

4.24 Electrolytes changes in rats treated with Phytol and Squalene

Figure 4.37 showed sodium ion concentration in normal control, diabetic untreated, Phytol, Squalene, and Metformin treated rats. There was significant decrease ($p<0.05$) in Na^+ concentration in diabetic untreated, diabetic + 3 mg/kg Squalene and 100mg/kg metformin treated groups when compared to the normal control and 250 mg/kg Phytol.

Figure 4.38 showed potassium ion concentration in normal control, diabetic untreated, Phytol, Squalene, and Metformin treated rats. K^+ significantly decreased ($p<0.05$) in diabetic untreated, diabetic + 3 mg/kg Squalene and diabetes +100 mg/kg metformin when compared to normal control and diabetes + 250 mg/kg Phytol.

Figure 4.39 showed calcium ion concentration in normal control, diabetic untreated, Phytol, Squalene, and Metformin treated rats. Ca^{2+} significantly decreased in diabetic untreated, diabetic + 3 mg/kg Squalene and when compared to normal control, diabetes + 250 mg/kg Phytol and diabetes +100 mg/kg metformin.

Figure 4.40 showed magnesium ion concentration in normal control, diabetic untreated, Phytol, Squalene, and Metformin treated rats. There was no significant difference in mg^{2+} concentration in diabetic untreated when compared to phytol, squalene and metformin treated groups.

Figure 4.41 showed bicarbonate ion concentration in normal control, diabetic untreated, Phytol, Squalene, and Metformin treated rats. There was significant decrease ($p<0.05$) in HCO_3^- in diabetic untreated, diabetes + 250 mg/kg Phytol, and diabetes +100mg/kg metformin when compared to normal control and diabetic + 3 mg/kg Squalene.

Figure 4.42 showed chloride ion concentration in normal control, diabetic untreated, Phytol, Squalene, and Metformin treated rats. There was significant decrease ($p<0.05$) in Cl^- in diabetic untreated when compared with normal control. Phytol administration at 250 mg/kg in diabetic rats caused significant increase ($p<0.05$) in Cl^- when compared to diabetic untreated, diabetic + 3 mg/kg Squalene, diabetes + 100 mg/kg metformin.

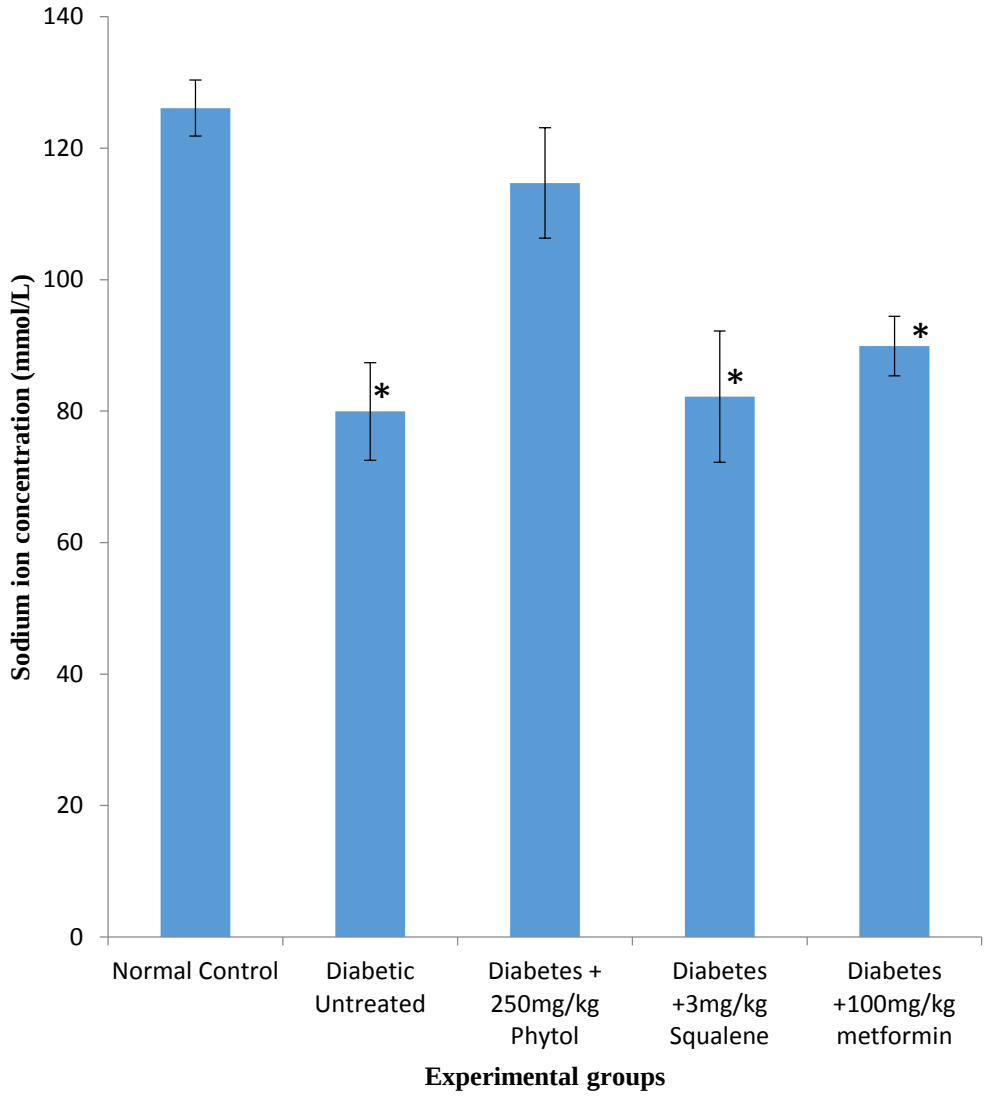

Figure 4.37 Effect of Phytol and Squalene on sodium ion concentration in experimental rats.

* indicates values significantly different from normal control, diabetes+ 250 mg/kg Phytol (n=5).

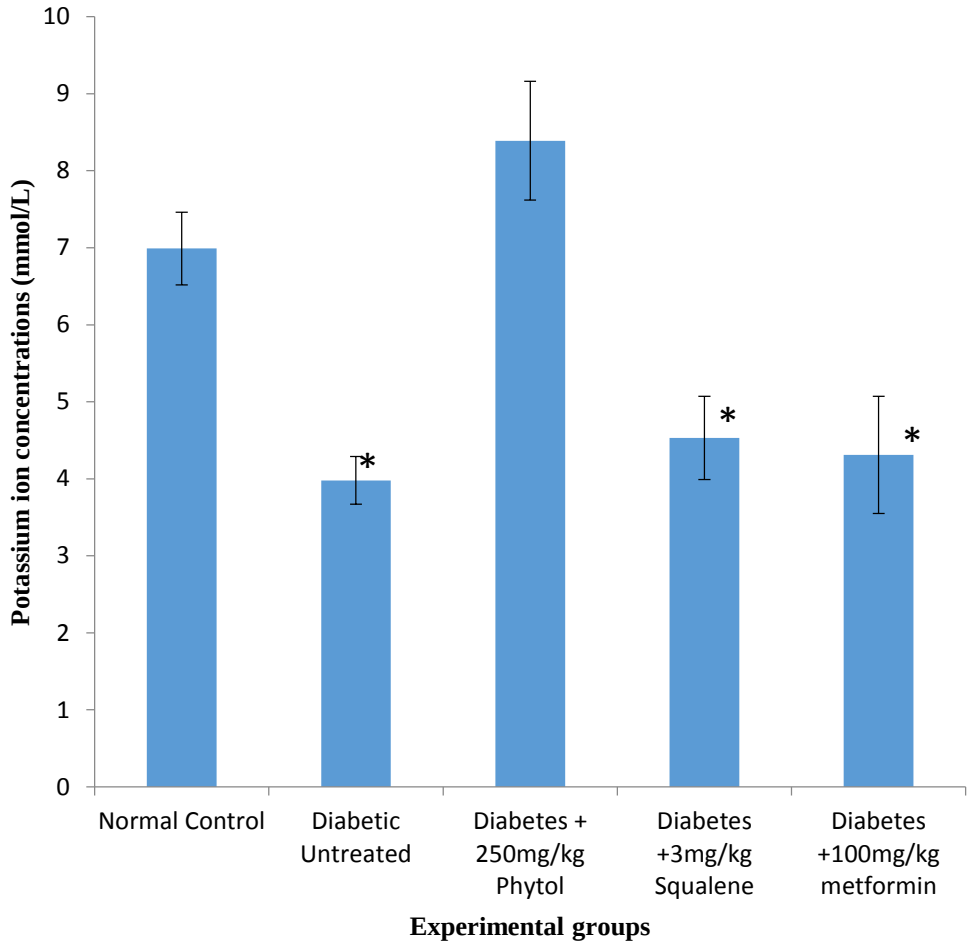

Figure 4.38 Effect of Phytol and Squalene on potassium ion concentration in experimental rats.

* indicates values significantly different from normal control, diabetes+ 250 mg/kg Phytol (n=5).

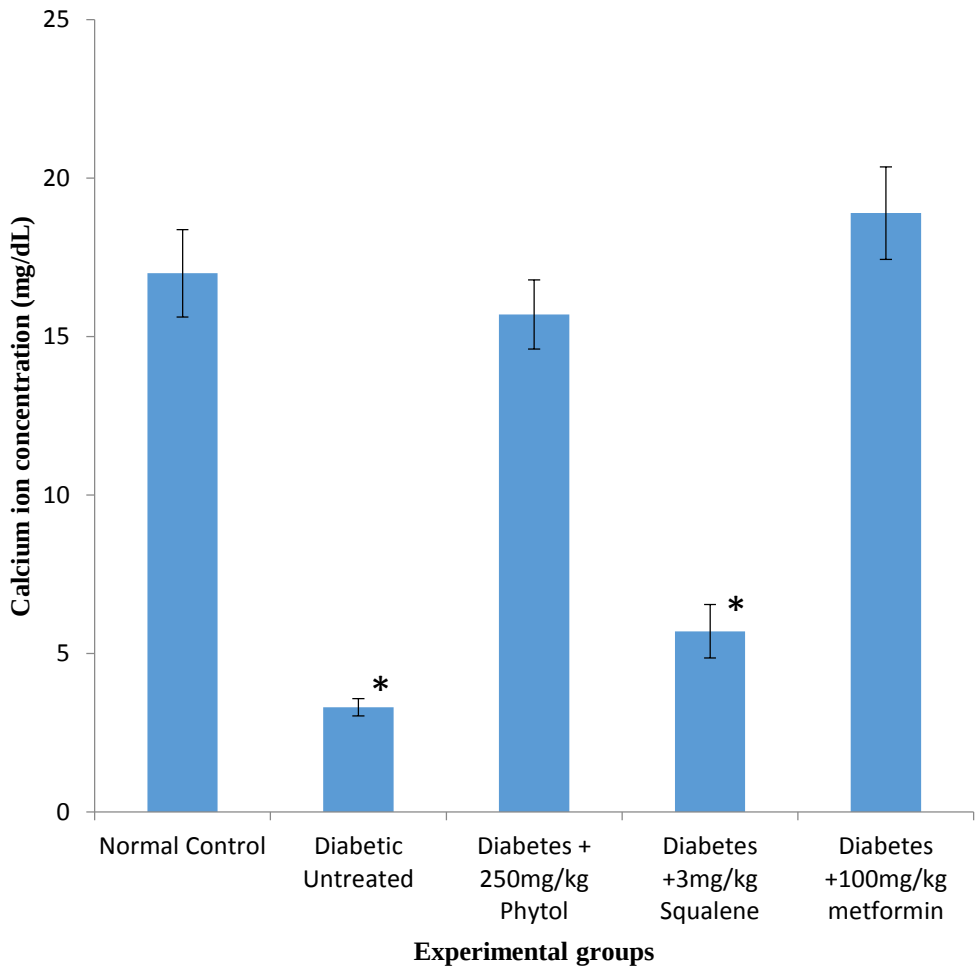

Figure 4.39 Effect of Phytol and Squalene on calcium ion concentration in experimental rats.

[a] indicates value significantly different from normal control, diabetic +250 mg/kg Phytol, diabetes+100 mg/kg metformin, (n=5).

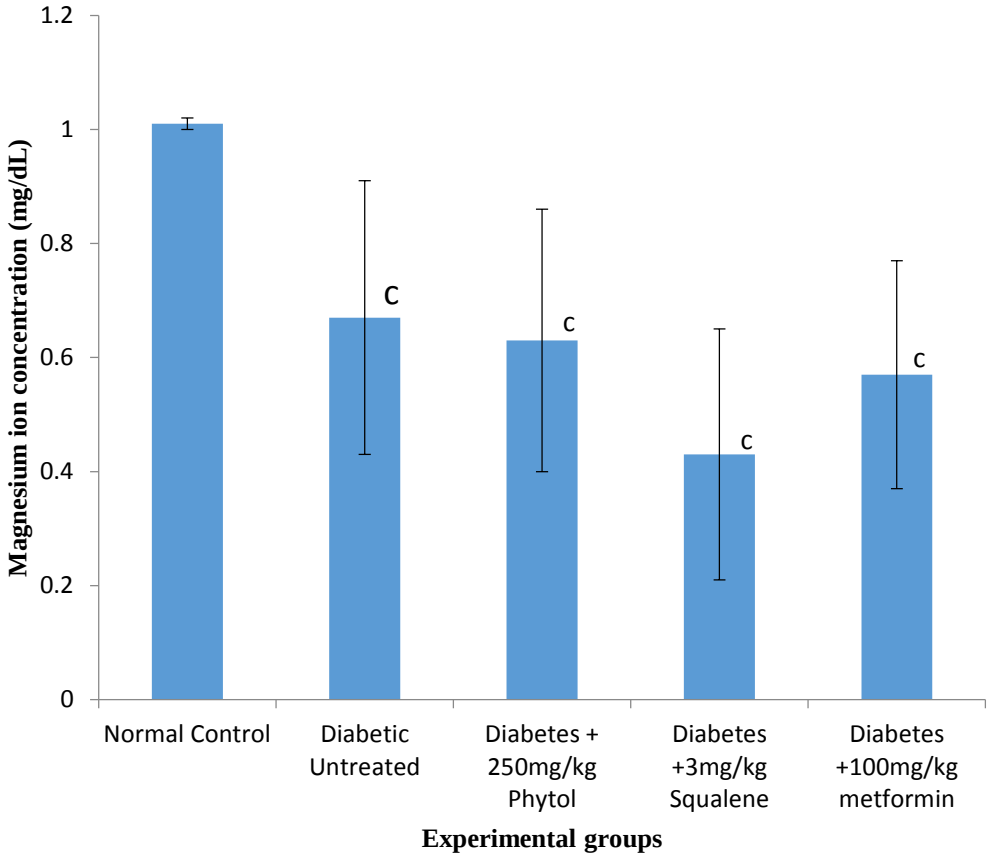

Figure 4.40 Effect of Phytol and Squalene on magnesium ion concentration in experimental rats.

[c] indicates value significantly different from normal control (n=5).

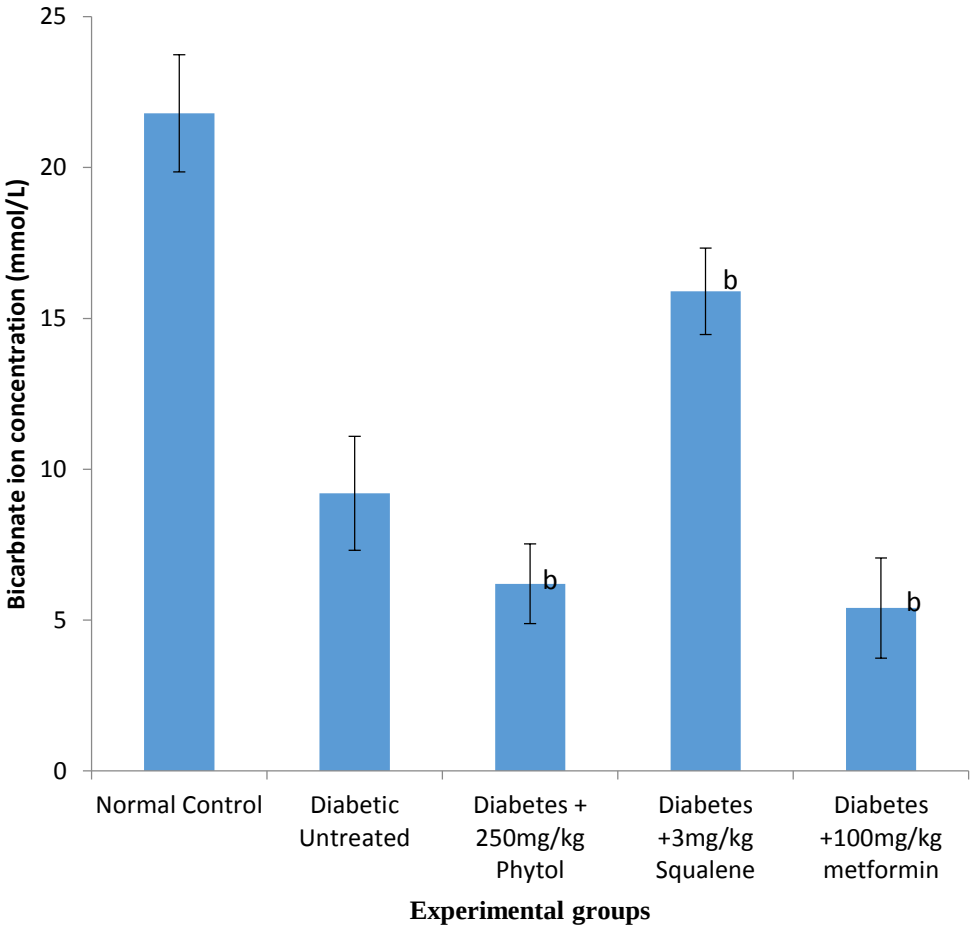

Figure 4.41 Effect of Phytol and Squalene on bicarbonate in experimental rats.

[b] indicates value significantly different from normal control, diabetic untreated (n=5).

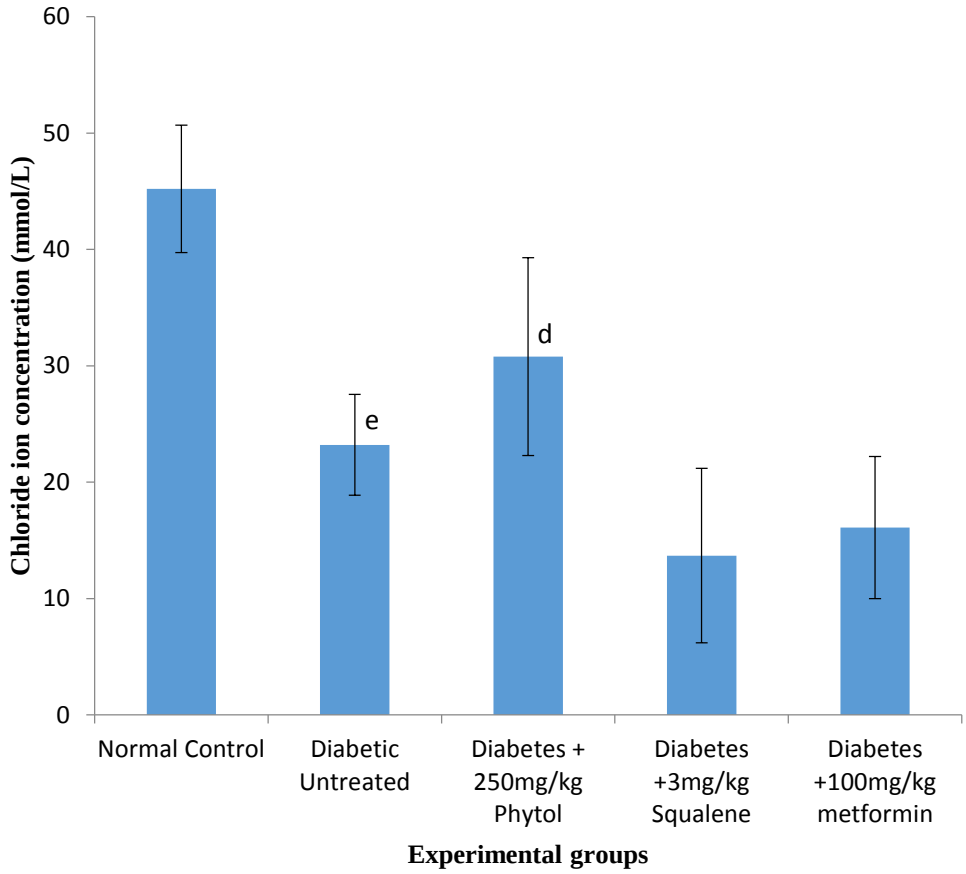

Figure 4.42 Effect of Phytol and Squalene on chloride ion concentration in experimental rats.

[e] indicates value significantly different from normal control, [d] indicates value significantly different from normal control, diabetic untreated, diabetes + 3 mg/kg Squalene and diabetes+100 mg/kg metformin diabetes+100 mg/kg (n=5).

4.25 Blood urea nitrogen and creatinine levels in rats treated with Phytol and Squalene

Figure 4.43 showed blood urea nitrogen (BUN) in normal control and diabetes treated with Phytol, Squalene and metformin. BUN significantly increased ($p<0.05$) in diabetic untreated when compared to normal control, diabetes treated with 250mg/kg Phytol, 3 mg/kg Squalene, and 100 mg/kg Metformin. However, no significant difference in BUN is observed in 250 mg/kg Phytol, 3 mg/kg Squalene, and 100 mg/kg Metformin when compared to normal control.

Figure 4.44 showed creatinine level in normal, Phytol, Squalene and Metformin treated groups. There was significant increase ($p<0.05$) in creatinine level in diabetic untreated group when compared to normal control, diabetes treated with 250 mg/kg Phytol, 3mg/kg Squalene and 100 mg/kg metformin. However, there was no significant difference in creatinine level in diabetes treated with 250 mg/kg Phytol, 3 mg/kg Squalene and 100 mg/kg Metformin.

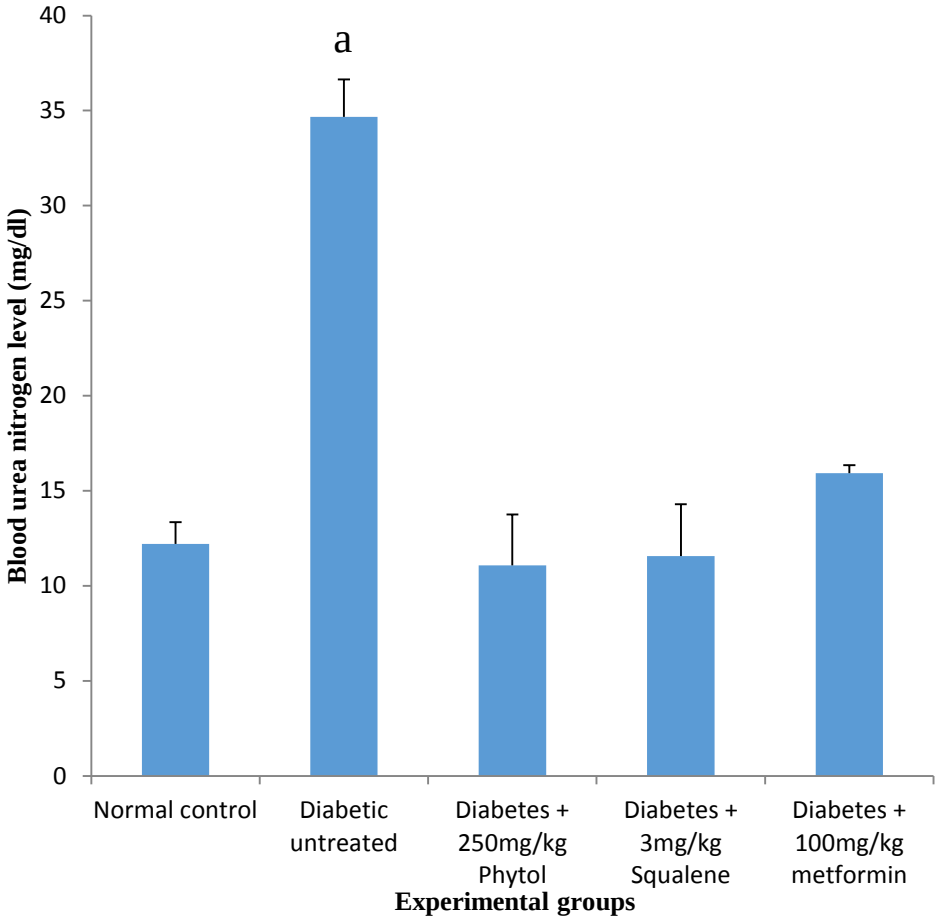

Figure 4.43 Effect of Phytol and Squalene on blood urea nitrogen in experimental rats

[a] indicate value significantly different from the normal control, Diabetes + 250 mg/kg Phytol, Diabetes + 3 mg/kg Squalene and Diabetes + 100 mg/kg metformin (n=5).

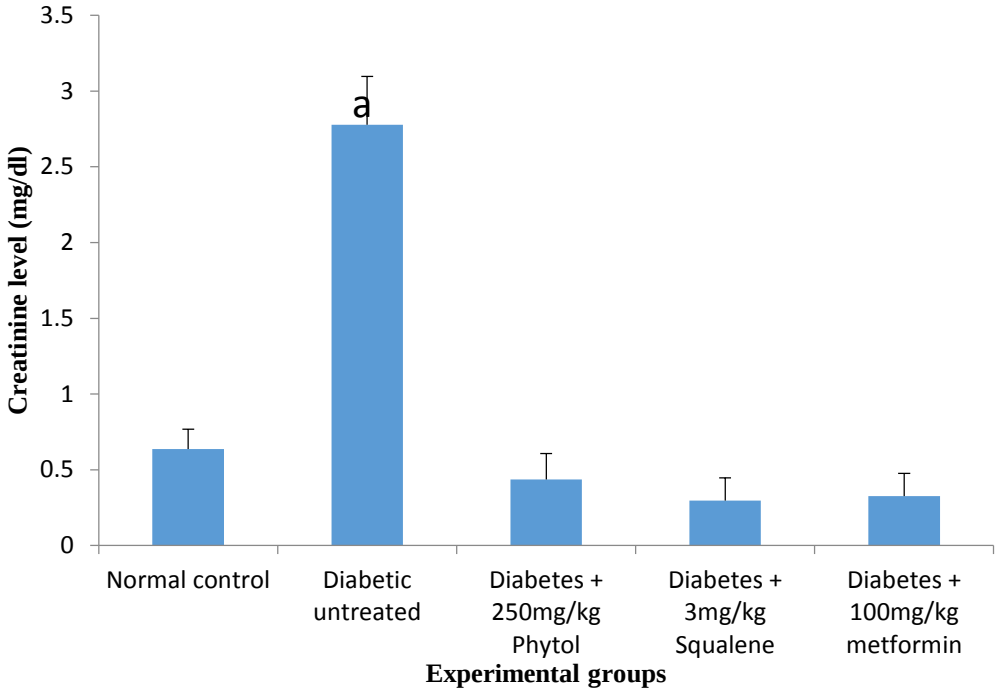

Figure 4.44 Effect of Phytol and Squalene on creatinine level in experimental rats

[a] indicates value significantly different from the normal control, Diabetes + 250 mg/kg Phytol, Diabetes + 3 mg/kg Squalene and Diabetes + 100 mg/kg metformin (n=5).

4.26 Histological changes in the kidney of rats treated with Phytol and Squalene

Plate 4.5 showed transverse section of the kidney in normal, diabetic untreated, diabetic treated with 250 mg/kg Phytol, diabetic treated with 3 mg/kg Squalene and diabetic treated with 100 mg/kg metformin respectively.

Photomicrographs of kidney sections stained by Periodic acid schiffs (PAS) showed normal architecture as seen with a high magnification x400, the renal cortex show normal glomeruli with normal mesengial cells and capsular spaces in the normal control and diabetic treated 250 mg/kg Phytol. Moreso, no visible pathological thickness of the Bowman's capsule was seen in normal control and diabetic treated 250 mg/kg Phytol.

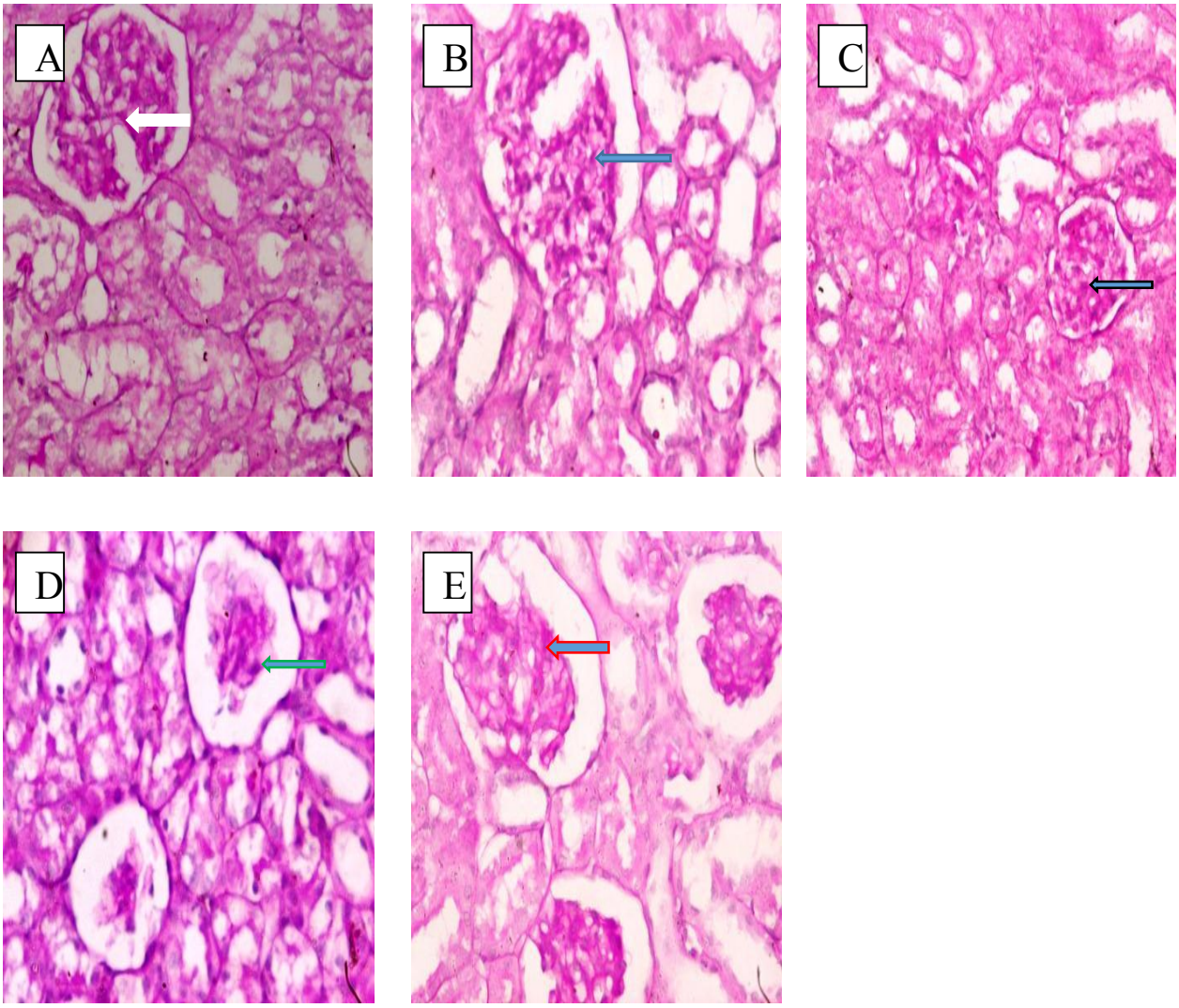

Plate 4.5 (A – E): Sections stained with PAS showing architecture of the kidney in A (Control), B (Diabetic untreated), C, D & E (Diabetes treated with 250 mg/kg Phytol, 3 mg/kg Squalene and 100 mg/kg Metformin respectively). A showed architecture of the kidney with normal glomerulus and basement membrane (white Arrow), B showed damaged glomerulus (Blue arrow), C showed normal basement membrane and capsular space after treatment with Phytol (Black arrow), D showed thickened basement membrane with normal capsular space (Green arrow) & E showed thickened basement membrane and mesangial expansion (Red arrow) X 400.

4.27 Diacylglycerol, protein kinase C alpha, mitogen activated protein kinase 8 and transforming growth factor beta in rats treated with Phytol and Squalene

Figure 4.45 showed diacylglycerol in rats treated with phytol and squalene. Diacylglycerol (DAG) increased significantly ($p<0.05$) in diabetic untreated when compared to normal control and diabetic treated with 250 mg/kg Phytol. Moreso, diacylglycerol increased significantly ($p<0.05$) in diabetic treated with 3mg/kg squalene when compared to normal control and diabetic treated with 250 mg/kg Phytol.

Figure 4.46 showed protein kinase C alpha in rats treated with phytol and squalene. Protein kinase C- alpha (PKC-α) significantly increase ($p<0.01$) in diabetic untreated and diabetic treated with 3 mg/kg squalene when compared to normal control and diabetes treated with 250 mg/kg phytol. However, there was no significant difference in PKC-α in diabetic treated with 3 mg/kg when compared to diabetic untreated.

Figure 4.47 showed mitogen activated protein kinase 8 in rats treated with phytol and squalene. There was significant increase ($p<0.05$) in mitogen activated protein kinase-8 (MAPK-8) in diabetic untreated, diabetes treated with 250 mg/kg Phytol and 3 mg/kg Squalene when compared to normal control.

Figure 4.48 showed transformin growth factor beta in rats treated with phytol and squalene. There was significant increase ($p<0.05$) in transformin growth factor-β (TGF-β) in diabetic untreated when compared to normal control, diabetic treated with 250 mg/kg Phytol and 3 mg/kg Squalene. TGF-β significantly increased ($p<0.05$) in diabetes treated with 250 mg/kg Phytol when compared to normal control. TGF-β significantly increased ($p<0.05$) in diabetes treated with 3mg/kg squalene when compared to normal control and diabetes treated with 250 mg/kg Phytol.

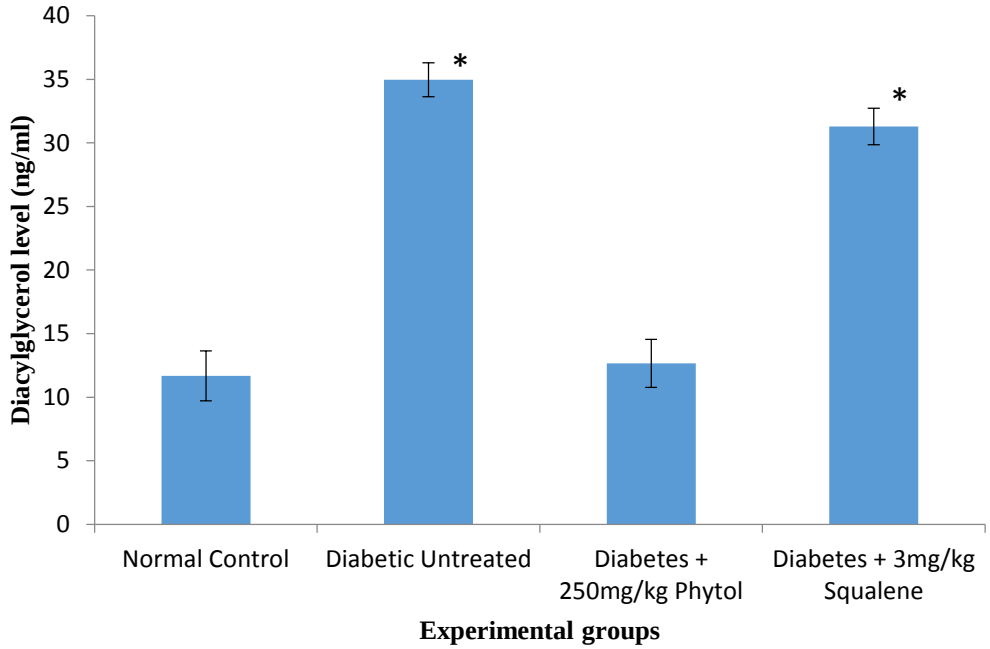

Figure 4.45 Effect of Phytol and Squalene on diacylglycerol level in experimental rats

*indicate values significantly different from the normal control, Diabetes + 250 mg/kg Phytol (n=5).

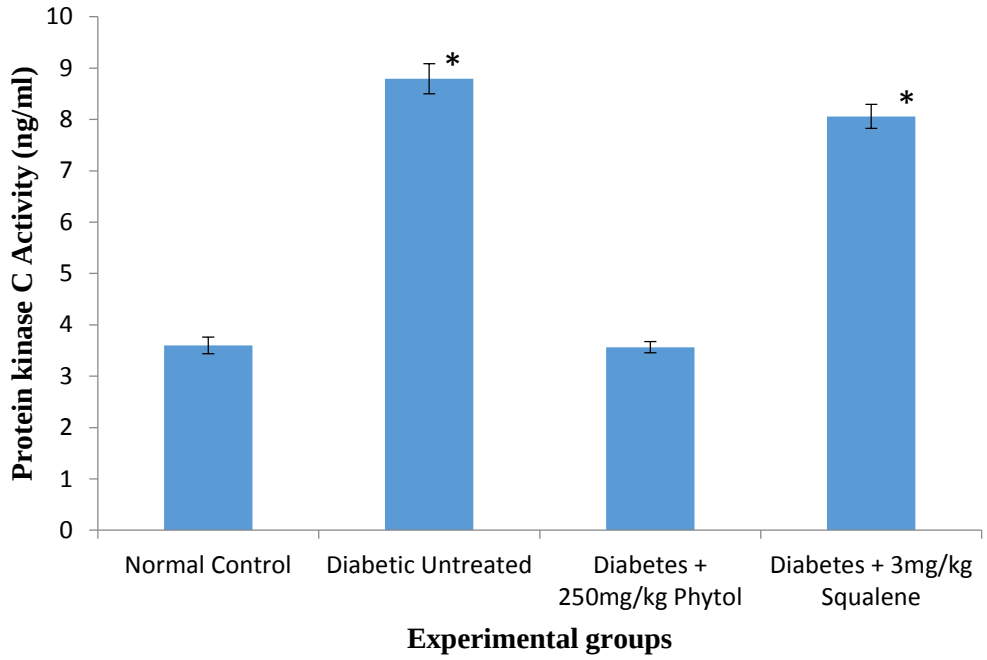

Figure 4.46 Effect of Phytol and Squalene on protein kinase C alpha activity in experimental rats

* indicate values significantly different from the normal control, Diabetes + 250 mg/kg Phytol (n=5).

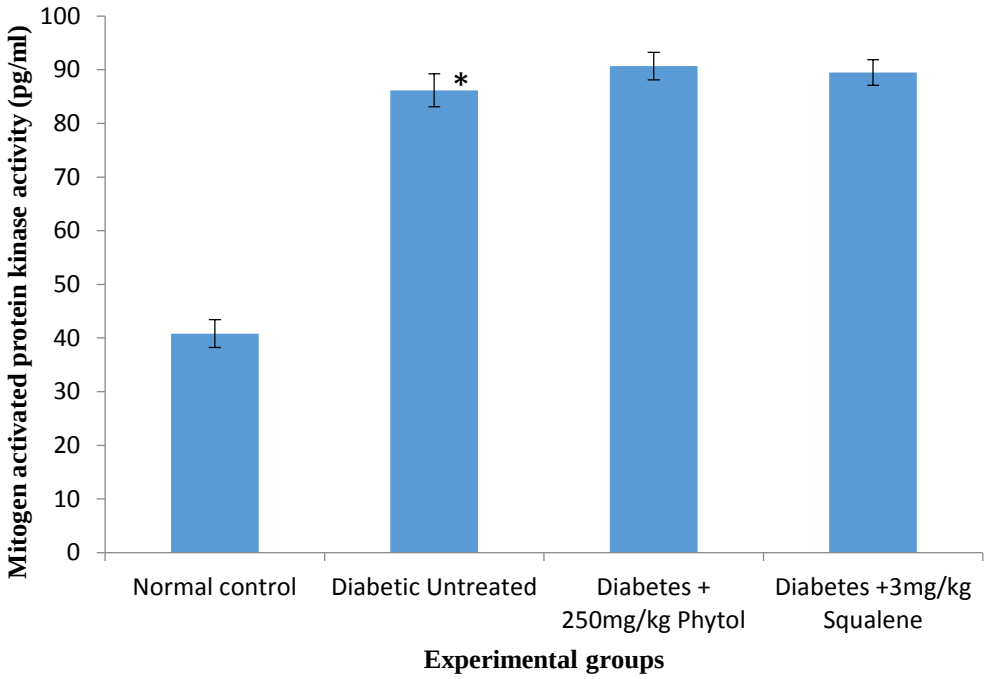

Figure 4.47 Effect of Phytol and Squalene on mitogen activated protein kinase-8 in experimental rats

*indicate values significantly different from the normal control (n=5).

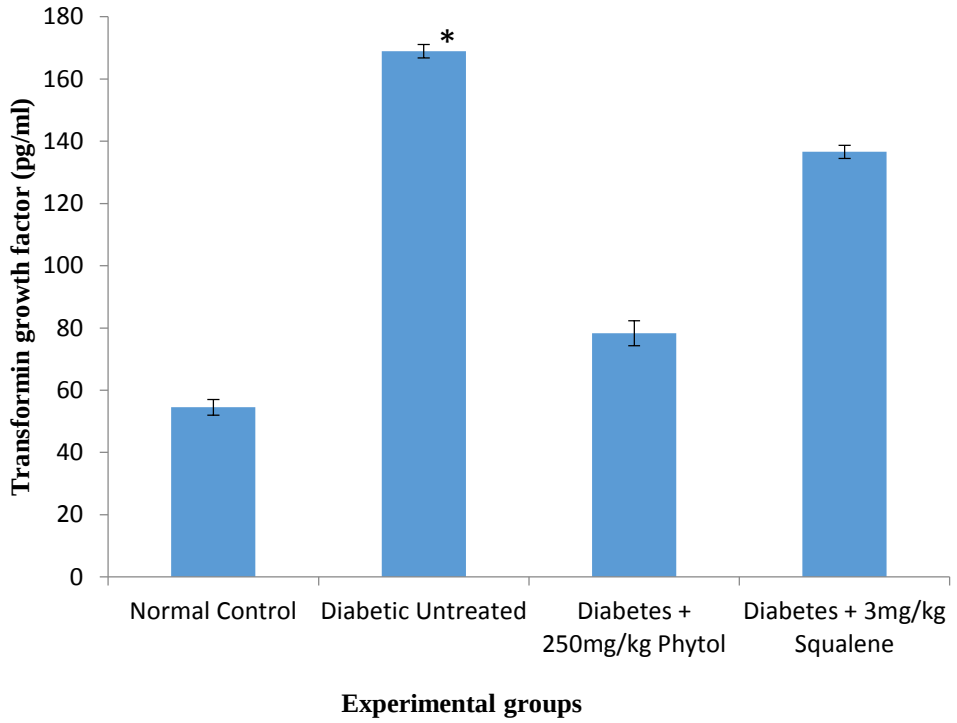

Figure 4.48 Effect of Phytol and Squalene on TGF-β in experimental rats

*indicate values significantly different from the normal control, Diabetes + 250 mg/kg Phytol and diabetes+ 3 mg/kg squalene (n=5).

4.28 Immunostaining of the kidney

Plates 4.6-4.9 showed transverse section of the kidney in normal control, diabetic untreated, diabetic treated with 250 mg/kg Phytol and diabetic treated with 3 mg/kg Squalene respectively.

Photomicrographs of a kidney section stained and showing expression of apoptotic proteins including p16, p53, Bcl-2 and COX-2. There was strong expression of p16, p53 and COX-2 in diabetic untreated and diabetic treated with 3 mg/kg Squalene. However, Bcl-2 proteins were strongly expressed in normal control and diabetic treated with 250 mg/kg Phytol.

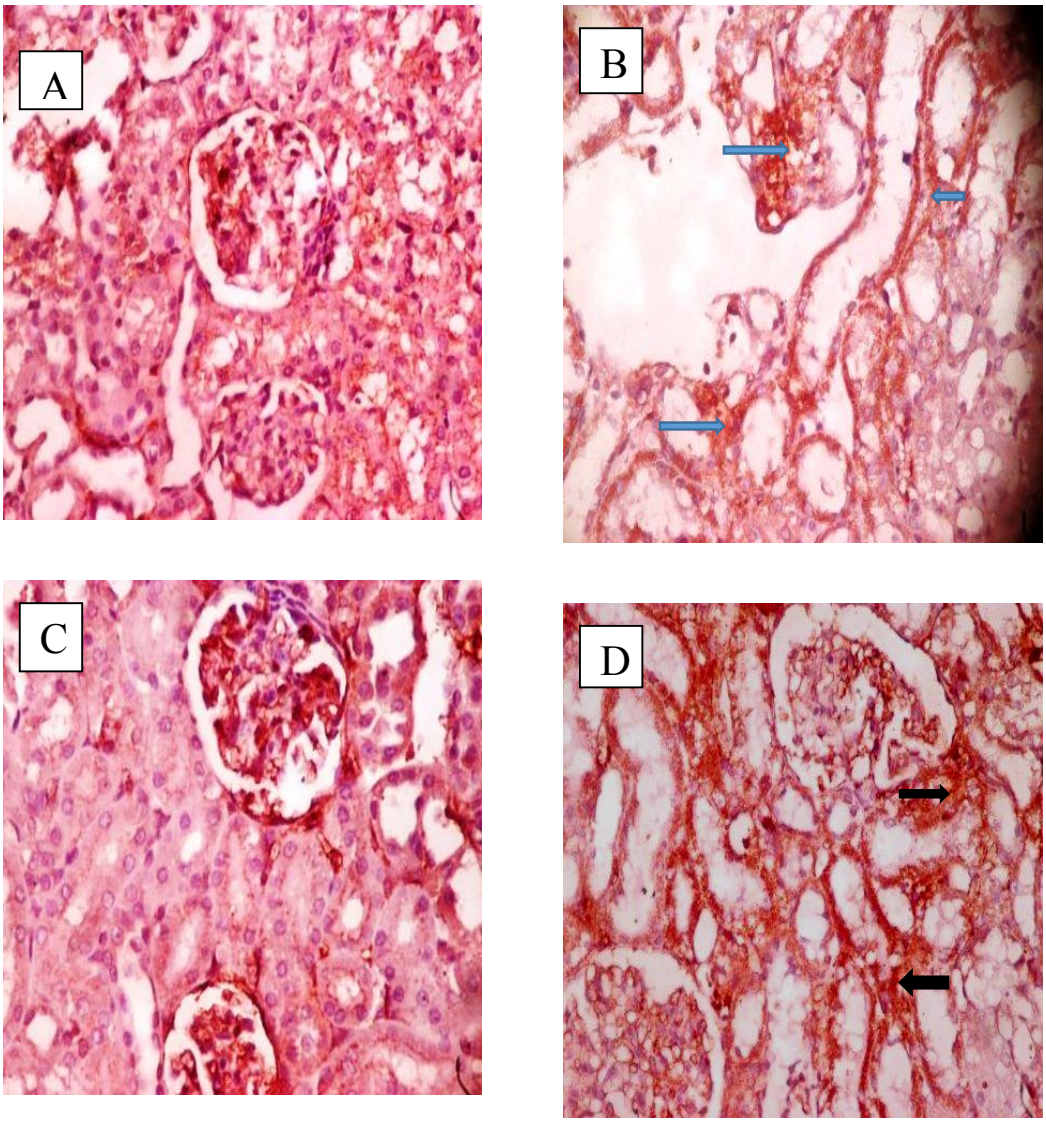

Plate 4.6 (A – D): Immunostained sections showing p16 expressions in the kidney, A (Control), B (Diabetic untreated), C & D (Diabetes treated with 250 mg/kg Phytol, 3 mg/kg Squalene respectively). A & C showed mild expression of p16 while B & D showed strong expression of p16 in the glomerulus and the surrounding basement membranes (Blue and Black arrows) X 400.

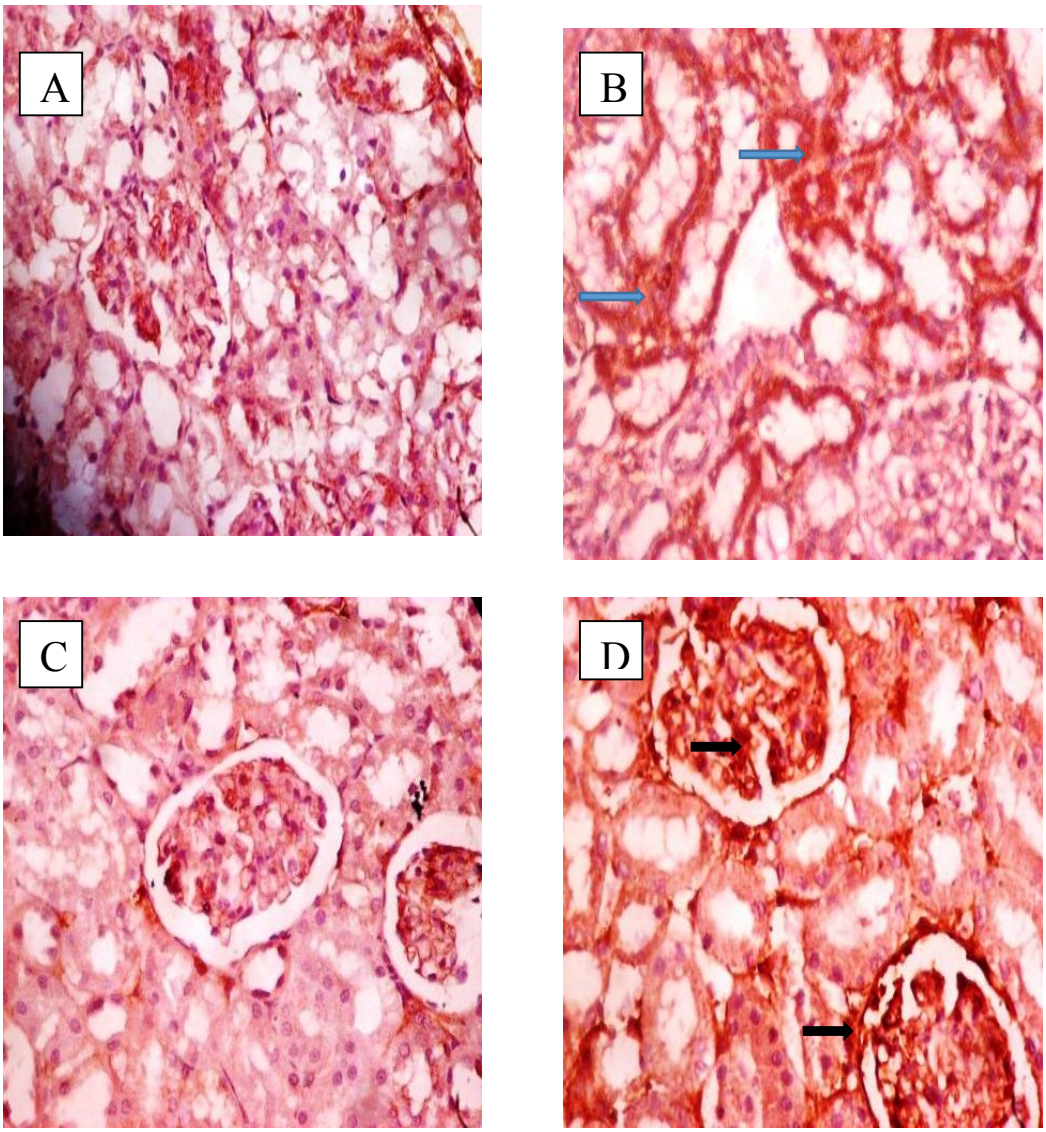

Plate 4.7 (A – D): Immunostained sections showing p53 expression in the kidney, A (Control), B (Diabetic untreated), C & D (Diabetes treated with 250 mg/kg Phytol, 3 mg/kg Squalene respectively). A & C showed mild expression of p53 while B & D showed significant expression of p53 in the glomerulus and the surrounding basement membranes (Blue and Black arrows) X 400.

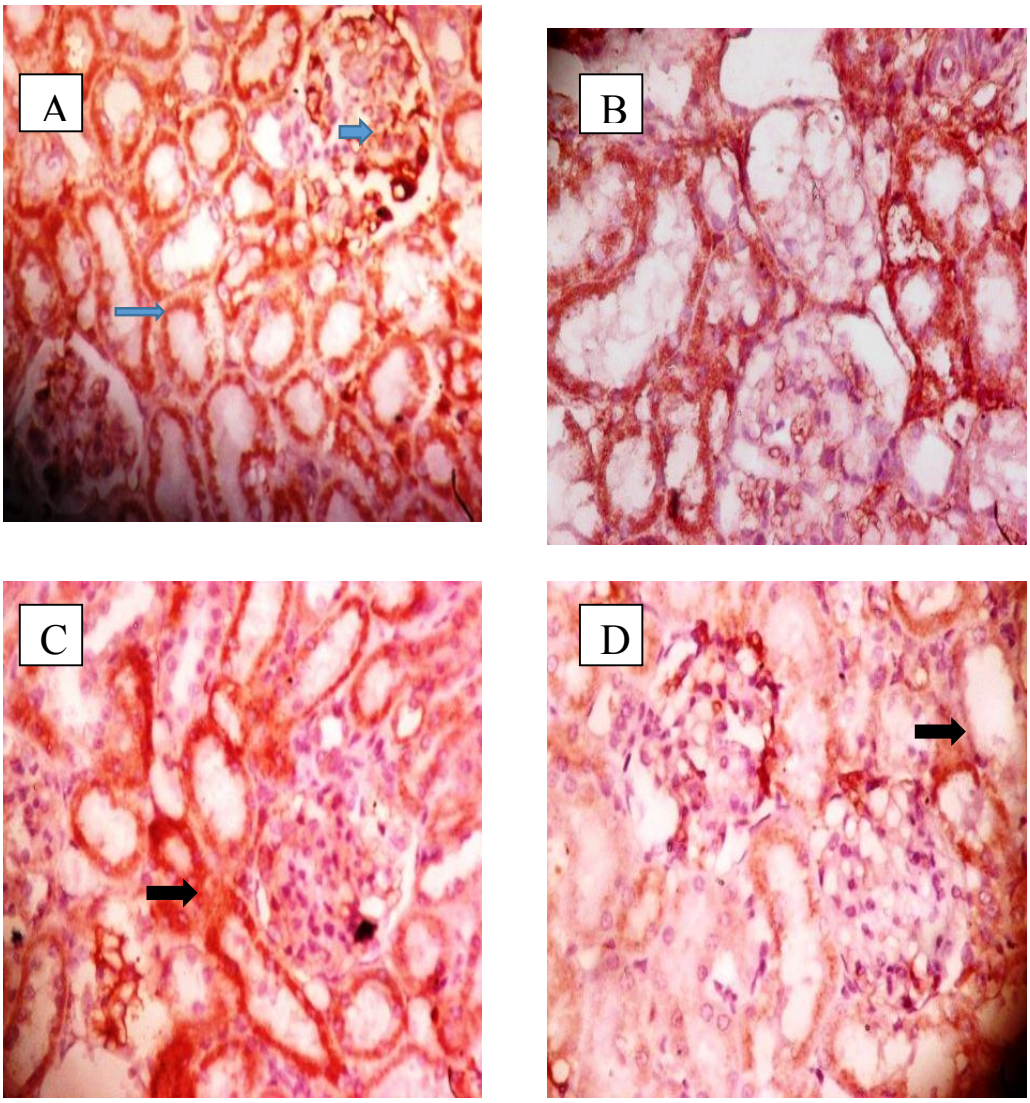

Plate 4.8 (A – D): Immunostained sections showing Bcl-2 expressions in the kidney, A (Control), B (Diabetic untreated), C & D (Diabetes treated with 250 mg/kg Phytol, 3 mg/kg Squalene respectively). A & C showed strong expression of Bcl-2 in the glomerulus and the surrounding basement membranes (Blue and Black arrows), D showed mild expression of Bcl-2 (Black arrow) while B showed no expression of Bcl-2 X 400.

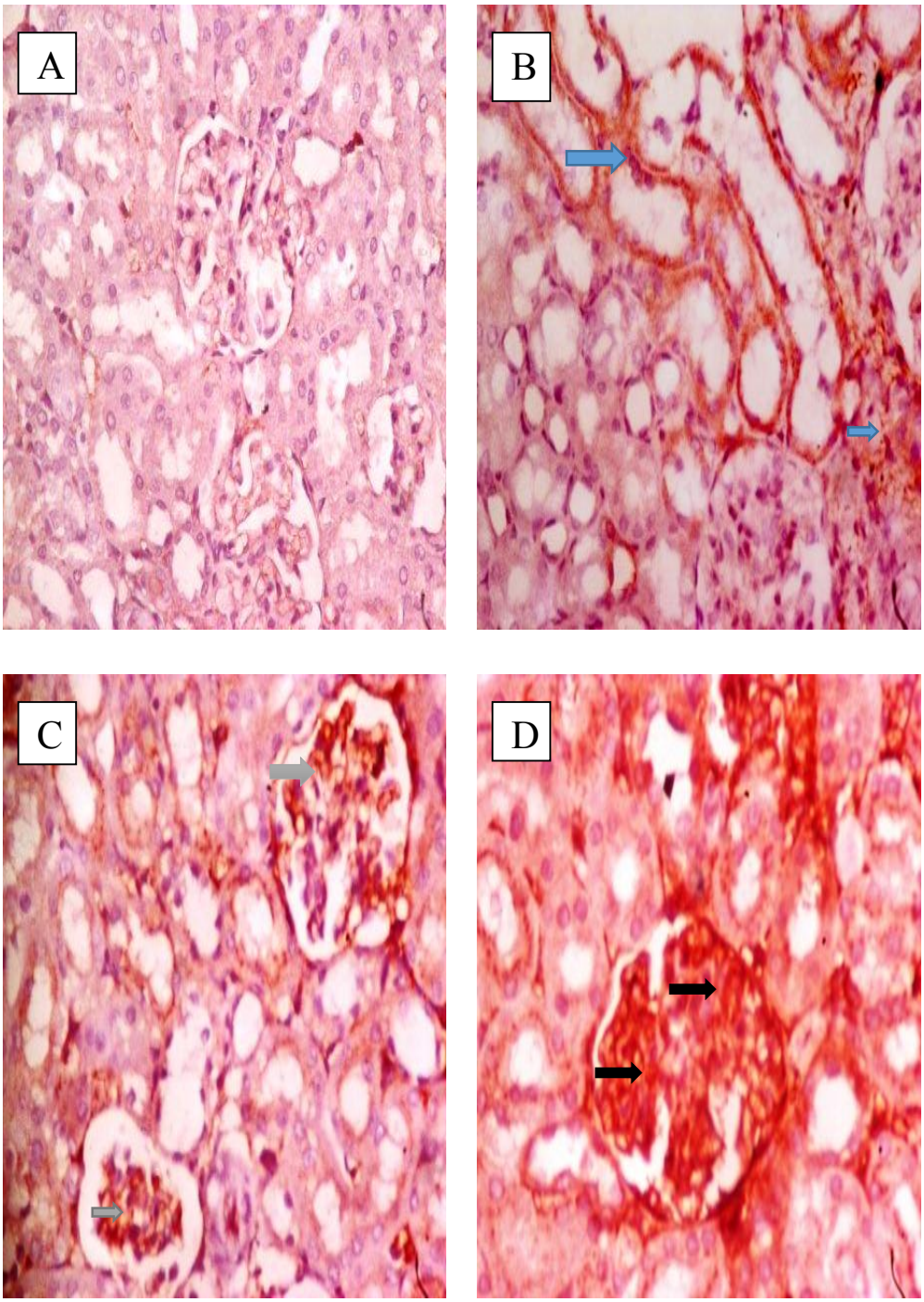

Plate 4.9 (A – D): Immunostained sections showing COX-2 expressions in the kidney, A (normal Control), B (Diabetic untreated), C & D (Diabetes treated with 250 mg/kg Phytol, 3 mg/kg Squalene respectively). A showed no expression of COX-2, C showed mild expression of COX-2 while B & D showed significant expression of COX-2 in the glomerulus and the surrounding basement membranes (Blue and Black arrows) X 400.

CHAPTER FIVE

5.0 Discussion and Conclusion

The present study revealed the presence of phytochemicals in methanol extract of *Parquetina nigrescens* some of which include alkaloids, cardenoloids and tannin among others. Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants like the *Parquetina nigrescens* are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube *et al*., 2008). The extraction of methanol extract of *Parquetina nigrescens* involves the separation of medicinally active portions of the plant using selective solvents through standard procedures. The products so obtained from it are relatively complex mixtures of metabolites in semisolid state and were intended for oral use. Plants metabolites could also be prepared in the form of decoctions, infusions, fluid extracts, tinctures, pilular (semisolid) extracts or powdered extracts. Such preparations have been popularly called galenicals, named after Galen, the second century Greek physician (Remington, 2000). Extraction methods used pharmaceutically involves the separation of medicinally active portions of plant tissues from the inactive/inert components by using selective solvents. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity (Ncube *et al*., 2008). The purpose of standardized extraction procedures for crude drugs (medicinal plant parts) is to attain the therapeutically desired portions and to eliminate unwanted material by treatment with a selective solvent known as menstrum. The extract obtained, may therefore be used as medicinal agent as such in the form of tinctures or fluid extracts or further processed to be incorporated in any dosage form such as tablets and capsules. These products contains complex mixture of many

medicinal plant metabolites, such as alkaloids, glycosides, terpenoids, flavonoids and lignans as observed from this study (Handa, 2008).

The Gas Chromatography-Mass Spectrometry analysis of Methanol extract of *Parquetina nigrescens* identified the presence of twenty-two chemical constituents in the extract. Gas chromatography has a very wide field of applications. But, its first and main area of use is in the separation and analysis of multi component mixtures such as essential oils, hydrocarbons and solvents (Pierangeli *et al*., 2008). Intrinsically, with the use of the flame ionization detector and the electron capture detector (which have very high sensitivities) gas chromatography can quantitatively determine materials present at very low concentrations. The second most important application area is in pollution studies, forensic work and general trace analysis. Because of its simplicity, sensitivity, and effectiveness in separating components of mixtures, gas chromatography is one of the most important tools in biomedicine. It is widely used for quantitative and qualitative analysis of mixtures, for the purification of compounds, and for the determination of thermo chemical constants as heats of solution and vaporization, vapor pressure, and activity coefficients (Andrew, 2009). A knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also because such information may be of great value in disclosing new sources of economic phytocompounds for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies.

The use of medicinal plants can have deleterious effects on health as reported in literature (Fall *et al*., 2011). Indeed, of about 1,500,000 plants investigated, most of them contain toxic substances (Ishii *et al*., 1984) like some secondary metabolites. Plants produce a variety of secondary metabolites that are subdivided into several classes based on their molecular structure. Among secondary metabolites are saponins, terpenoids, cyanogenic, tannins, toxic amino acids, glycosides, alkaloids (Fall *et al*., 2011). It has been shown that the toxicity of a given plant depends on various factors, including the strength of secondary metabolites, the quantity consumed, the time of exposure, different parts of the plant (root, oil, leaves, stem bark and seeds), individual body chemistry, climate and soil, and genetic differences within the species (Tülay, 2012). Medicinal plants should therefore be used with precautions and toxicology studies should be conducted to increase the knowledge on the plant or plants preparation given to populations. Both in-vitro and in-vivo models are available to study the toxicity of

medicinal plants. Regardless of the type of extract, the parts of plant used, the concentration of the extract, the mode of administration, and the organism under consideration, the lethal dose 50 (LD_{50}) that represent the dose that kill 50% of a tested population is used to appreciate the toxicity of the plant (Amy *et al*., 2002). In addition, for chronic or sub-chronic toxicity histological orgenetic modifications are the most relevant indicators. The toxicological test performed in the experimental rats using MEPN showed no case of mortality in various groups treated and this suggests that MEPN is non-toxic even at higher doses administered. In line with Hodge and Sterner scale of toxicity, a plant extract with an LD_{50} < 2000 mg/kg is said to be toxic and since the LD_{50} of MEPN from this study was >5000 mg/kg therefore, MEPN is said to belong to a non-toxic category of medicinal plant. However, this observation is in contrast with report of Lyon *et al*., (2006) who had earlier reported that the latex part of the plant is toxic and could even be used in making poison.

Effect of Methanol extract of *Parquetina nigrescens* on antidiabetic indices in experimental rats

A fundamental principle of nutrition and metabolism is that body weight change is associated with an imbalance between the energy content of food eaten and energy expended by the body to maintain life and to perform physical work. Such an energy balance framework is a potentially powerful tool for investigating the regulation of body weight. However, there is need for a better understanding of the components of energy balance and their interactions over various time scales to explain the natural history of conditions such as obesity and to estimate the magnitude and potential success of therapeutic interventions. In the present study, the weight gain in the body, kidney and the liver observed in diabetic group treated with MEPN could be due to the ability of the extract to decrease basal metabolic rate and stimulate adipocyte cell differentiation and fat distribution in the body (Saltzman, 2008).

Apart from the first few days of life, normal fasting blood glucose concentrations are kept within a narrow physiological range of 3.5–5.5 mmol/L. Continuous blood glucose monitoring shows that blood glucose concentrations may ‘flicker’ on either side of these two values (especially post meal) but, then rapidly and spontaneously revert to within this normal range. Fasting and postprandial normal blood glucose levels are maintained

within this narrow range by a complex interplay of hormones which control glucose production and glucose utilization. The mechanism through which circulating blood glucose is regulated in the diabetic Wistar rats after treatment with Methanol extract of *Parquetina nigrescens* (MEPN) was investigated. The rise in fasting blood glucose observed in diabetic untreated is in accordance with the report of Mrozikiewicz *et al*. (1994) who had earlier reported an increase in fasting blood glucose in diabetic animals after alloxan monohydrate (120 mg/kg *i.p*) was injected intraperitoneally. The hyperglycaemia observed in diabetic untreated group might be because of the damage of the Islet cell thereby causing unavailability of insulin at the tissue level to facilitate glucose uptake (Marchetti *et al*., 2004). However, the hypoglycaemic effect observed after treatment with MEPN at 100 and 200 mg/kg corroborate the reports of Saba *et al*. (2010) who had earlier reported the hypoglycemic properties of aqueous extract of *Parquetina nigrescens* in alloxan induced diabetic animals. Research has shown that beta cells show remarkable potential for regeneration hence, MEPN may have stimulated the regeneration of the beta cells and insulin production to exert its hypoglycaemic effects (Juárez-Rojop *et al*., 2012). Moreso, metformin has been reported to cause hypoglycaemia by stimulating cellular uptake of glucose and therefore it may also be acting via this mechanism (Pernicova *et al*., 2014).

Glycogen is a branched polymer of glucose that contains a minor amount of phosphate and glucosamine. In the linear chains, the glucose residues are connected by α-1,4-glycosidic linkages while α-1,6-glycosidic bonds create the branch points. Branches within normal glycogen are distributed at even intervals resulting in a structure with spherical shape. The source and function of phosphate and glucosamine in human glycogen are unclear. The glycogen particle consists of up to 55.000 glucose residues. Glycogen can be identified by electron microscopy inside the cells (Chkwana *et al*., 2013). The synthesis of glycogen requires the coordinated action of a number of enzymes Glucose enters the cells via glucose transporters, being phosphorylated to glucose 6-phosphate by hexokinase isoenzymes. The next step is the isomerization of glucose 6-phosphate into glucose 1-phosphate by phosphoglucomutase-1. Then, uridine 5′-diphosphate (UDP)-glucose pyrophosphorylase catalyzes the formation of UDP-glucose from glucose 1-phosphate. UDP-glucose is the immediate glucose donor for glycogen construction. Glycogenin initiates the synthesis of glycogen by autoglycosylation transporting glucose from UDP-glucose to itself and forming a short

linear chain of about 10–20 glucose moieties. The elongation of this initial glycogen sequence is catalyzed by glycogen synthase that transfers a glycosyl moiety from UDP-glucose to the growing glycogen strand, providing the α-1,4-glycosidic linkages between glucose residues. The branching enzyme introduces branch points in the glycogen particle, by creating α-1,6 glycosidic bonds at regular intervals. Diabetics experience depletion or reduction in hepatic glycogen storage (Anusha *et al*., 2017) which is said to be due to insulin deficiency or reduction in the activity of glycogen synthase (Ben *et al.,* 2017). Studies have shown that insulin substrate is necessary for hepatic glucose uptake (Abel *et al*., 2001) therefore; the decrease in the liver glycogen content in diabetic untreated rats may be as a result of insulin deficiency due to the damaged islet cells of the pancreas. Glycogen synthase is a rate–limiting enzyme in hepatic glycogen production. Reduction or inhibition in the activities of this enzyme may lead to reduced glycogen content (Dewalkar *et al*., 2014) and further accumulation of glucose in blood (Daisy *et al*., 2009). The significant increase in liver glycogen content observed with MEPN (100 and 200mg/kg) in this study suggests possible potentiation of hepatic glucose uptake or activities of glycogen synthase by MEPN. This effect of MEPN may be due to the phytochemical constituents in the plant extracts e.g alkaloids, cardenolides, anthraquinones, terpenoids, tannins and flavonoids. The increased glycogen in metformin treated rats, reflect its ability to upregulate glycogen synthase activities hence, improving glycogen storage (Kinkar *et al*., 2016). The liver is a glucose regulatory organ. It was not attacked by alloxan administration and that was why the liver cells in diabetic untreated was not compromised. Furthermore, the liver cells were preserved in MEPN and metformin treated groups and this further confirmed that MEPN was non-toxic at the various doses administered in the animals (Plate 4.0). The integrity of the hepatocytes and the granules were also maintained.

Complex carbohydrates reaching the small intestine must be hydrolyzed to monosaccharides such as glucose or galactose in order to be transported across the intestinal mucosa. The classical pathway of glucose absorption is across the intestinal - border membrane, which was predominantly mediated by SGLT1, a membrane protein that couples two Na^+ together with one molecule of glucose. The passive movement of glucose across the basolateral surface of enterocytes is mediated by facilitated-diffusion glucose transporter (GLUT2) which allows glucose to move from the IEC into the extracellular medium near the blood capillaries (Roder, 2014). The absorption of glucose

may be adjusted by other transporters (Chaudry, 2012). Translocation of GLUT2 from cytoplasmic vesicles into the apical membrane markedly increases the capacity of glucose uptake by the enterocyte (Grefner, 2015). Thus, any factor that influences the activities of SGLT1 and GLUT2 will also alter the absorption and metabolism of glucose. Modulation of intestinal glucose absorption has been shown as a promising way to reduce glucose transport from intestinal lumen into the enterocytes (Lihong chen *et al*., 2016). Facilitated diffusion of glucose from the enterocytes into general circulation can also be targeted to reduce blood glucose level (Roder *et al*., 2014). According to Lichongchen *et al*., (2006), duodenal and jejunal glucose absorption depends on the activities of sodium glucose transporters and some ions channels. The ability of MEPN (100 and 200 mg/kg) to decrease duodenal and jejunal glucose absorption may depend on its ability to act on SGLT2 transporters which are responsible for glucose transport into the enterocytes from the intestinal lumen. Furthermore, it's possible that the extract may have inhibited K^+ ion channels in the apical membrane of the small intestine, Na^+/Ca^{2+} exchanger and Ca^{2+} ion channels that facilitate glucose transport across the enterocyte in order to reduce glucose absorption (Wu *et al*., 2015). MEPN may also have inhibited the redistribution and translocation of GLUT2 transporters from the cytoplasmic vesicles of enterocytes into the basolateral membrane and the subsequent release of glucose into circulation.

Compliance with the insulin therapy is important in preventing the adverse clinical eff ects of diabetes. Insulin treatment in type I and type II diabetes has come a long way since its discovery by Banting and Best in 1922 (Heinemann, 1992). The β cells of pancreatic islets synthesize insulin from a single-chain precursor of 110 amino acids termed preproinsulin. After translocation through the membrane of the rough endoplasmic reticulum, the 24- amino-acid N-terminal signal peptide of preproinsulin is cleaved rapidly to form proinsulin. Thereafter, proinsulin folds and the disulfide bonds form. During conversion of human proinsulin to insulin, four basic amino acids and the remaining connector or C peptide are removed by proteolysis. This gives rise to the A and B peptide chains of the insulin molecule, which contains one intrasubunit and two intersubunit disulfide bonds. The A chain usually is composed of 21 amino acid residues and the B chain has 30, the molecular mass is thus about 5808 daltons. There is a single insulin gene and a single protein product in most species. However, rats and mice have two genes that encode insulin and synthesize two molecules that differ at two amino acid

residues in the B chain. The significant decrease in insulin concentration and pancreatic beta cell function in diabetic untreated group may be due to alloxan administration which is known to act by selectively destroying the insulin producing islet cells of the pancreas therefore, depleting the release of insulin into the circulation as observed in this study (Mrozikiewicz *et al*., 1994). Insulin resistance was not affected and this indicates that type 1 diabetes was induced and the peripheral cells may still responds normally to available insulin (Ahre´ *et al*., 2004). The significant increase in insulin concentration and beta cell function in treatment groups may be due to the effect of MEPN to stimulate the few surviving cells of the islet and improve the release of insulin into circulation. This effect observed with MEPN was comparable with diabetic group treated with metformin. The pancreas is an organ with both endocrine and exocrine functions. Among the endocrine cells of the pancreas is the beta Islet cells which produces insulin. The loss of Islet cell in diabetic untreated could be due to the effect of alloxan monohydrate which work by destroying these insulin cells (Plate 4.1B). However, the hypertrophy of the Islet cells observed in MEPN and metformin treated groups could be due to the protective effects of MEPN and also the increase demand by the peripheral tissue to produce insulin.

Effect of Methanol extract of *Parquetina nigrescens* on antioxidative parameters in experimental rats

It is a universal truth that oxygen is the major factor that has made life finite. It is one of the important components of aerobic life. However in some circumstances, this oxygen may be a killer of cells when it generates reactive species that causes necrosis and ultimately the cell death. RNS and RCS also cause oxidation by the generation of certain mechanism that interferes with the normal physiological processes inside the cell (Weseler and Bast, 2010). ‘‘Oxidative stress’’ can be defined as any disturbance in the balance of antioxidants and pro-oxidants in favor of the later due to different factors such as aging, drug actions and toxicity, inflammation and/or addiction (Sies, 1985). It is in general, excess formation or/and insufficient removal of highly reactive molecules such as reactive nitrogen species (RNS) and reactive oxygen species (ROS) (Johansen et al., 2005). Oxygen is highly reactive specie that has the ability to become part of potentially harmful and damaging molecules (Free Radicals). Oxidative stress causes healthy cells of the body to lose their function and structure by attacking them. Oxidative stress has been implicated in the pathogenesis of diabetes mellitus (Moussa *et al*., 2008).

A low Glucose-6-phosphate dehydrogenase (G6PDH) activity has been said to increase oxidative stress because less NAPDH is produced during pentose phosphate pathway (Adinortey *et al*., 2011). The significant decrease in G6PDH activity observed in diabetic untreated is consistent with the reports of Wan *et al*., 2002 who showed a that positive correlation exist between a reduced G6PDH activity and diabetes mellitus. In diabetic untreated, less NADPH is likely produced to reduce the oxidized glutathione in the pentose phosphate pathway leading to altered glucose tolerance (Mehta *et al*., 2000). The increase in G6PDH observed in 200mg/kg MEPN group indicates that more reduced glutathione may have been produced to mop up free radicals that may have been generated in diabetes mellitus (Gaskin *et al*., 2001). The increase in G6PDH observed in metformin treated group also suggests that it may also have the potential of reducing oxidative stress as well (Mehta *et al*., 2000). Cellular redox processes such as oxidative phosphorylation may lead to excess electrons in solution. As cells have reasonable oxygen concentrations, superoxide radical ($O_2^{\bullet-}$) can be rapidly formed by attachment of the electron. Superoxide radical is not a particularly strong reductant or oxidant, so it is rather unreactive with the amino acids that comprise the protein backbone, with the notable exceptions of the sulfur-containing amino acids, cysteine and methionine. It is, however, very reactive with some transition metal complexes and the corresponding aquated ions, particularly copper, iron and manganese. Additionally, evidence has accumulated for the existence of a number of targets for $O_2^{\bullet-}$ reactivity in vivo. These are mainly soluble proteins containing [Fe-S] clusters, such as aconitase, dehydrases, and fumarases. Superoxide dismutases (SODs) are enzymes that function to catalytically convert $O_2^{\bullet-}$ to oxygen (O_2) and hydrogen peroxide (H_2O_2). Based on the metal co-factor they harbor, SODs can be classified into four groups: iron SOD (FeSOD), manganese SOD (MnSOD), copper-zinc SOD (CuZnSOD), and nickel SOD (NiSOD). The decrease in SOD activity in diabetic untreated could be due to the damaging effects of superoxide radicals present within the cells. However, MEPN increased the activity of SOD in the cell in order to mop the superoxide anions in the cells. MEPN could also be acting as a cofactor to potentiate SOD activity**.**

Catalase is a tetrameric protein of 244 kda with molecular 222 symmetry, comprising four identical subunits of 59.7 kda. Each subunit contains 527 amino acid residues, one haem group, namely iron (III) protopor-phyrin IX, and a tightly bound molecule of NADPH (Safo *et al*., 2001). The decrease observed in Catalase (CAT) activity in

diabetic untreated may indicate that more hydrogen peroxide was produced and if this is allowed to accumulate may be potentially toxic at high concentration (Goth *et al*., 2016). However, the increase observed in the treatment groups indicates that more hydrogen peroxide may have been broken down to harmless water and oxygen (Goth *et al*., 2016).

Albumin is a multifunctional protein with 585 amino acids and one reduced cysteine residue (Cyst 34) and a molecular weight of 66kDa (Marjolaine *et al*., 2008). The significant decrease in albumin in diabetic untreated may likely indicates a decrease in the quantity of the reduced Cyst 34 residues in albumin to scavenge hydroxyl radicals (Gutteridge *et al*., 1986). However, the increase observed in albumin level in the MEPN groups suggests an increase in reduced cyst 34 in albumin which is converted to sulfenic acid that is important in redox modulation of reactive species (Oettl *et al*., 2007). It is also likely that MEPN may have reversed methionine sulphoxide that may have been produced in diabetic untreated back to methionine residue in albumin which is highly susceptible to oxidative damage (Bourdon *et al*., 2005). These effects observed in 100mg/kg and 200 mg/kg MEPN were comparable to metformin treated group.

Effect of Methanol extract of *Parquetina nigrescens* on renoprotective indices in experimental rats

The body contains a large variety of ions, or electrolytes, which perform a variety of functions. Some ions assist in the transmission of electrical impulses along cell membranes in neurons and muscles. Other ions help to stabilize protein structures in enzymes. Still others aid in releasing hormones from endocrine glands. All of the ions in plasma contribute to the osmotic balance that controls the movement of water between cells and their environment. Electrolytes in living systems include sodium, potassium, chloride, bicarbonate, calcium, phosphate, magnesium, copper, zinc, iron, manganese, molybdenum, copper, and chromium. In terms of body functioning, six electrolytes are most important: sodium, potassium, chloride, bicarbonate, calcium, and phosphate.

Electrolyte imbalance is a consistent observation in diabetes mellitus (Palmer *et al*., 2015). Maintaining electrolyte balance translates to either preventing loss in urine or alterations of transport enzyme to prevent morbidity. Na^+-K^+ ATPase is a ubiquitous

enzyme which ensures that the transmembrane gradients of sodium and potassium concentrations are maintained. Alteration in these transport enzymes is associated with pathogenesis and complications of diabetes mellitus (Totan *et al*., 2002). Reduced level of Na^+ and k^+ ions in diabetes mellitus as observed in this study has been shown to imply disturbances in membrane depolarization and nerve conduction (Ruiz-Guttierez *et al.,* 1993). Significant increase in Na^+ and k^+ ions in MEPN treated groups (100 and 200mg/kg) implies improvement in renal tubular reabsorption of Na^+ and stimulation of Na^+ - k^+ ATPase activities to increase influx of K^+ into the cell.

Calcium ion is one of the prominent intracellular cations which carry out several biochemical reactions (Reinhart *et al*., 1988). Diabetes is also associated with calcium reduction (Djurhuus *et al.,* 2000) as observed in this study. Its decrease is associated with loss of 1, 25 dihydroxycholecalciferol which is necessary for bone resorption and calcium absorption from the intestinal mucosa cells (Takiishi *et al*., 2012). Findings from this study showed that MEPN may have increased Ca^{2+} ion level by stimulating intestinal calcium absorption, renal tubular calcium reabsorption and osteoclastic activities of bone cells resulting in bone resorption.

The problem for the body is that normal metabolism is associated with continuous production of hydrogen ions (H^+) and carbon dioxide (CO_2), both of which tend to reduce pH. The mechanism which overcomes this problem and serves to maintain normal blood pH (i.e. preserve acid-base homeostasis) is a complex synergy of action involving chemical buffers in blood, the red cells (erythrocytes), which circulate in blood, and the function of three organs: lungs, kidneys and brain. After prolonged hyperglycaemia, the body switch to utilizing protein and fats for energy production. This releases many ketones in the body meanwhile large amount of energy is produced in the process. The release of ketones in the blood decreases the pH of the blood making it to be more acidic. The decrease in Cl^- and HCO_3^- in diabetic untreated is an indication of acidosis in the blood. However, treatment with MEPN ameliorated acidosis by increasing the production of chloride and bicarbonate. The mechanism of this increase could be reabsorption of bicarbonate and increase secretion of hydrogen ion into the renal tubule.

Blood urea nitrogen (BUN) are nitrogenous waste products produced from breakdown of protein into ammonia which undergoes deamination by liver enzymes to produce urea which are excreted through the kidneys. The increase in BUN in diabetic untreated rats may be due to damage to the glomerulus of the renal tubular cells from uncontrolled hyperglycemia (Molitori *et al*., 2007) as shown by the photomicrograph (4.2B). However, oral administration of MEPN at 100 mg/kg and 200 mg/kg significantly decrease urea nitrogen and this implies that MEPN could also prevent damage to renal tubular cells and increase excretion of urea nitrogen in the urine as shown by the photomicrographs (4.2 C&D).

Creatinine is formed from muscle creatine as metabolic waste product and excreted through the kidneys (Stryer *et al*., 1997). The rise in creatinine in diabetic rats is an indication of kidney damage, sclerosis, inflammation and decreased glomerular filtration rate (Henry *et al*., 2001). This observation is consistent with previous reports which identified creatinine as a basic marker of renal dysfunction in diabetes mellitus (Cholongitas *et al*., 2007). However, the significant decrease in creatinine level in 100 mg/kg and 200 mg/kg MEPN was comparable to that of metformin treated group and this implies that MEPN may have the ability to improve kidney functions in diabetic condition so that more waste products can be filtered from the blood and excreted in urine as shown by the photomicrograph (4.2 C&D) (Meyer *et al*., 2007).

The kidney is a gluconeogenic organ producing glucose from non-carbohydrate sources. However, chronic hyperglycaemia could damage the renal tubular cells of the kidney due to increase production of reactive oxygen species as it was also observed in diabetic untreated group. However, MEPN was able to maintain the integrity of the renal cell and prevent the damage that could result from prolonged hyperglycaemia. This is in contrast to metformin treated group which was unable to prevent the damage to the renal tubule as shown by the photomicrograph (4.2E).

Effect of phytol and squalene on antidiabetic indices in experimental rats

A fundamental principle of nutrition and metabolism is that body weight change is associated with an imbalance between the energy content of food eaten and energy expended by the body to maintain life and to perform physical work. Such an energy balance framework is a potentially powerful tool for investigating the regulation of body

weight. However, we need a better understanding of the components of energy balance and their interactions over various time scales to explain the natural history of conditions such as obesity and to estimate the magnitude and potential success of therapeutic interventions. In the present study, the weight gain in the body, kidney and the liver observed in diabetic group treated with phytol and squalene could be due to the ability of the extract to stimulate adipocyte cell differentiation and fat redistribution in the body (Saltzman, 2008).

Diabetes mellitus is characterized with persistent and uncontrolled hyperglycaemia capable of causing deleterious effects on tissues and organs of the body. The rise in fasting glucose seen in diabetic untreated could be as a result of selective destruction of beta cells from alloxan administration (Manosroi *et al*., 2011). Treatment with Phytol and Squalene significantly lowered fasting glucose, this could be due to the ability of these compounds to stimulate the surviving beta cell of the pancreas to produce insulin for glucose uptake (Junod *et al*., 1969). These effects were comparable with diabetic treated with 100 mg/kg metformin.

Some of the functions of the liver include glycolysis, glycogenesis, gluconeogenesis and glycogenolysis. Endogenous glucose formation is augumented by the liver in diabetes mellitus due to poor pancreatic function (Schillinger *et al*., 2002). However, the significant increase in liver glycogen observed after treatment with Phytol and Squalene could be due to their ability to up-regulate key enzymes that control glycolysis, glycogenolysis and gluconeogenesis (Hikino *et al*., 1989). The liver histology showed no sign of toxicity and this means that Phytol and Squalene are potentially non-toxic compounds at doses administered and could further improve glycogen storage or cause the pancreas to secrete insulin for glucose uptake in the liver

Glucose absorption occur in the enterocytes of small intestine. The major route of glucose from the intestinal lumen into enterocyte is through SGLT even though GLUT-2 may also play role in its exit into circulation (Gorboulev *et al*., 2011). Meftormin caused significant decrease in duodenal and jejunal glucose absorption by regulating the activities of these transporters according to the reports of Abbasi *et al*. (1998). However, no significant difference was observed with Phytol and Squalene which showed that they may not be acting via these mechanisms.

Insulin is a major substrate produced from the pancreas Islets cells and responsible for glucose uptake in organs like the liver, muscles and adipose tissues (Koster *et al*., 2005). The significant increase in insulin level and sensitivity after treatment with Phytol suggests it has ability to stimulate islet cell to produce insulin (Kumagai *et al*., 1993). This was consistent with the decrease in fasting blood glucose observed in this study. Metformin also showed increased insulin sensitivity by acting through similar mechanism with Phytol. Treatment with Phytol improves beta cell function and prevents insulin resistance. Phytol may be acting by stimulating the activities of voltage-gated Ca^{2+} and K^{+} ATP sensitive ion channels on beta cell membrane which is capable of regulating glucose level and improving the cell activities (Koster *et al*., 2005). In addition, Phytol increases insulin concentration by improving the sensitivity and signalling from insulin receptors to glucose transporters of peripheral tissues (Waters *et al*., 1993). The loss of pancreatic islet cells in diabetic untreated may be due to alloxan administration however, the pancreatic islet cells hypertrophy in phytol, squalene and metformin treated groups may be due to the increase demand from the peripheral cells to produce insulin. Degeneration was prevented in the various groups treated with Phytol, Squalene and Metformin. The pancreas is an organ with both endocrine and exocrine functions. Among the endocrine cells of the pancreas is the beta islet cells which produces insulin.

The loss of Islet cell in diabetic untreated could be due to the effect of alloxan monohydrate which works by destroying these insulin cells. However, the hypertrophy of the Islet cells observed in phytol, squalene and metformin treated groups could be due to their protective effects and also the increase demand by the peripheral tissues to produce insulin.

Effect of phytol and squalene on antioxidative parameters in experimental rats

Glucose 6 phosphate dehydrogenase (G6PDH) deficiency plays role in the pathogenesis of diabetes mellitus. NADP, a coenzyme in the monophosphate shunt is necessary to generate reduced glutathione (GSSH) to improve glucose tolerance in diabetes. A decreased NADPH production may cause G6PDH deficiency which may results in oxidative stress damage of macromolecules as it was observed in diabetic untreated group (Meloni *et al*., 1992). Phytol, Squalene and Metformin upregulated the level of reduced NADPH produced in the Hoxose monophosphate shunt pathway hence, increasing the level of G6PDH. Moreso, NADPH is a cofactor for endothelial nitric oxide synthase that produces NO necessary for vasodilation (Meloni *et al*., 1992). A reduced level of NADPH in diabetic untreated is also an indication of endothelial dysfunction and diabetic vascular damage (Leopold *et al*., 2003). However, treatment with Phytol improves vascular functions and these was comparable with normal control and diabetic group treated with 100mg/kg metformin.

Hydrogen peroxide (H_2O_2) is a highly reactive small molecule formed as a natural by-products of energy metabolism. Excessive concentration of H_2O_2 may lead to significant damages to DNA, RNA and lipids. Therefore, enzyme catalase (CAT) breakdown the H_2O_2 into water and oxygen (Goth *et al.*, 2012). The significant decrease in CAT activities is an indication that more H_2O_2 is produced during metabolism. However, Phytol and Squalene may possess the abilities to potentiate CAT activities so that more H_2O and O_2 can be produced.

Glutathione peroxidase (GPx) is a ubiquitous enzyme which detoxifies H_2O_2 and in the process convert reduced glutathione (GSH) to oxidized glutathione (GSSG) which act as cofactor (Seghrouchi 2002). A significant decrease in GPx in diabetes untreated may be an indication of H_2O_2 accumulation (Gibson, 2012). Treatment with Phytol increased GPx activities which suggests that more H_2O_2 may likely be broken down to H_2O and O_2. The effect was comparable to metformin treated group.

There was no significant increase in albumin (ALB) after treatment with Phytol and Squalene, this mean that other active ingredient might have been responsible for the observed changes in albumin level.

Cellular redox processes such as oxidative phosphorylation may lead to excess electrons in solution. As cells have reasonable oxygen concentrations, superoxide radical ($O_2^{\bullet-}$)

can be rapidly formed by attachment of the electron. Superoxide radical is not a particularly strong reductant or oxidant, so it is rather unreactive with the amino acids that comprise the protein backbone, with the notable exceptions of the sulfur-containing amino acids, cysteine and methionine. It is, however, very reactive with some transition metal complexes and the corresponding aquated ions, particularly copper, iron and manganese. Additionally, evidence has accumulated for the existence of a number of targets for $O_2^{\bullet-}$ reactivity in vivo. These are mainly soluble proteins containing [Fe-S] clusters, such as aconitase, dehydrases, and fumarases. Superoxide dismutases (SODs) are enzymes that function to catalytically convert $O_2^{\bullet-}$ to oxygen (O_2) and hydrogen peroxide (H_2O_2). Based on the metal co-factor they harbor, SODs can be classified into four groups: iron SOD (FeSOD), manganese SOD (MnSOD), copper-zinc SOD (CuZnSOD), and nickel SOD (NiSOD). The decrease in SOD activity in diabetic untreated could be due to the damaging effects of superoxide radicals present within the cells. However, Phytol and Squalene increased the activity of SOD in the cell in order to mop the superoxide anions in the cells. Phytol and could also be acting as a cofactor to potentiate SOD activity**.**

Effect of phytol and squalene on renoprotective parameters in experimental rats

The body contains a large variety of ions, or electrolytes, which perform a variety of functions. Some ions assist in the transmission of electrical impulses along cell membranes in neurons and muscles. Other ions help to stabilize protein structures in enzymes. Still others aid in releasing hormones from endocrine glands. All of the ions in plasma contribute to the osmotic balance that controls the movement of water between cells and their environment. Electrolytes in living systems include sodium, potassium, chloride, bicarbonate, calcium, phosphate, magnesium, copper, zinc, iron, manganese, molybdenum, copper, and chromium. In terms of body functioning, six electrolytes are most important: sodium, potassium, chloride, bicarbonate, calcium, and phosphate.

Hyponatraemia is a common electrolyte disorder associated with increased morbidity and mortality (Liamis *et al*., 2015). Decreased serum sodium level are occasionally observed in patient with diabetes mellitus due to hyperglycaemia, hypervolumia, chronic kidney disease and drug ingestion (Liamis *et al*., 2015) which was also consistent with this study. However, treatment with Phytol restores Na^+ level and blood volume. Studies have shown that serum K^+ significantly lowered in diabetes mellitus (Gunanithi *et al*.,

2016), which may be due to gastrointestinal loss, bacteria overgrowth, chronic diarrhea state and renal loss due to osmotic diuresis as was evident from this study (Adrogue *et al*., 1986). Treatment with Phytol ameliorates hypokalaemia and this may be due to reduction in renal loss of K^+ to osmotic diures.

Patients with diabetes mellitus have increased risk of developing acute renal failure which is capable of reducing phosphorus excretion leading to hyperphosphatemia (Liamis *et al*., 2015). This condition induces hypocalcemia by causing excess phosphate to bind to ionized calcium in order to remove it from the blood stream (Blaine *et al*., 2015). This report is consistent with our observation in diabetic untreated in which rats were hypocalcaemic. However, treatment with Phytol elevated calcium level which suggest that Phytol may improve phosphorus excretion in the kidney thereby preventing calcium binding. These effect of Phytol was comparable with normal control and diabetic treated with 100 mg/kg metformin.

The problem for the body is that normal metabolism is associated with continuous production of hydrogen ions (H^+) and carbon dioxide (CO_2), both of which tend to reduce pH. The mechanism which overcomes this problem and serves to maintain normal blood pH (i.e. preserve acid-base homeostasis) is a complex synergy of action involving chemical buffers in blood, the red cells (erythrocytes), which circulate in blood, and the function of three organs: lungs, kidneys and brain. After prolonged hyperglycaemia, the body switch to utilizing protein and fats for energy production. This releases many ketones in the body meanwhile large amount of energy is produced in the process. The release of ketones in the blood decreases the pH of the blood making it to be more acidic. The decrease in Cl^- and HCO_3^- in diabetic untreated is an indication of acidosis in the blood. However, treatment with Phytol and Squalene ameliorated acidosis by increasing the production of chloride and bicarbonate. The mechanism of this increase could be reabsorption of bicarbonate and increase secretion of hydrogen ion into the renal tubule.

Blood urea nitrogen (BUN) is a major nitrogenous end product of protein and amino acid catabolism (Henry, 2013) while creatinine (CRT) is a breakdown product of creatine phosphate in muscle (Abdel, 2006). Blood urea nitrogen and Creatinine are good indicators of renal function and are excreted in urine through the kidneys. Elevated values of both BUN and Creatinine may indicate kidney dysfunction as observed in

diabetic untreated. Treatment with Phytol and Squalene improves renal loss of BUN and CRT in urine. One of the complications of diabetes mellitus is irreversible damage to the renal tubule. Moreso, the disease could also activate the deposition of extracellular cell matrix and thickening of the wall of the renal tubule. The phytol was able to prevent sclerosis and damage to the renal tubule. Although, squalene and metformin prevented damage to the renal tubule, they could not prevent glomerusclerosis which eventually results in the thickening of the renal tubule.

Effects of phytol and squalene on diacylglycerol-protein kinase C activation pathway and apoptotic proteins.

Diabetic nephropathy is the most common cause of end stage renal disease and one of the leading causes of morbidity and mortality in patients with diabetes (Bruno *et al*., 2003) and it's characterized with several pathological changes in renal glomeruli of the kidney (Sharma *et al*., 2013). An uncontrolled hyperglaemia could results in diabetic complications through several pathways including the polyol pathway, advance glycated end product pathway, diacylglycerol-protein kinase C pathway (DAG-PKC) and hexosamine pathway. DAG-PKC activation pathway is the major culprit in the formation of diabetic nephropathy (Mazzucco *et al*., 2002).

Diacylglycerol (DAG) is a second messenger and a physiologic activator of protein kinase C (PKC) (Lyons *et al*., 1990). The observed increased in DAG level in diabetic untreated could be due to increase de novo synthesis of DAG by hyperglycaemia from glycolytic intermediates and metabolism of phosphatidylcholine by phospholipase D and glycerol-3- phosphate (Abdel-Nabey *et al*., 1992). However, Phytol decreases DAG level by inhibiting these intermediate products of the glycolytic pathway.

The increased PKC activity observed in diabetic untreated group could be due to increased level of DAG as observed in this study. This finding is consistent with the report of (Noh *et al*., 2007) who had also earlier reported an overexpression of protein kinase C in the glomerular and mesangial cells in animal model of diabetes. Treatment with Phytol, decreased PKC activity by also reducing DAG level as observed in this study.

Activation of protein kinase C has been shown to activate several cytokines at the signal transduction level which promote formation of cytoskeletal materials within the glomerulus. Mitogen activated protein kinase activity (MAPK) was upregulated in

diabetic untreated whereas Phytol and Squalene exert no effect on MAPK-8 activity and this indicates that MAPK-8 is unaffected in preventing structural changes in chronic kidney disease (Adhikary *et al.*, 2004).

Deposition of extracellular cell matrix in the glomerulus and renal tubule could decrease the velocity and pressure of blood flow in the kidney leading to renal hypertension due to persistent activation of the rennin-angiotensin aldosterone system (Moschella *et al*., 2007). Phytol down-regulates the level of transformin growth factor (TGF) which means that it prevents the accumulation of extracellular cell matrix (ECM) deposition within the messangial cell which could lead to hypertrophy, glomerulosclerosis and haemodynamic abnormalities (Adhikary *et al*., 2004).

The p16 and p53 are guardian of genome and tumour suppressor proteins. In most tumours, cells possess mutations of p16 and p53 gene which code for their synthesis therefore, enhancing proliferation of tumour cells (Denaro *et al*., 2011). In diabetes mellitus however, most cells are susceptible to senescense and apoptosis due to prolonged DNA damage which was also consistent with our findings in diabetes untreated group (Carlos *et al*., 2004) (Plate 4.7). The p16 and p53 proteins were not expressed in normal control and and 250mg/kg Phytol treated groups and this means that phytol possesses the ability to inhibit p16 and p53 expressions which in turn deactivate pro-apoptotic proteins like Bax, PUMA, NOXA which trigger programmed cell death (Shinohara *et al*., 2014).

The Bcl-2 is encoded by Bcl-2 gene and it's the founding member of the Bcl-2 family of protein which plays a role in regulating apoptosis (Alavian *et al*., 2011). The Bcl-2 is specifically considered as an anti-apoptotic protein. The significant reduction in Bcl-2 presence in diabetic untreated may be due to inhibition of Bcl-2 and increase in the pro-apoptotic protein which causes the loss of cell and total degeneration of the glomerulus and surrounding basement membrane of the renal tubular cells (Eckenrode *et al*., 2010). However, the overexpression of Bcl-2 in normal control and 250 mg/kg Phytol treatment enhances cell survival by surppressing apoptosis in cells by inhibiting the proapoptotic proteins (Kalverda *et al*., 2009)

Inflammation is an adaptive response of the body elicited as a principal component of tissue repair to deal with injuries and microbial infections (Pimson *et al*., 2014). It can

be elevated in chronic conditions such as peripheral neuropathy, retinopathy, nephropathy and fatty liver (Lontchi *et al*., 2013). Diabetes mellitus is characterized with chronic inflammation which causes the release of cytokines capable of activating different cyclooxygenase isoforms. Cyclooxygenase (COX) is the rate limiting step in the synthesis of prostanoids, a large family of arachidonic acid metabolites comprising prostaglandins, prostacyclin, and thromboxanes (Montserrat *et al*., 2014). The presence COX-2 in diabetes is consistent with our findings in diabetic untreated rats and this suggests that COX-2 may have been upregulated and cause the formation of prostanoids which are involved in inflammatory reactions in tissues especially the kidney and brain (Curry *et al*., 2005). However, treatment with Phytol reduces COX-2 to a level comparable to the normal control. This effect of Phytol may be due to its ability to inhibit COX-2 in the kidney and prevent formation of prostanoid metabolites. Phytol may have acted through mechanisms similar to non-steroidal anti-inflammatory drugs (NSAIDs) in reducing COX-2 expressions in the kidney (Emanuela *et al*., 2011)

5.1 Findings

The result of this study has revealed that MEPN, Phytol and Squalene exert anti-hyperglycaemic effects. This might be due to their ability to potentiate the regeneration of few surviving islet cells of the pancreas to produce insulin for glucose uptake. Furthermore, they caused increase hepatic glycogen storage, an effect which may be due to their ability to upregulate the activity of glycogen synthase enzyme. The decrease in intestinal glucose absorption may be due to the ability of methanol extract of *Parquetina nigrescens* to inhibit the glucose transporters (SGLT and GLUT) on apical and basolateral membrane of enterocytes respectively.

The methanol extract of *Parquetina nigresens*, Phytol and Squalene caused increased G6PDH activity; an effect which may be due to their ability to convert oxidized glutathione to reduced glutathione. They also increased catalase activity which increased the breakdown of hydrogen peroxide. Similarly, methanol extract of *Parquetina nigresens* caused increased albumin level by increasing the availability of more cysteine 34 amino acid residues for redox modulation of free radicals.

The methanol extract of *Parquetina nigresens*, Phytol and Squalene restored electrolyte imbalance caused by hyperglycaemia. They caused decrease in the level of creatinine and blood urea nitrogen. These effects may be due to the ability to prevent damage to renal tubular cells, reduce thickening of glomerulus and improve ultrafiltration.

Phytol decreased DAG, PKC-α and TGF-β while it increased Bcl-2 expression. Phytol could have prevented the following; nephromegaly, mesangial expansion, glomerulosclerosis and haemodynamic abnormalities that may results from diabetic nephropathy.

5.3 Conclusion

In conclusion, the results of the anti-diabetic studies have shown that methanol extract of *Parquetina nigrescens* and Phytol could help in ameliorating diabetes mellitus and its complications. These effects of methanol extract of *Parquetina nigresens* may be due the presence of phytochemicals and the active compounds present in the plant extract as shown by the results of phytochemical screening and Gas chromatography-mass spectrometry analysis.

5.2 Recommendations

The effects of the methanol extracts of *Parquetina nigrescens* and the chemical constituents (Phytol and Squalene) in diabetes are unknown; nevertheless, considering these findings in rat model of diabetes, *Parquetina nigrescens* could be a good source of diabetic drug and phytol could be useful in preventing renal damage in diabetic patients. However, this medicinal plant should be subjected to further research to determine its mechanisms of actions and further validate its properties.

5.3 Contributions to knowledge

1. This study has identified the chemical constituents of methanol extract of *Parquetina nigrscens*

2. Previous studies have reported that *Parquetina nigrescens* causes decrease in blood glucose. However, this study has shown that this decrease may be due to the ability of MEPN, phytol and squalene to increase insulin production which is necessary for glucose uptake or to increase hepatic glycogen storage which is necessary to remove glucose from circulation or decrease intestinal glucose absorption which is necessary to reduce the movement of glucose from the lumen of the intestine across the enterocyte into the blood.

3. Previous studies have shown that the diabetes mellitus is characterized with hypoglycemia with increase oxidative stress damage. This study has shown that *Parquetina nigrescens*, phytol and squalene could be useful in reducing oxidative stress by increasing the activities of antioxidant enzymes which are necessary to mop up free radicals which include superoxide anions, hydrogen peroxide, hydroxyl radicals and many others.

4. Previous studies have shown that diabetes mellitus is associated with long-term damage to several organs most especially the kidneys. However, this study has shown that *Parquetina nigrescens*, and phytol could improve kidney functions in diabetes mellitus by maintaining serum electrolyte concentrations and increasing the excretion of urea and creatinine metabolites in urine.

5. Previous studies have shown that hyperglycemia which occur in diabetes could result in the development of diabetic nephropathy through diacylglycerol-protein kinase C activation pathway. However, this study has shown that phytol, a component of methanol extract of *Parquetina nigrescens* could prevent nephropathy by down-regulating DAG, PKC and TGF which are responsible for nephromegaly, mesangial expansion, glumerulosclerosis, ischemia and hemodynamic abnormalities which occur in diabetes nephropathy.

5.4 Further studies

Although, this study has revealed the various antidiabetic mechanisms of *Parquetina nigrescens*, further investigations that may be carried out in this area of physiology using the medicinal plant extract are as follows;

1. The effects of the other bioactive compounds in methanol extract of *Parquetina nigrescens* in diabetic wistar rats.
2. Morphological and biochemical changes in the muscle after treatment with methanol extract of *Parquetina nigrescens*.

3. The effects of methanol extract of *Parquetina nigrescens* on the mechanism of insulin secretion.
4. The effect of methanol extract of *Parquetina nigrescens* on the mechanism of glucose uptake in the intestine, liver and the muscles.

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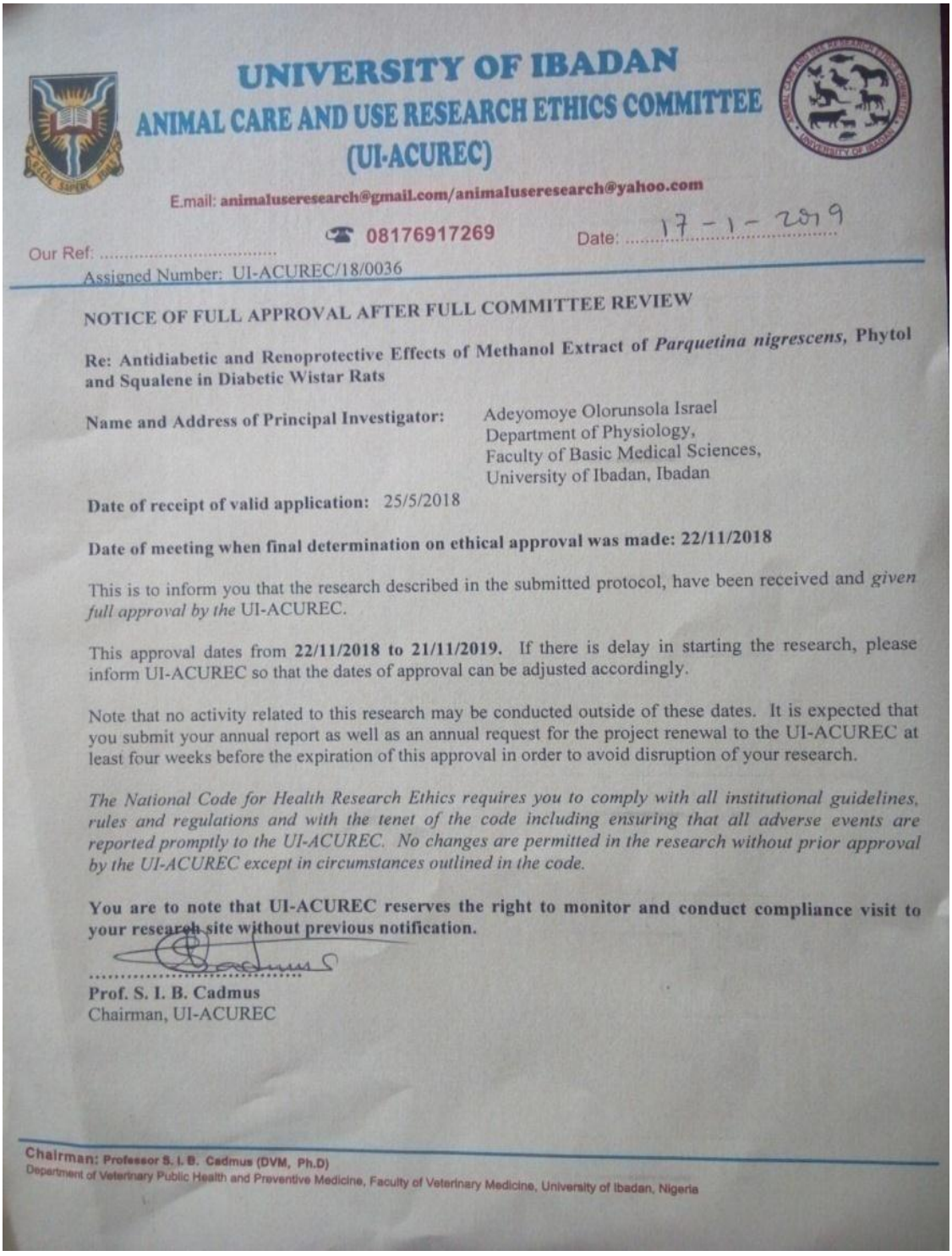
APPENDICES



UNIVERSITY OF IBADAN
ANIMAL CARE AND USE RESEARCH ETHICS COMMITTEE
(UI-ACUREC)

E.mail: animaluseresearch@gmail.com/animaluseresearch@yahoo.com

☎ 08176917269

Our Ref: .....

Date: 17-1-2019

Assigned Number: UI-ACUREC/18/0036

NOTICE OF FULL APPROVAL AFTER FULL COMMITTEE REVIEW

Re: Antidiabetic and Renoprotective Effects of Methanol Extract of *Parquetina nigrescens*, Phytol and Squalene in Diabetic Wistar Rats

Name and Address of Principal Investigator: Adeyomoye Olorunsola Israel
Department of Physiology,
Faculty of Basic Medical Sciences,
University of Ibadan, Ibadan

Date of receipt of valid application: 25/5/2018

Date of meeting when final determination on ethical approval was made: 22/11/2018

This is to inform you that the research described in the submitted protocol, have been received and *given full approval by the* UI-ACUREC.

This approval dates from **22/11/2018 to 21/11/2019.** If there is delay in starting the research, please inform UI-ACUREC so that the dates of approval can be adjusted accordingly.

Note that no activity related to this research may be conducted outside of these dates. It is expected that you submit your annual report as well as an annual request for the project renewal to the UI-ACUREC at least four weeks before the expiration of this approval in order to avoid disruption of your research.

The National Code for Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenet of the code including ensuring that all adverse events are reported promptly to the UI-ACUREC. No changes are permitted in the research without prior approval by the UI-ACUREC except in circumstances outlined in the code.

You are to note that UI-ACUREC reserves the right to monitor and conduct compliance visit to your research site without previous notification.

.....

Prof. S. I. B. Cadmus
Chairman, UI-ACUREC

Chairman: Professor S. I. B. Cadmus (DVM, Ph.D)
Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Ibadan, Nigeria

Figure 3.2: ACUREC'S certificate

ESSENTIAL OILS

Data Path : C:\msdchem\1\sequence\ESSENTIAL OIL .M\
Data File : PNTadeyomoye.D
Acq On : 17 Feb 2016 00:04
Operator : supervisor
Sample : PNTadeyomoye

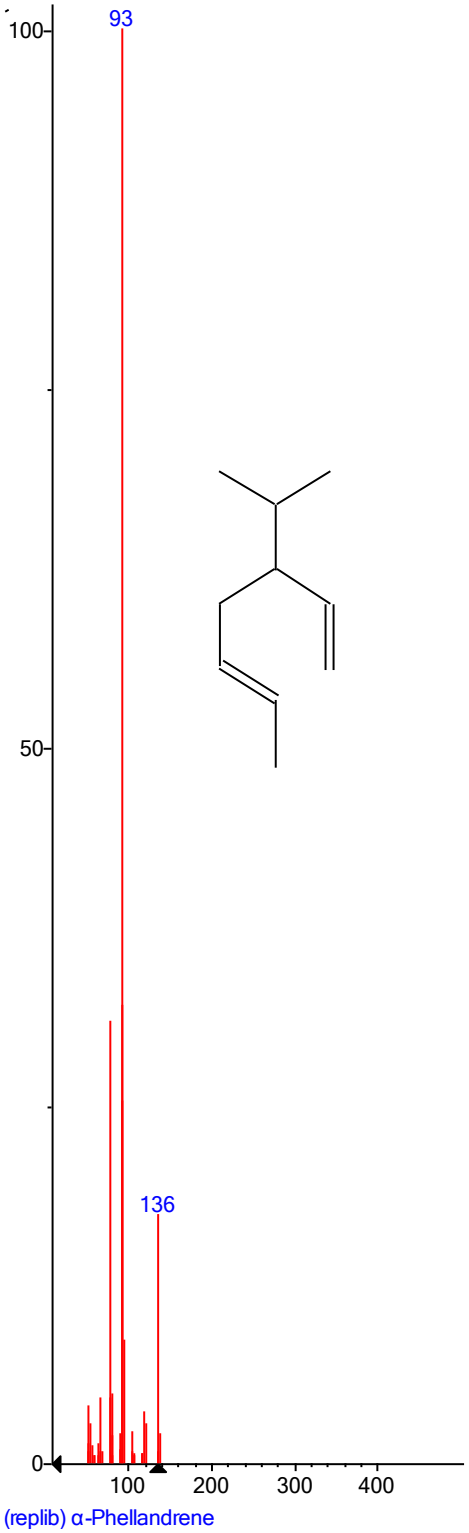
Method : C:\msdchem\1\sequence\ESSENTIAL OIL .M
Title :

Signal : TIC: PNTadeyomoye.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	% max.	% of total
1	5.820	56	128	168	BV	491896	20968434	7.68%	1.726%
2	6.115	168	180	226	VV	465494	20896664	7.65%	1.720%
3	12.005	1186	1209	1215	VV 4	188731	9009544	3.30%	0.742%
4	12.126	1215	1230	1246	VV	441586	21124996	7.73%	1.739%
5	12.264	1246	1254	1266	VV 5	237131	9305504	3.41%	0.766%
6	12.443	1266	1286	1310	VV 2	241668	9113538	3.34%	0.750%
7	12.992	1353	1382	1400	BV 2	111405	2895998	1.06%	0.238%
8	15.033	1699	1738	1746	VV 2	109983	11917853	4.36%	0.981%
9	15.286	1759	1783	1806	VV 9	352643	29296532	10.72%	2.412%
10	15.477	1806	1816	1826	VV 7	265020	11321982	4.14%	0.932%
11	15.576	1826	1833	1847	VV 7	190422	12033889	4.41%	0.991%
12	15.867	1847	1884	1898	VV 9	318463	39137506	14.33%	3.222%
13	16.275	1898	1955	1966	VV 3	784621	94329014	34.53%	7.765%
14	16.438	1966	1984	2023	VV 5	622785	82770716	30.30%	6.814%
15	16.813	2023	2049	2144	VV 2	3642967	259701819	95.06%	21.378%
16	17.711	2198	2206	2211	VV 5	311215	7845668	2.87%	0.646%
17	17.772	2211	2217	2223	VV 2	376007	10738769	3.93%	0.884%
18	18.184	2223	2235	2257	VV 2	1404158	57968811	21.22%	4.772%
19	18.274	2257	2305	2319	VV 7	3237404	273185816	100.00%	22.488%
20	18.388	2319	2325	2466	VV 5	1236540	112580600	41.21%	9.268%
21	20.222	2587	2645	2767	BB 5	136727	21583038	7.90%	1.777%
22	21.021	3945	4009	4097	BB 3	1033870	97060757	35.53%	7.990%

Sum of corrected areas: 1214787446

STRUCTURES AND SPECTRAL ANALYSIS OF ESSENTIAL OILS IN METHANOL EXTRACT OF *Parquetina nigrescens*



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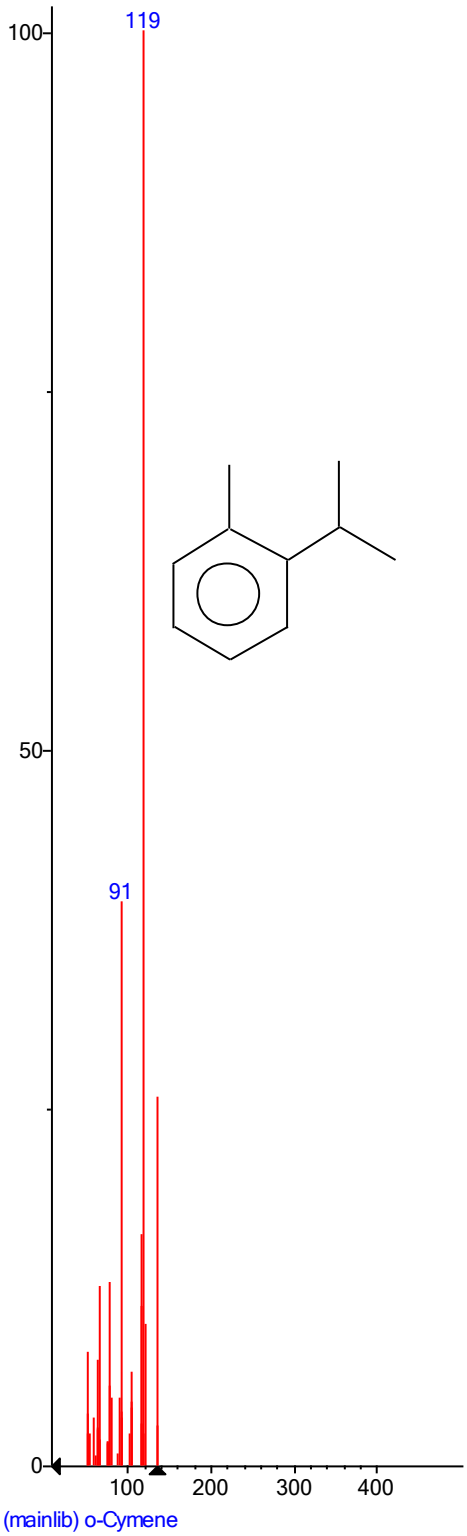

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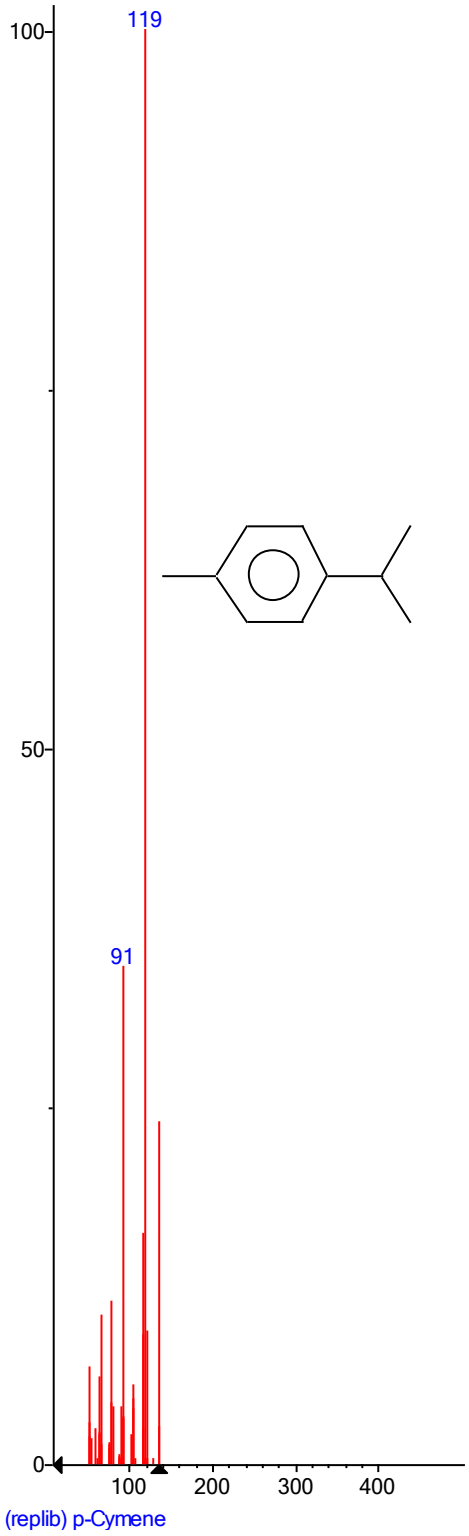
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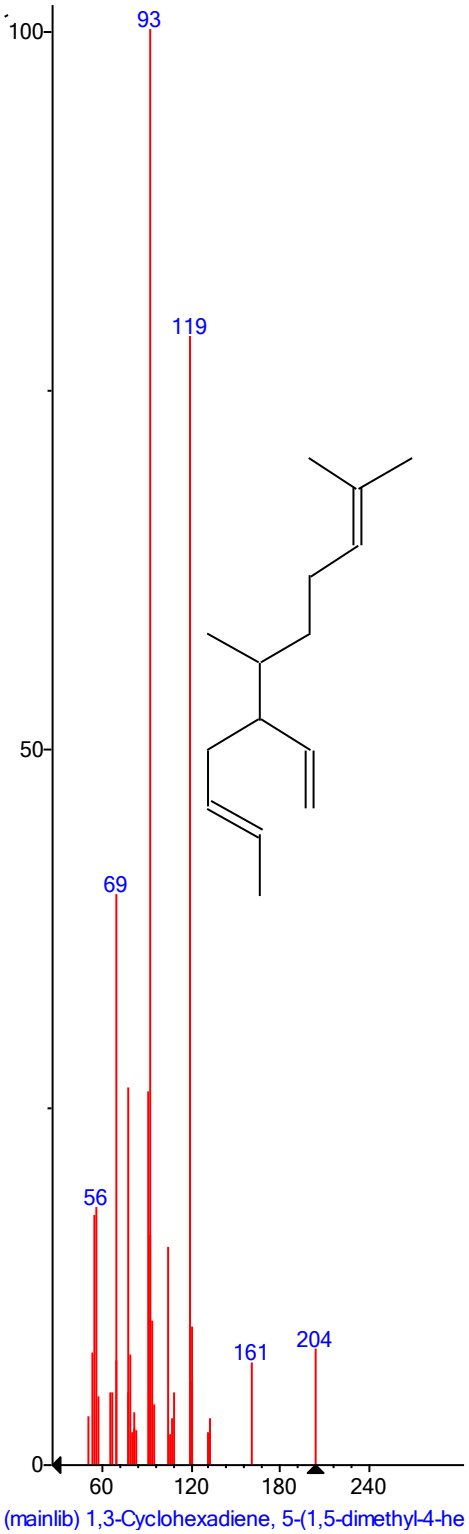
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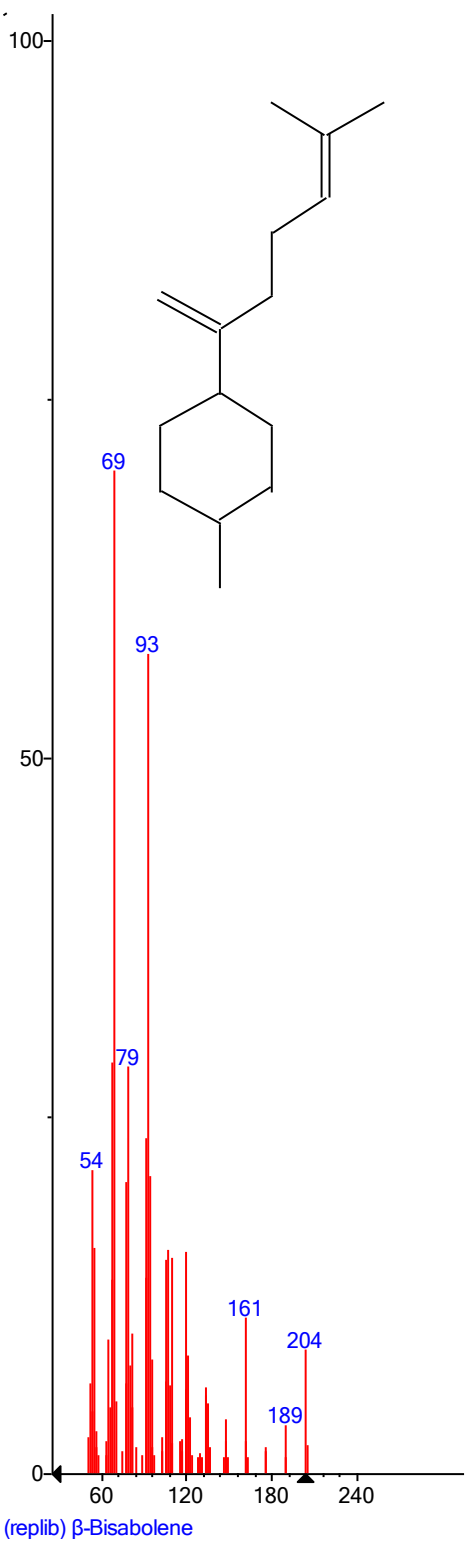

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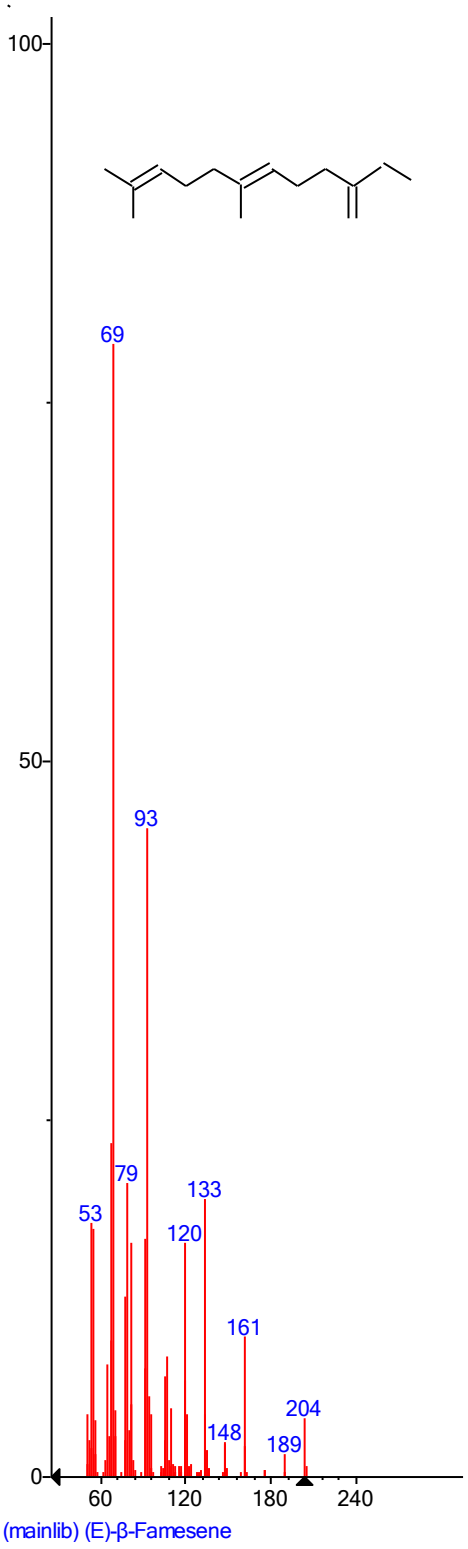

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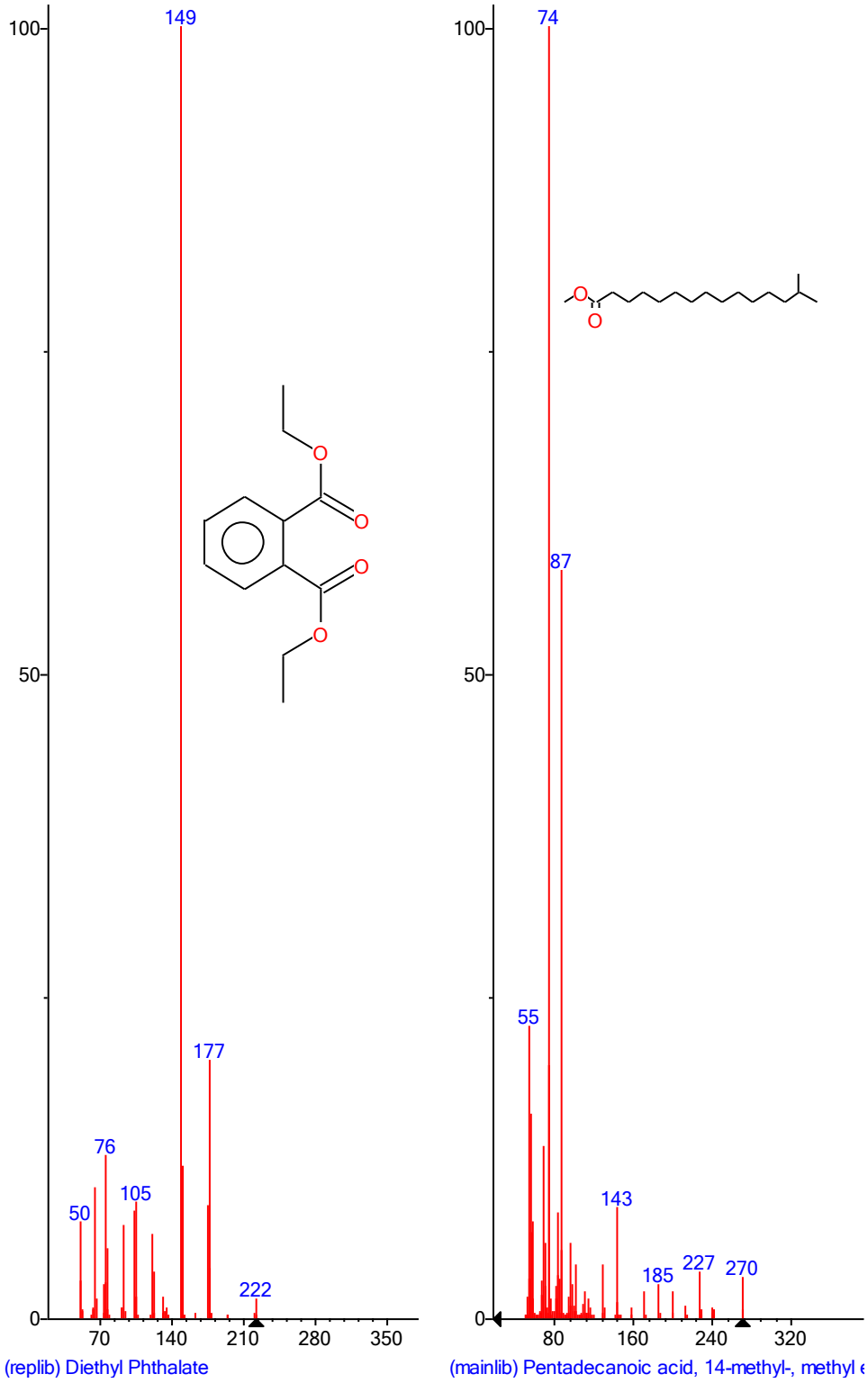
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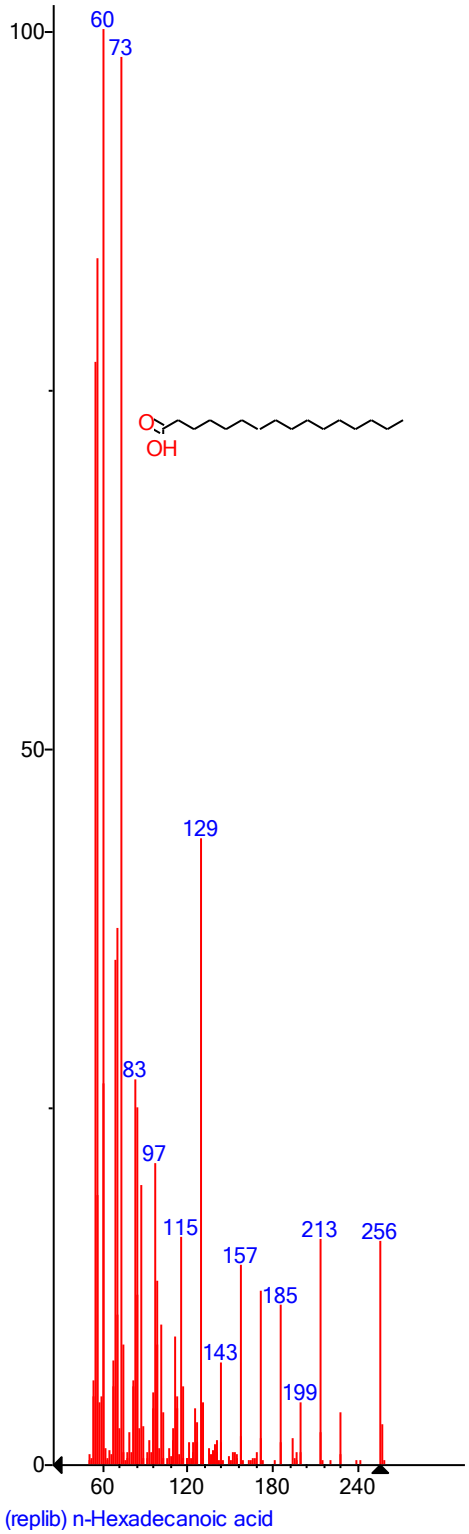
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OH
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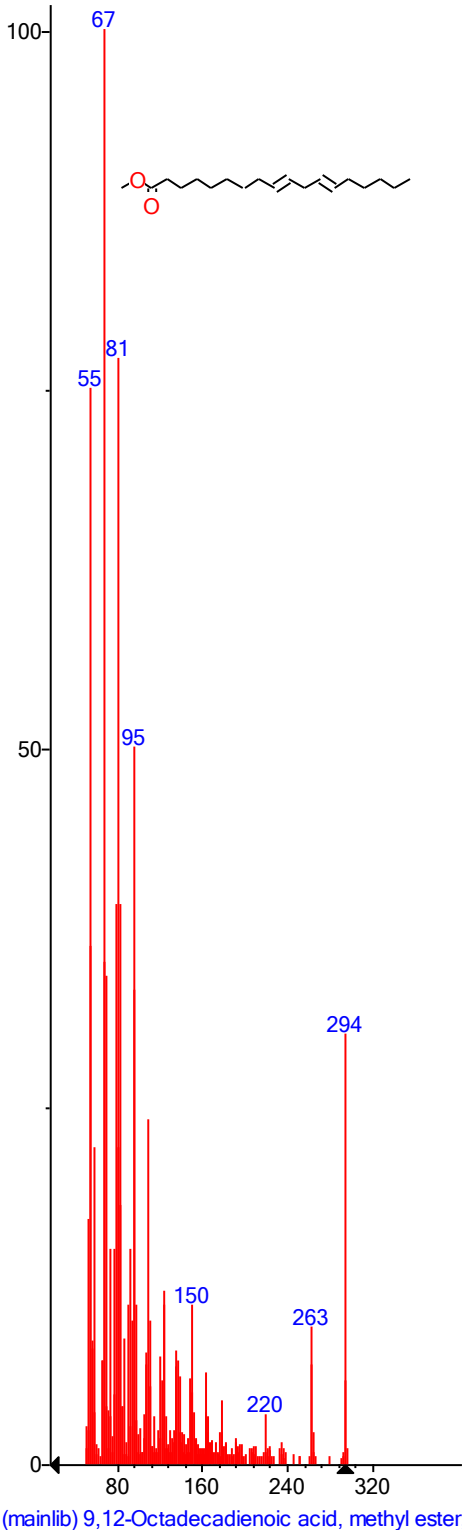

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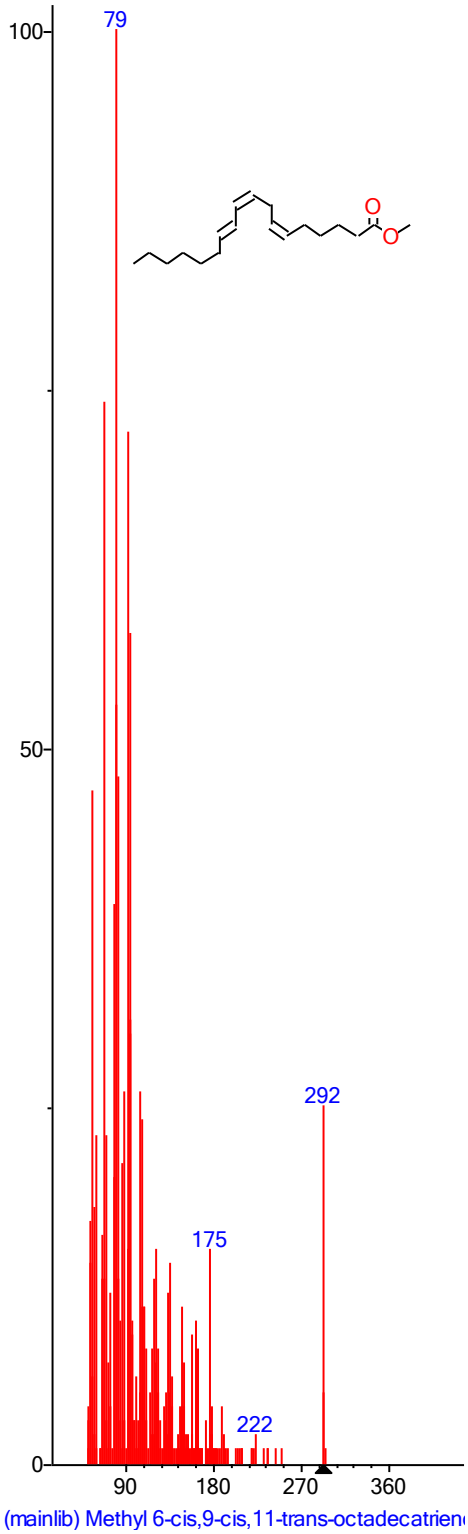

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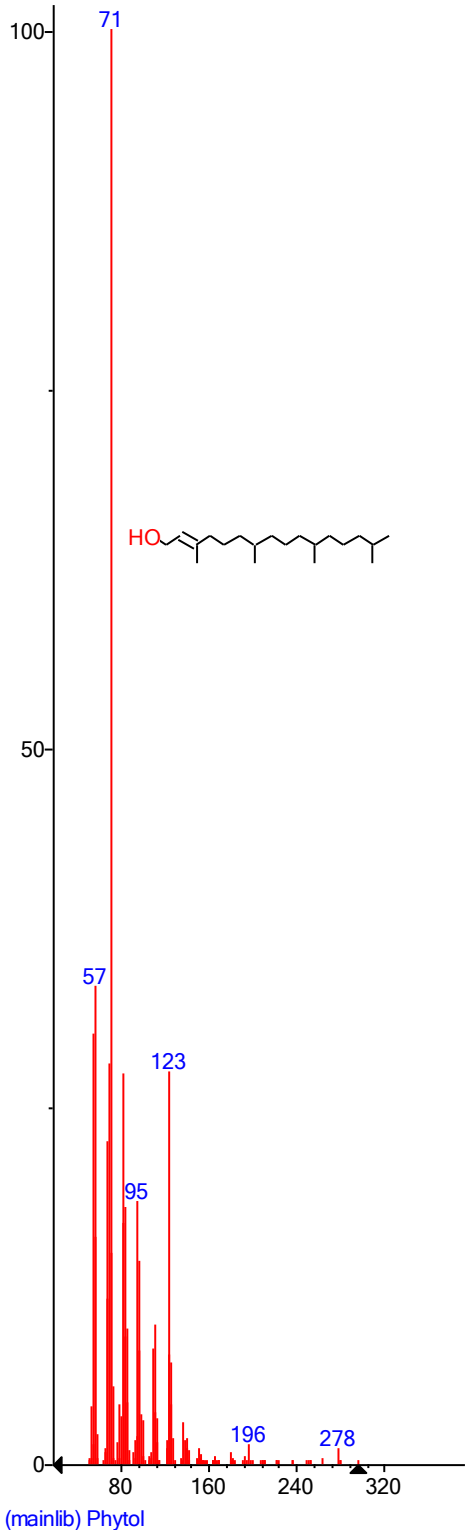
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240
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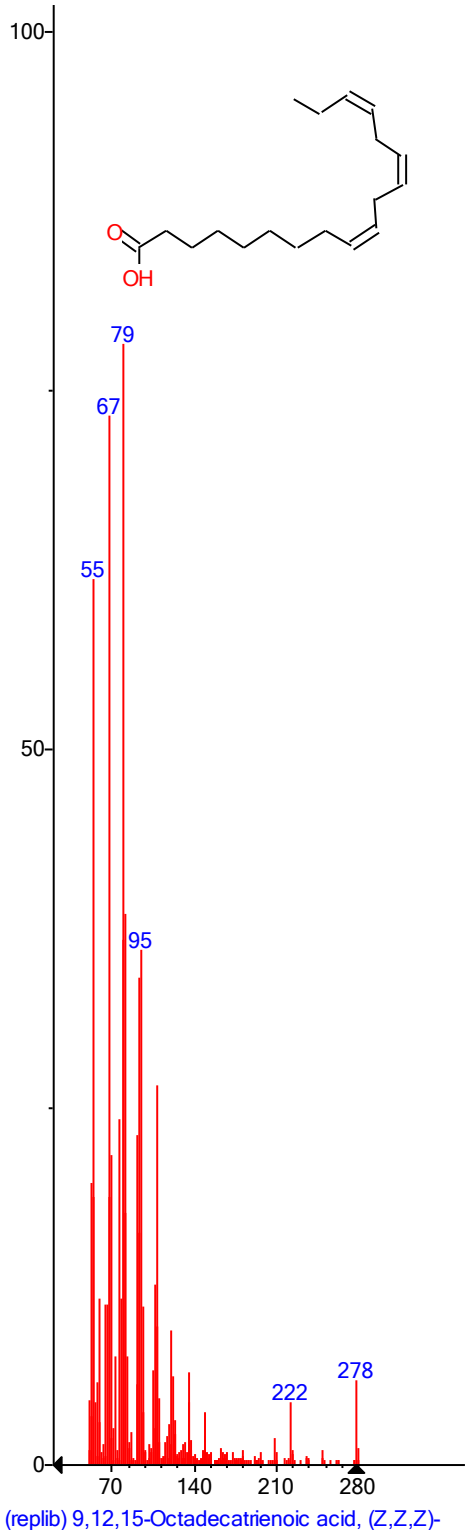

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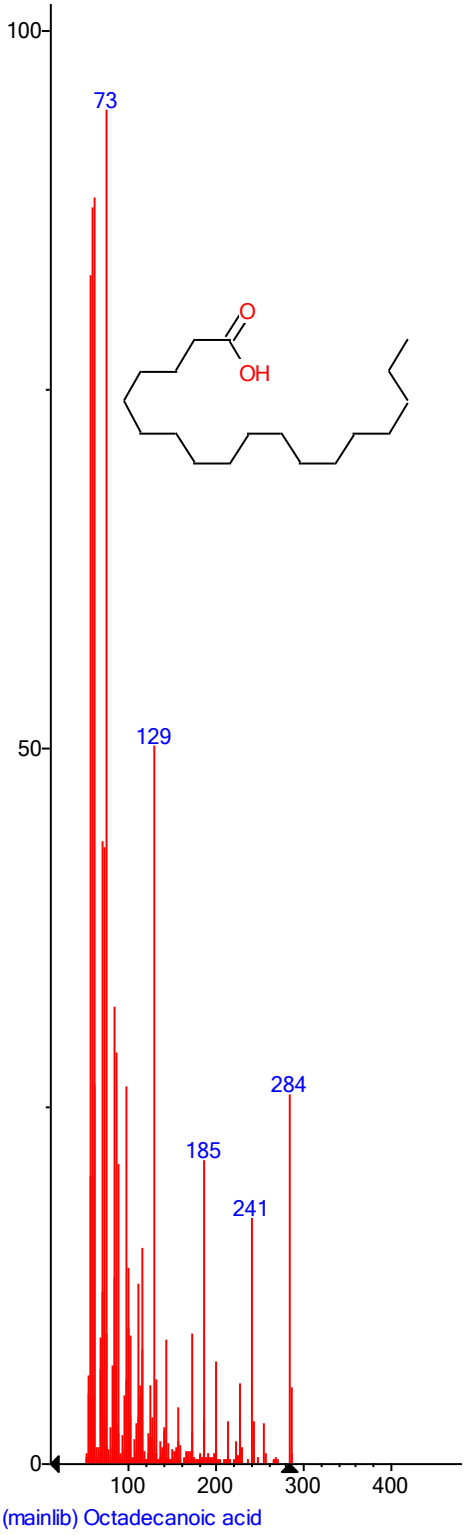

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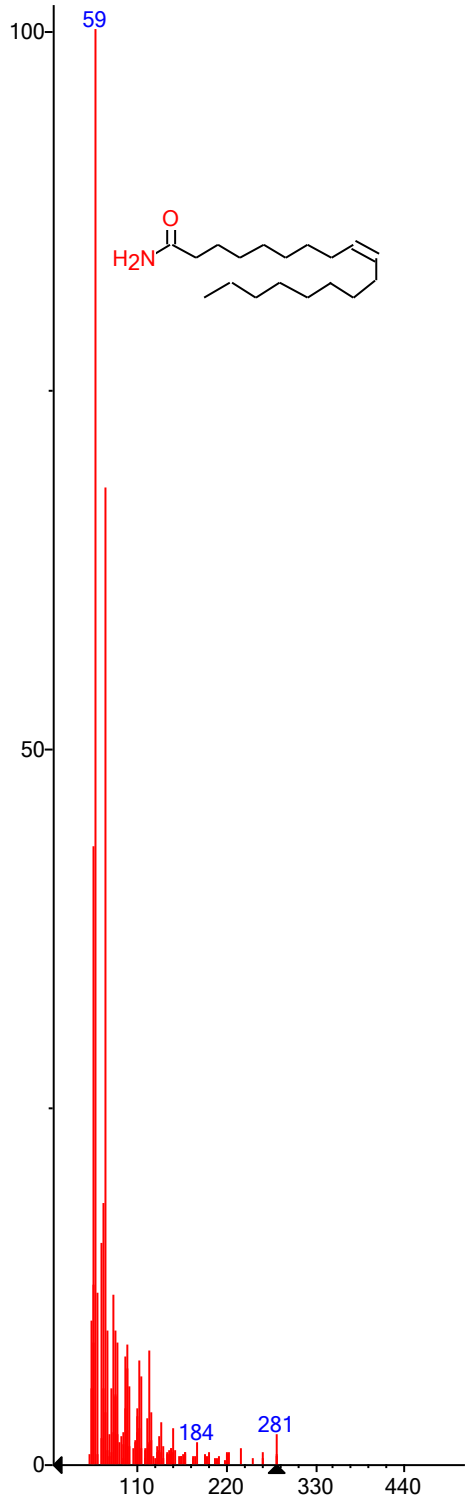

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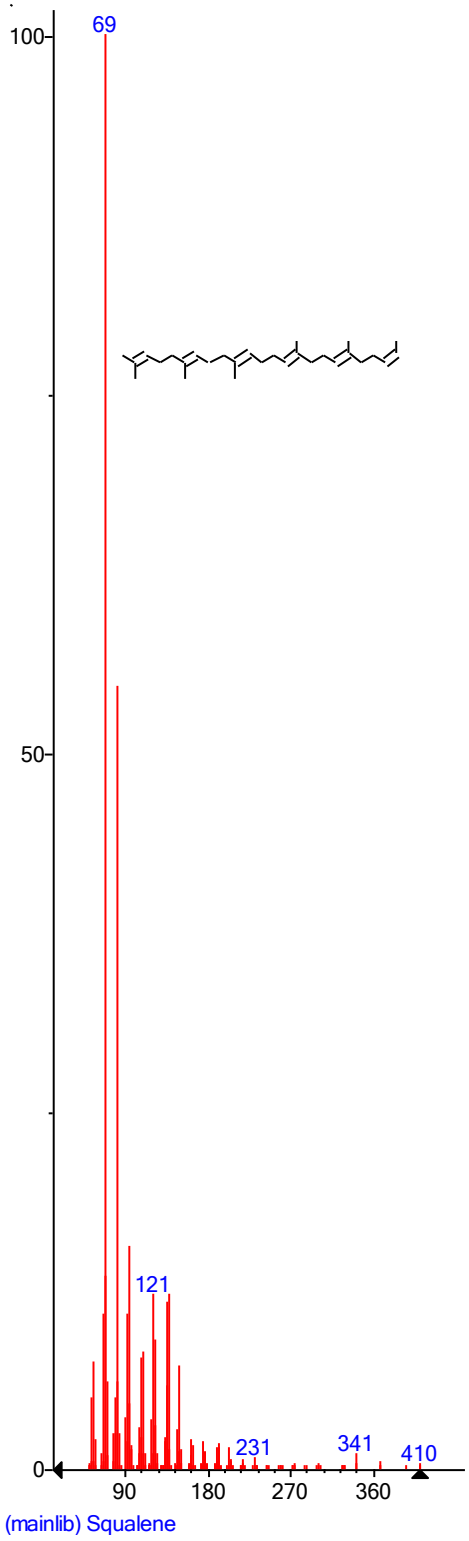
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50
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121
231
341
410
90
180
270
360
(mainlib) Squalene