UNIVERSITY OF GHANA

COLLEGE OF HEALTH SCIENCES

SCHOOL OF PHARMACY

DEPARTMENT OF PHARMACOLOGY & TOXICOLOGY

EFFECT OF CELLGEVITY®, A GLUTATHIONE-ENHANCER DIETARY SUPPLEMENT, ON STREPTOZOTOCIN-INDUCED TYPE-2 DIABETES AND NEPHROTOXICITY IN RAT

BY

BOADU AUGUSTINE ASARE (10344477)

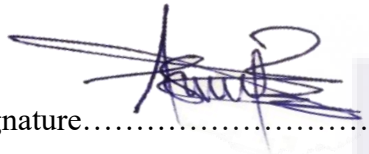
THIS THESIS/DISSERTATION IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF MPHIL IN PHARMACOLOGY DEGREE.

JULY, 2019

DECLARATION

DECLARATION BY CANDIDATE

I hereby declare that except for references to work of other researchers, which have been duly referenced, this project is the product of my own research carried out under supervision in accordance with regulations of the School of Graduate Studies, University of Ghana. I further declare that this dissertation has neither in whole nor in part been presented for another degree elsewhere, and that I am solely responsible for any residual flaws in this work.



Signature……

Date…29th April, 2020……..

Boadu Augustine Asare (10344477)

DECLARATION BY SUPERVISORS

We declare that the practical work and presentation of this thesis were supervised by us in accordance with guidelines on supervision of thesis laid down by the University of Ghana.

Principal supervisor:

Signature…  …… Date……07/05/2020……..……

Dr. Kwame Benoit Nguessan Banga

Co-supervisor:

Signature…  …… Date……07/05/2020…..……

Dr. Joseph Adusei Sarkodie

DEDICATION

This research is dedicated to the late Miss Constance Asare and Nana Opuni Mohammed for their immense contributions and encouragements towards this project.

UNIVERSITY OF GHANA

ACADEMIC AFFAIRS DIRECTORATE

RESIT REGISTRATION FORM

NAME: SARAH ACKAAH

INDEX NUMBER: 10746565 **PROGRAMME: DIPLOMA IN LIBRARIANSHIP**

NATIONALITY:

EMAIL: SACKAAH003@ST.UG.EDU.GH **TEL NO:**

COURSE CODE(S)	CREDITS
INFS005 - PRESERVATION OF DOCUMENTARY MATERIALS	3

Total Amount GHC: 105

Date: November 20, 2019

TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

PAGE

LIST OF ABBREVATIONS

ACR ……,,,,,,……..Albumin- to – creatinine ratio

ADA……...……...American Diabetes Association

b.wt ……..…….. Body weight

CCr ……..……..Creatinine Clearance

g/L…… Gram per liter

GBM ……..…Glomerular Basement Membrane Thickening

GFR……..……..……..Glomerular Filtration Rate

GLU……..……Glucose

HbA1c……..……...Glycated haemoglobin

HDL……..…..High Density Lipoprotein

HRP……..…..Horseradish Peroxidase

HSL……..……Hormone sensitive lipase

IDA……..….International Diabetes Association

kg/m^2……Kilogram per meter square

LDL……..……...…Low Density Lipoprotein

mg/kg……...……. Milligram per kilogram

mL.....……..……...Milliliters

mmol/L……..……Millimole per liter

NDMRC……..…National Diabetes Management and Research Center

^{o}C……..……..…Degree Celsius

%.....Percentage

PKA……..……Protein kinase A

PKC……..……Protein kinase C

SD……...…….Standard Deviation

STZ-NA ……Streptozotocin-nicotinamide

T1DM……..…….Type one diabetes mellitus

T2DM……..…….Type two diabetes mellitus

T.CHOL…….…...……Total Cholesterol

TRIG…….…….....…….…….Triglyceride

T.PROT……..…….……Total Protein

UK……...……..….United Kingdom

WHO……..……..……World Health Organization

µL……...……....Microliters

β-cell……..……beta cell (pancreatic)

CDC…….……Centre for Disease Control

cAMP ……..…..….Cyclic adenosintriphosphate

DKA……..……...Diabetes ketoacidosis

DM……Diabetes Mellitus

DN ……..……....Diabetic Nephropathy

EDTA……..Ethylene diamine tetra-acetic acid

FBG……..…Fasting Blood Glucose

FFA……Free fatty acid

Fig……..Figure

ABSTRACT

Background: Diabetic nephropathy (DN), a diabetes-induced nerve damaging effect on the kidney structure and function due to hyperglycaemia is a major microvascular complication. Hyperglycaemia causes uremia, hypercreatininemia, declined glomerular filtration rate, and decreased levels of serum protein and albumin. This condition leads to progressive decline in renal function resulting in renal insufficiency and End-Stage Renal Disease. The prevalence of DN in Africa has increased significantly in recent years, emphasising the importance of developing new preventive and treatment therapies. Despite the fact that there are different medications used to manage DN, most of these drugs are associated with serious unwanted side effects that contributes to the many complications observed in DN patients. This has paved way for the search for newer and better therapeutic agents that can better manage the disease and, at the same time, causing very less side effects. It was hypothesised that Cellgevity® could provide protection from diabetes and its complications.

Aim: To evaluate the hypoglycaemic and nephroprotective potentials of Cellgevity® in healthy and type-2 diabetic nephropathy rat models.

Methods: Seventy (70) male Sprague-Dawley rats with an average weight of 200 g were grouped into10 groups of seven rats each (n = 7). All rats were subjected to an overnight fast for 12 hours after which type-2 diabetes mellitus (T2DM) was induced using streptozotocin (STZ) (60 mg/kg b.wt) and nicotinamide (NA) (110 mg/kg b.wt). After 24-hours of STZ-NA injection, rats with fasting blood glucose (FBG) greater than 11.1 mmol/L were considered diabetic and divided into 8 groups. Group 1 of the diabetic rats received distilled water (diabetic negative control). Groups 2, 3, and 4 were orally administered with varying doses of Cellgevity® (40 mg/kg b.wt; 80 mg/kg b.wt; 160 mg/kg b.wt respectively); Group 5 and 6

received 15 mg/kg b.wt and 20 mg/kg b.wt of glibenclamide (Glib) and captopril (Cap) respectively. Group 7 and 8 of the diabetic rats received combinations of either Cellgevity® and Glib or Glib and Cap. Two additional groups (non-diabetic rats) served as normal controls. Blood samples were taken by tail snip and FBG were measured at specific days (1, 3, 6, 9, 12, 15, 22 and 29) following induction of T2DM.

At the end of the experiment (29th day), the animals were sacrificed, and their blood, kidneys and pancreas harvested for haematological, biochemical, and histological studies.

Results: Diabetic rats showed a significant increase in FBG (30.33 mmol/L, $p<0.001$), serum creatinine (81.20%) and urea (110%) with a corresponding decrease in total protein (47.94%) and albumin (75.46%) when compared to the normal control rats. The Cellgevity® doses, 40 mg/kg b.wt and 80 mg/kg b.wt significantly decreased ($p<0.001$) FBG from 30.33 to 17.33±3.69 mmol/L and 17.35±9.07 mmol/L respectively. The Glib dose (15 mg/kg b.wt) reduced levels of FBG from 30.33 to 9.85±3.96 mmol/L within the 28-day treatment period. Also, there was a significant increase ($p<0.01$) in serum albumin (ALB, 176.52%), total proteins (T.PROT, 79.13%) and white blood cell count (WBC, 154.98%) in diabetic rats administered with Cellgevity® when compared to the diabetic control rats. Serum ALB levels in diabetic rats administered with single therapy of Glib and combined form (Glib plus Cap) increased significantly ($p<0.01$) by 109.30% and 210.83% respectively in comparison with the diabetic control rats. Furthermore, Glib and Glib plus Cap markedly increased T.PROT by 27.10% and 61.87% respectively when compared to the diabetic control. Administration of Cellgevity® (low dose) resulted in 68.78% and 55.87% decrease in serum CREA and urea respectively. Similarly, the groups that were administered with Glib and Glib plus Cap had a

decrease in serum CREA by 56.61% and 58.69% respectively in comparison with diabetic control rats.

Kidney histological sections (40X) showed noticeable alterations (shrank glomeruli tuft, ballooned bowman space) in the diabetic control rat in comparison with the diabetic positive control rats.

Conclusion: The study demonstrated that administration of Cellgevity® (40, 80 mg/kg b.wt) reduces FBG level and protects against nephrotoxicity in STZ-NA induced type-2 diabetic rats. This study could justify the use of Cellgevity® in the management of nephropathy in diabetic patients. Further study (clinical trial) should be conducted in humans to confirm this finding.

CHAPTER ONE

INTRODUCTION

1.1 Background

The American Diabetes Association (ADA) (2012) defined Diabetes Mellitus (DM) as a Non-Communicable Disease (NCD) characterised by high blood glucose levels and impaired carbohydrates, lipids, and proteins metabolism caused by insulin deficiency, insulin resistance or both. Lowy and Williams (1967) were the first authors who reported anomaly in the function of the pancreas in it production of insulin, as a major contributory factor to the development of diabetes mellitus. Insulin is responsible for blood glucose regulation of fat and carbohydrate metabolism. Under normal condition, pancreatic insulin release correlates with the blood glucose level in the body (Grodsky *et al*., 1963).

Primarily, there are two main forms of DM, type 1 diabetes mellitus (caused as a result of auto-immune destruction of the pancreatic beta cells) and type-2 diabetes mellitus (caused as a result of the body's inability to respond to insulin produced in the blood) (WHO, 2018). Type-2 diabetes mellitus (T2DM) is the most predominant form of DM (Tripathi & Srivastava, 2006). In T2DM, there is deficient action of insulin on target tissues that brings about elevated blood glucose level, antioxidants decrease and abnormal glucose homeostasis (Hink *et al*., 2001; Jay *et al*., 2006).

Hyperglycaemia is associated with increased urination (polyuria), increased thirst (polydipsia), weight loss, increased serum creatinine (hypercreatininemia), uremia, sometimes with increased hunger (polyphagia), and decreased serum albumin and proteins.

For the past three decades, people with diabetes mellitus have increased fourfold and it is reported to be the ninth major cause of death worldwide (WHO, 2017). In one study, it was reported that, about 1 in 11 adults (18 years and above) have diabetes mellitus with 90% to 95% of all diabetic patients having T2DM (Tripathi & Srivastava, 2006). In another study, it was documented that T2DM is expected to rise to about 439 million people by 2030 (Chen *et al*., 2011). Zheng *et al*. (2018) reported that Africa and Asia are the two major areas with rapid emergence of the disease, with China and India being the top two epicentres. In Ghana, the National Diabetes Association in 2016 revealed that not less than 4 million Ghanaians are currently suffering from diabetes with three out of every nine adults suffering from it across the country (NDA, 2016). The report further indicated that about 5, 000 deaths are recorded every year making it a life-threatening condition. Boateng (2007) also reported that the disease is the main cause of protracted ill-health in majority of the populace and pose a threat to about 50 percent of all Ghanaian patients with genetic predisposition.

Although, genetic constituent of an individual is a risk factor to the development of T2DM, delayed diagnosis, unhealthy diet, and sedentary lifestyles have been reported to be pivotal in the current global epidemic (Wu *et al*., 2014; ADA, 2017). T2DM patient may be predisposed to one or more vascular complications such as diabetic nephropathy (DN), renal insufficiency, early kidney failures, cardiovascular ailments, blindness, sexual impotence and gangrene of the feet which often leads to amputation (Deshpande *et al*., 2008; Wu *et al*., 2014). DN continues to be the leading cause of ESRD, and non-traumatic lower limb amputations; these tend to be the major cause of morbidity and mortality in diabetic patients globally (Cade, 2008; Dugbartey, 2017).

Free radicals play a major role in the pathogenesis of DM (Hayden and Tyag, 2002; Wiernsperger, 2003). Free radicals are naturally produced in the body by aerobic cells and their production increases under conditions of cell injury (Valko *et al*., 2007). It is exogenously produced via excessive smoking, alcohol intake, and sedentary lifestyle. Over generation of free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) cause oxidative stress and reduces antioxidants in the body (Kurutas, 2016). Oxidative stress was shown to initiate diabetes due to the fact that the B-cells on the pancreas have relatively low antioxidant potential (Lenzen *et al*., 1999). Formation of lipid peroxides by the action of free radicals on unsaturated fatty acids have been implicated in the pathogenesis of the disease. Several researches done on DM so far indicate that there is an increased state of oxidative stress beyond the usual levels in diabetic models due to hyperglycaemia (Kaeney & Loscalzo, 1999; Hayden & Tyag, 2002; Wiernsperger 2003; Ozbek 2012). ROS play an important role in the signalling pathways and is a key determinant for cell survival. However, an excess of ROS formation causes cell damage and death (Le Bras *et al*., 2005). The irreversible cell damage, induced by free radicals is prevented by an adaptive response, consisting of a compensatory upregulation of antioxidants like GSH peroxidase, superoxide dismutase, and catalase, aimed at restoring the redox homeostasis (Landriscina *et al*., 2009). Sadowska-Bartosz & Bartosz (2014) reported that as one ages, there is a negative correlation with the amount of antioxidants in the body. A number of studies have also reported that, glutathione deficiency is implicated in T2DM patients (Whiting *et al*., 2008; Sundaram *et al*., 1996; Vijayalingam *et al*.,1996; Memisogullari *et al*., 2003). Several studies suggest that supplementing with glutathione precursors and acetyl glutathione, ameliorate adverse hyperglycaemic conditions in

patients via restoration of their glutathione, which consequently reduces free radicals generations and protect cells against oxidative damage (Fang *et al*., 2002; Dragan *et al*., 2015; Arbid, 2017).

Good glycaemic control has been shown in several studies to decrease the risk of microvascular complications (Di Landro *et al*., 1998; Molitch, 1997). It is evident that a good glycaemic control (one that brings blood glucose to normal without causing significant hypoglycaemia) plays a critical role in stabilizing and slowing nephropathy even with established renal damage (Giaccari *et al*., 2009). Currently, the main treatments for kidney disease caused by diabetes include the use of biguanides, sulphonylureas, Renin-Angiotensin-Aldosterone Inhibitors, and controlling hypertensive, hyperglycaemic and dyslipidemia disease conditions (Pálsson & Patel, 2014). Unfortunately, these drugs which are currently the most frequently prescribed oral antidiabetic drugs for diabetic nephropathy patients worldwide, seems to be associated with increased mortality and cardiovascular risk (Hong *et al*., 2013; Azimova *et al*., 2014). Other management strategies aside chemotherapy includes early diagnosis, lifestyle modifications, social support, and the use of dietary supplements (Shrivastava *et al*., 2013; Ofori & Unachukwu, 2014).

The use of dietary supplements and herbs is very common among type-2 diabetic patients globally due to the high cost and numerous side effects associated with the use of chemotherapy (Bastaki, 2005; Halat and Dennehy, 2003; Chang *et al*., 2013). Dietary supplements that are usually used contain vitamins, minerals, precursors of amino acids, fatty acids or other substances. These dietary supplements are present in the form of pills, tablets, capsules and liquids (Halsad, 2003; Kourkouta *et al*., 2016; Yarandi *et al*., 2011).

Glutathione dietary-enhancer supplements act as potent antioxidants that help to decrease ROS generation in cells (Poljšak & Fink, 2014). In Ghana, one good example of this dietary-enhancer supplement is Cellgevity® (a product of Max International). It is used to manage various diseases including diabetes and kidney diseases. Some of its constituents includes resveratrol, quercetin, grape seed extract, lipoic acid, and curcumin that have been reported to contain high antioxidant and antihyperglycaemic properties (Mozafari *et al*., 2015; Adam *et al*., 2016; Awodele *et al*., 2018). Several studies reported that dietary supplements replenish endogenous glutathione levels and reduces oxidative stress in animal models with T2DM (Awodele *et al*., 2018; Rodrigues & Percival, 2019; Straub *et al*., 2019). Although the antioxidant functions of Cellgevity is well established, with emphasis on its chemical composition, studies on this dietary supplement in type-2 diabetic nephropathy rat models is limited.

In the light of these findings, this research seeks to evaluate the hypoglycaemic and nephroprotective potentials of Cellgevity® in streptozotocin-nicotinamide induced type-2 diabetic rat models.

1.2 Problem statement

Type-2 diabetes mellitus is a major health concern that affect the lives of many people across the globe, and it is anticipated that every 1 in 11 adults has the disease (WHO, 2014). T2DM has been a high health burden to individuals, families and nations in terms of its life-threatening complications. It is currently the leading cause of ESRD, contributing to high morbidity and mortality in diabetic patients (Molitch *et al*., 2004; Gunzler *et al*.,

2013, Dugbartey, 2017). In Ghana, there are few renal centres for renal replacement therapy due to the capital-intensive nature of renal units and machines. More so, kidney transplant which is also the main alternative for patients diagnosed with ESRD is very expensive to undergo, resulting often in an increased mortality. This emphasises the need for a more affordable agent for management of the disease, and at the same time has nephroprotective potential.

Hyperglycaemia which increases ROS generations in cells can also cause oxidative stress, and inflammation (Yin *et al*., 2015). Oxidative stress is a major molecular factor underlying the pathogenesis and progression of diabetic nephropathy (Kashihara *et al*., 2010; Loeffler, & Wolf, 2019).

Challenges associated with the use of current therapies in treating and managing DM, as well as the cost involved, make it necessary to explore other efficient, relatively cheaper alternatives with less side effects. Sulfphonylurea (example glibenclamide) used in the management of T2DM causes side effects such as hypoglycaemia and excessive weight gain with meglitinides causing headache, diarrhoea, nausea, vomiting, and bloating due to the build-up of gas (Lamos *et al*., 2013).

Previous studies conducted on Cellgevity focused on its antioxidant potentials on cells and organs with few data on its hypoglycaemic, antihyperglycaemic and protective effects on the kidney (Nagasawa, 2015; N'guessan *et al*., 2018; Awodele *et al*., 2018). The research sought to investigate the antidiabetic and nephroprotective potentials of Cellgevity®.

1.3 Justification

Several studies in animal models have shown that oral GSH, administered either in the diet or by gavage, increases GSH levels in plasma and tissue (Meister *et al*., 1991; Owen *et al*., 2010; Chung *et al*., 1991; Hayes *et al*., 2005; Huenchuguala *et al*., 2014). Other studies have shown promising results in the effectiveness of dietary supplements for the management of diseases induced by oxidative stress (Rodrigues & Percival, 2019; Straub *et al*., 2019; Chinaka *et al*., 2018). Oxidative stress has long been implicated in diabetes development and progression, suggesting that antioxidant treatment could provide protection from diabetes and its complications (Wright *et al*., 2006; Sasaki & Inoguchi, 2012; Feng *et al*., 2013; Bajaj & Khan, 2012; Wu, Tang, & Chen, 2014). However, there has not been any study to evaluate the hypoglycaemic and nephroprotective properties of Cellgevity®.

In recent times, metformin and glibenclamide which are currently the most frequently prescribed oral anti diabetic drug for managing DN seems to be associated with increased mortality and cardiovascular risk compared with dietary supplements (Hong *et al*., 2013; Azimova *et al*., 2014; Xu & Rajaratnam, 2017; Bronzato & Durante, 2018). Dietary supplements are globally acceptable adjunct therapies for NCDs emphasising on their natural modes of healing, coupled with less side effects (Cheung *et al*., 2007; Afolayan & Wintola, 2014). Several studies have shown the use of dietary supplements for the managements of NCDs (Afolayan & Wintola, 2014; Siddharthan *et al*., 2015; Forouhi *et al*., 2018). However, not all dietary antioxidant supplements have nephroprotective effects

(Poljšak & Fink, 2014), thus the findings from this study will throw more insight on the antidiabetic and nephroprotective activities of Cellgevity®.

1.4 Hypothesis

Cellgevity®, a glutathione-enhancer dietary supplement will reduce hyperglycaemia and protect against STZ-induced diabetic nephropathy via its antioxidant property.

1.5 Aim

The research is aimed at evaluating the hypoglycaemic and nephroprotective effects of Cellgevity® in a rat model of type-2 diabetic nephropathy.

1.6 Specific objectives

1. To determine the effect of varying doses (low, medium, and high) of Cellgevity® on fasting blood glucose in STZ/NA-induced diabetic nephropathy rat models.
2. To evaluate and compare the hypoglycaemic activity (acute and subacute phases) of Cellgevity® in combination with some selected conventional drugs in STZ/NA-induced type-2 diabetic nephropathy rat models.
3. To determine the nephroprotective activity of Cellgevity® in STZ/NA-induced type-2 diabetic nephropathy rat models.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Glucose Metabolism and Endocrine Actions

Glucose is a fundamental metabolic substrate of all mammalian cells used as a cellular source of energy in tissues (muscle, adispose) and organs (brain) (Rui, 2014). Skeletal muscle accounts for approximately seventy-five percent (~75%) of total body insulin-stimulated glucose uptake, malfunctions in this tissue may play a key role in glucose regulation (Bjornholm & Zierath, 2005; Honka *et al*., 2018). There are two main sources of glucose namely exogenous and endogenous. The exogenous carbohydrate presented to the cell for energy production is D-glucose whereas endogenous glucose is obtained from the liver and kidneys via glycogenolysis (breakdown of glycogen to glucose) and gluconeogenesis (synthesis of glucose) (Szablewski, 2011; Triplitt, 2012; Han *et al*., 2016).

Glucose is transversely transported through the walls of the intestine to the hepatic portal vein and then to liver cells and other tissues. The liver cells convert glucose to glycogen and then released later during starvation (glycogenolysis). Organs such as the brain and tissues use the body's glucose as source of energy. The amount of glucose in the blood needs to be regulated as low blood concentration (hypoglycaemia) can cause seizures, loss of consciousness, and death whilst long lasting elevation of blood glucose concentration (hyperglycaemia) can result in blindness, renal failure, nephropathy, neuropathy, and vascular complications (Szablewski, 2011). Therefore, the concentrations of blood glucose need to be regulated and sustained at a steady-state level, a process known as glucose homeostasis (DeFronzo, 1988). The normal fasting blood glucose (FBG) and post prandial

blood glucose (PBG) level ranges for humans are 4.4 – 6.1 mmol/L and at most 7.8 mmol/L respectively. However, Wang *et al*. (2010) reported that Sprague-Dawley rats have normal FBG and PBG values of 3.95 – 5.65 mmol/L and 5.69 - 6.61 mmol/L respectively.

2.1.1 Mechanisms of glucose homeostasis / hormonal interplays

Blood glucose homeostasis is accomplished through hormone-mediated peripheral glucose uptake by muscles, adipocytes, hepatic cells, and the loss of glucose via kidney tubules (Szablewski, 2011; Röder *et al*., 2016). Other mechanism of glucose regulation is by hepatic glucose production (gluconeogenesis) and glycogenolysis (Fig.2.1). Insulin and glucagon are the two major hormones secreted by the body to regulate blood glucose levels. These two hormones are antagonistic in function (Aronoff *et al*., 2004) and are released into the blood at different threshold (Gerich, 1993).

During periods of elevated blood glucose (≥ 5 mmol/L), the β-cells of the pancreatic islets of Langerhans secrete more insulin into the blood that counter a number of hyperglycaemia-generating hormones (glucagon) to maintain low blood glucose levels (Szablewski, 2011). Insulin can suppress hepatic glucose production by inhibiting glycogenolysis and gluconeogenesis (Szablewski, 2011). Conversely, periods of hypoglycaemia (≤ 3 mmol/L) stimulate the α-cells of the pancreas to secrete glucagon into the blood. Glucagon increases blood glucose by stimulating hepatic glucose production via glycogenolysis and gluconeogenesis (Triplitt, 2012). Once any of these two hormones is secreted into the blood, glucose molecules can enter the cells either through the transmembrane channel transporters (glucose transporters) by facilitated diffusion or by secondary active transport via sodium glucose transporters, and Na^+/K^+ ion pump

transporters (using ATP). There are ten subtypes of glucose transporters (GLUT); GLUT 1 and GLUT 3 located in all cells; GLUT 2 in pancreatic beta cells, and GLUT 4 on skeletal muscles as well as adipocytes (Navale & Paranjape, 2016).

Amylin and incretins are other two hormones known to regulate blood glucose concentrations (Triplitt, 2012). Amylin contributes to reduction in postprandial glucagon levels whilst incretin aids in stimulation of insulin during elevated blood glucose levels and activate G-protein coupled receptors in plasma membranes of β-cells to promote insulin secretion. When mechanisms such as those discussed above become impaired, the individual stand to be at high risk of chronic hyperglycaemia.

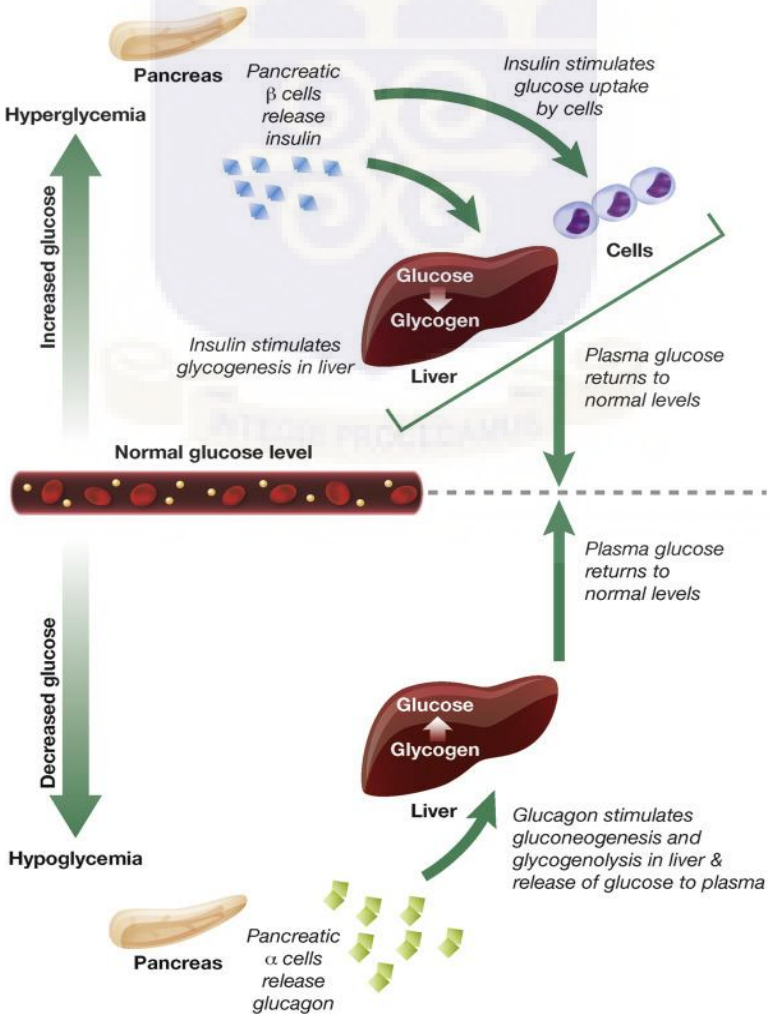


Figure 2.1: Regulation of glucose homeostasis

Source: https://ars.els-cdn.com/content/image

2.2 Diabetes Mellitus and classifications

Diabetes mellitus (DM) is one of the clinical signs of impaired glucose regulation in the body (defects in insulin secretion, insulin action, or both) leading to long-term chronic hyperglycaemia, usually involving multiple organs and hormonal pathways (ADA, 2009). Diabetic mellitus patients usually present with polyuria (increased urination), polydipsia (increased thirst), polyphagia (increased hunger) and unexplained weight loss with elevated blood glucose concentrations (WHO, 2014).

There are two main forms of DM, type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). In type 1 diabetes mellitus (T1DM), the pancreas fails to produce enough insulin into the blood to transport the glucose through the glucose transporters (GLUT-2, GLUT-4) (Macfarlane *et al*., 1997; Charron *et al*., 1990). T2DM is caused as a result of the body's inability to respond to insulin produced in the blood. It is the most predominant form of DM with 90% to 95% of all diabetic patients having T2DM (Tripathi & Srivastava, 2006). T2DM is expected to increase to about 439 million people by 2030 (Chen *et al*., 2011), and may lead to the onset of nephropathy which increases the risks of fatal vascular events (Pálsson & Patel, 2014).

T2DM is linked with both microvascular and macrovascular complications affecting multiple organs. Nephropathy, neuropathy, retinopathy, (microvascular) and cerebrovascular disease, peripheral vascular disease, ischemic heart disease, being macrovascular. The leading cause of mortality and morbidity in type-2 diabetic patients is attributed to vascular complications and this is linked to its anatomical, structural, and functional changes in implicated organs (Deshpande *et al*., 2008; Rahman *et al*., 2007).

2.3 Diabetic Nephropathy

Diabetic nephropathy (DN) is a protracted microvascular complication of type-1 and type-2 diabetes mellitus and is mainly attributed to chronic hyperglycaemia-mediated oxidative stress. Long-lasting peripheral blood glucose plays a vital role in the aetiology of DN, and this is attributable to the many metabolic and structural derangements, including the production of advanced glycation end products (AGE), abnormal activation of signalling cascades (p38 MAPK), over generation of reactive oxygen species (ROS) and abnormal stimulation of hemodynamic regulation systems (like the renin-angiotensin system [RAS]) (Cade, 2008). It is either characterised by persistent albuminuria ($> 300\ mg/d$) that is confirmed on at least two occasions, three to six months apart or a progressive decline in the glomerular filtration rate (GFR) accompanied by increased blood pressure (Vecihi, 2018).

Some associated risk factors include hyperglycaemia, age of onset, late diagnosis, dyslipidemia, hypertension, tobacco use, and obesity (Table 1.1) (Hasslacher *et al*., 1989). Patients with DN usually manifest systemic symptoms and signs of impaired renal function such as oedema, fluid overload, electrolyte abnormalities, high serum creatinine and blood urea nitrogen (McPhee *et al*., 2006).

2.3.1 Epidemiology of diabetic nephropathy

According to Harjutsalo & Groop (2014), almost half of patients with diabetes mellitus develops DN. Patients diagnosed with the disease have renal manifestations such as abnormalities in urine volume or composition (for example RBCs, ketones, and proteins in

urine). DN is linked with high mortality rate, ESRD, fluid overload, electrolyte abnormalities, high serum creatinine, dyslipidemia (i.e, raised serum LDL, TG, and low HDL levels), high blood urea nitrogen, anaemia, cardiovascular diseases, and escalating health-care costs due to the need for renal replacement therapy (dialysis and kidney transplantation) (McPhee *et al*., 2006; Harjutsalo & Groop, 2014).

Diabetic nephropathy affects both males and females equally and it is projected that about 11 % of the adult population globally has diabetic nephropathy that leads to chronic kidney diseases (CKD) (Coresh *et al*., 2003; Hill *et al*., 2016). It usually affects patients who have had diabetes for a period of 10-20 years. Pavkov *et al*. (2006) reported that the mean age of patients who reach ESRD is about 60 years. There is high incidence in the rise of the disease due to diseases such as diabetes mellitus, hypertension, and glomerular disorders such as focal segmental glomerulosclerosis (Rosenberg & Kopp, 2017). Black people, Hispanic people, and those with blood tides to patients who have a diagnosis of kidney disease stand a higher prevalence than the overall populace (Freedman *et al*., 1997). Moreover, individuals with incidence of acute kidney injury are most likely to be at risk for long-lasting kidney injury and ESRD in the future (Coca, 2012).

In Ghana, the prevalence of DN is gradually increasing at a fast pace along with the diabetes epidemic due to factors like obesity, population growth, urbanization, ageing, and sedentary lifestyles (lack of exercise, consumption of monosodium salts and high fat diet).

2.3.2 Pathophysiology of DN

2.3.2.1 Role of hyperglycaemia in DN pathophysiology

The key pathophysiologic determinant for DN are basement membrane damage, shrinking of the glomeruli tuft, and ballooning of the bowman'space (Fig. 2.3) due to mesangial expansion directly linked to hyperglycemia (Sabbatini *et al*., 1992; Crowley *et al*., 1991). Hyperglycaemia plays a crucial role in the development of DN because of its effects on glomerular and mesangial cells as well as tubule in the kidney (Thomas *et al*., 2005; Dronavalli *et al*., 2008; Vallon, 2011). This damage occurs as a result of a non-enzymatic glycosylation of plasma and glomerulus basement membrane proteins bringing about pathologic change in mesangial and vascular cells, formation of AGEs, accumulation of polyols via the aldose reductase pathway, and activation of protein kinase C. The figure (2.2) below is a schematic diagram for the pathogenesis of diabetic nephropathy.

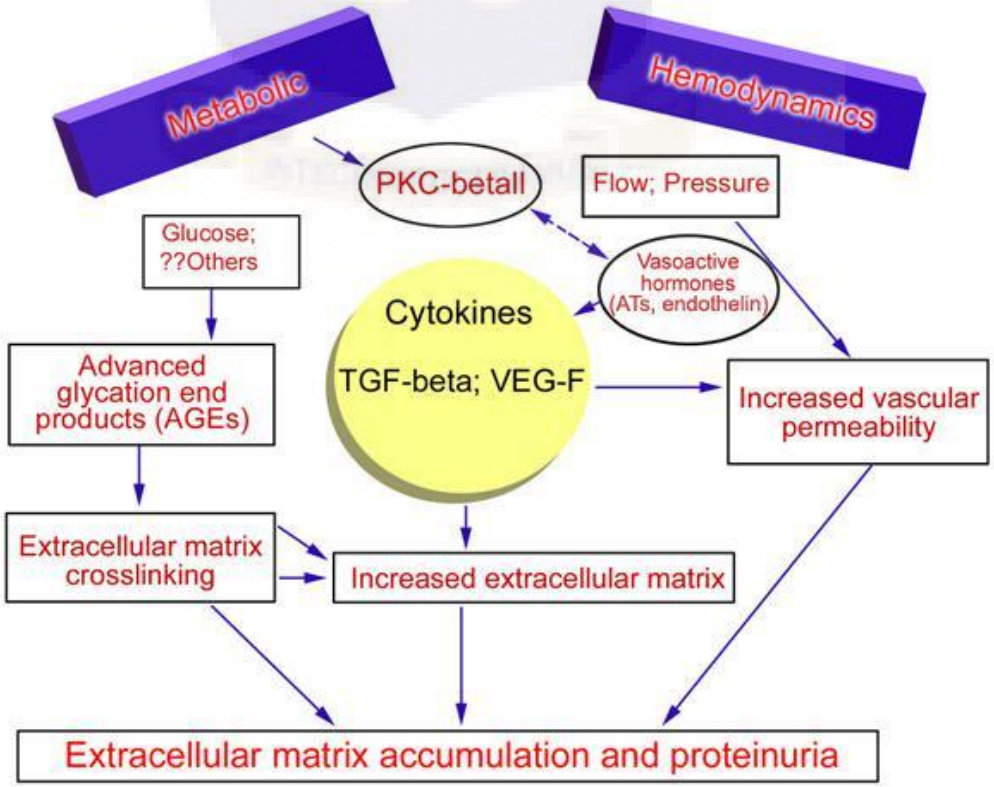


Figure 2.2: Hyperglycaemia-induced metabolic and haemodynamic changes leading to PKC activation ***Source: medscapestatic.com***

Within the glomerulus are endothelial cells which become affected by hyperglycaemia and that causes anomaly in blood flow (Brownlee, 2005). This phenomenon reduces the number of vasodilators such as nitric oxide and reversely increases the production of endothelin-1 and angiotensin II, which are potent vasoconstrictors (Brownlee, 2001). This brings about glomerular hyperfiltration. Glomerular hyperfiltration is an early physiologic abnormality coupled with the onset of microalbuminuria, the earliest practical sign of renal involvement in diabetes (Pourghasem *et al*., 2015).

To add with, intracellular hyperglycaemia mediates increased vascular permeability leading to over-expression of vascular endothelial growth factor (VEGF). Viollet *et al*., (2012) reported that, the expression of VEGF due to hyperglycaemia-mediated actions upregulate reactive oxygen species production in podocytes that markedly changes glucose levels in cells of the proximal tubules due to decrease glucose transport. High blood glucose affects the glomerular filtrate and output and thereby increasing glucose load within the tubules (Vallon, 2011). Later stages may also be accompanied by clinically significant albuminuria and or proteinuria, oedema, and nephrotic syndrome. Ultimately, the distinctive clinical picture of renal failure set in (Pourghasem *et al*., 2015).

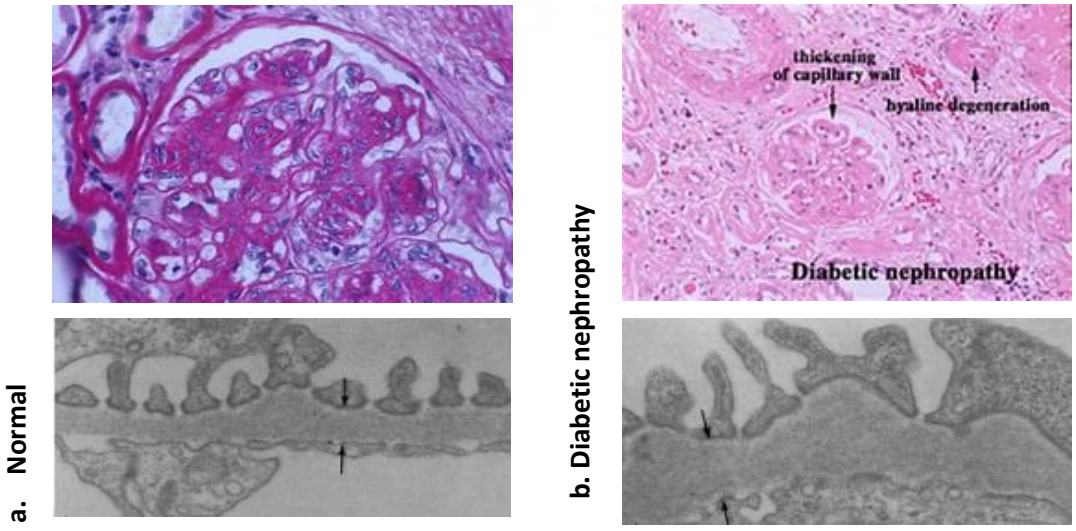


Figure 2.3: Morphometry of capillary basement membrane thickness in normal and diabetic nephropathy rat ***Source: https://tse4.mm.bing.net***

2.3.2.2 The role of renin angiotensin-aldosterone system in DN pathophysiology

The renin angiotensin-aldosterone system (RAAS) also called renin angiotensin system (RAS) is known to be implicated in the pathophysiological changes linked with diabetic nephropathy due to the production of angiotensin II (AII) (Wolf, 2004; Ziyadeh & Wol, 2008). It plays an important mechanism in regulating physiological blood pressure (BP); fluid and electrolyte balance through coordinated effects on the heart, blood vessels, and Kidneys. It is reported to mediate cardiovascular and renal remodelling (Wiecek *et al*., 2003; Hostettert, 2004).

AII, the main effector of the RAS (Aros & Remuzzi, 2002) is a potent vasoconstrictor on the post glomerular arterioles which increases the glomerular hydraulic pressure as well as the ultrafiltration of plasma proteins (Yoshiokat *et al*., 1987). This effect is reported to cause the onset and progression of kidney damage (Remuzzi & Bertani, 1998). AII causes haemodynamic changes (Fig. 2.6) by releasing growth factors or cytokines that activate secondary messengers which may lead to the over production of reactive oxygen species (ROS) in cells, cause inflammation that have damaging effects on podocytes, etcetera (Ruiz *et al*., 2001; Wolf, 2004).

Over-production of AII is linked with increased glomeruli permeability as well as interstitial fibrosis (Wiecek *et al*., 2003) (Fig.2.4). Interventions that restrain the activity of the RAS are renoprotective and may slow or even stop the progression of chronic nephropathies (Anderson *et al*., 1986; Zojac *et al*., 1998). Angiotensin converting enzyme (ACE) inhibitors and AII receptor antagonists can be used in combination to maximize RAS inhibition and more effectively reduce proteinuria and GFR decline in diabetic and

nondiabetic renal disease. Recent evidence suggests that, the addition of aldosterone antagonist in therapy may further increase renoprotection, but may also enhance the risk hyperkalemia (Anderson *et al*., 1993; Zojac *et al*., 1998).

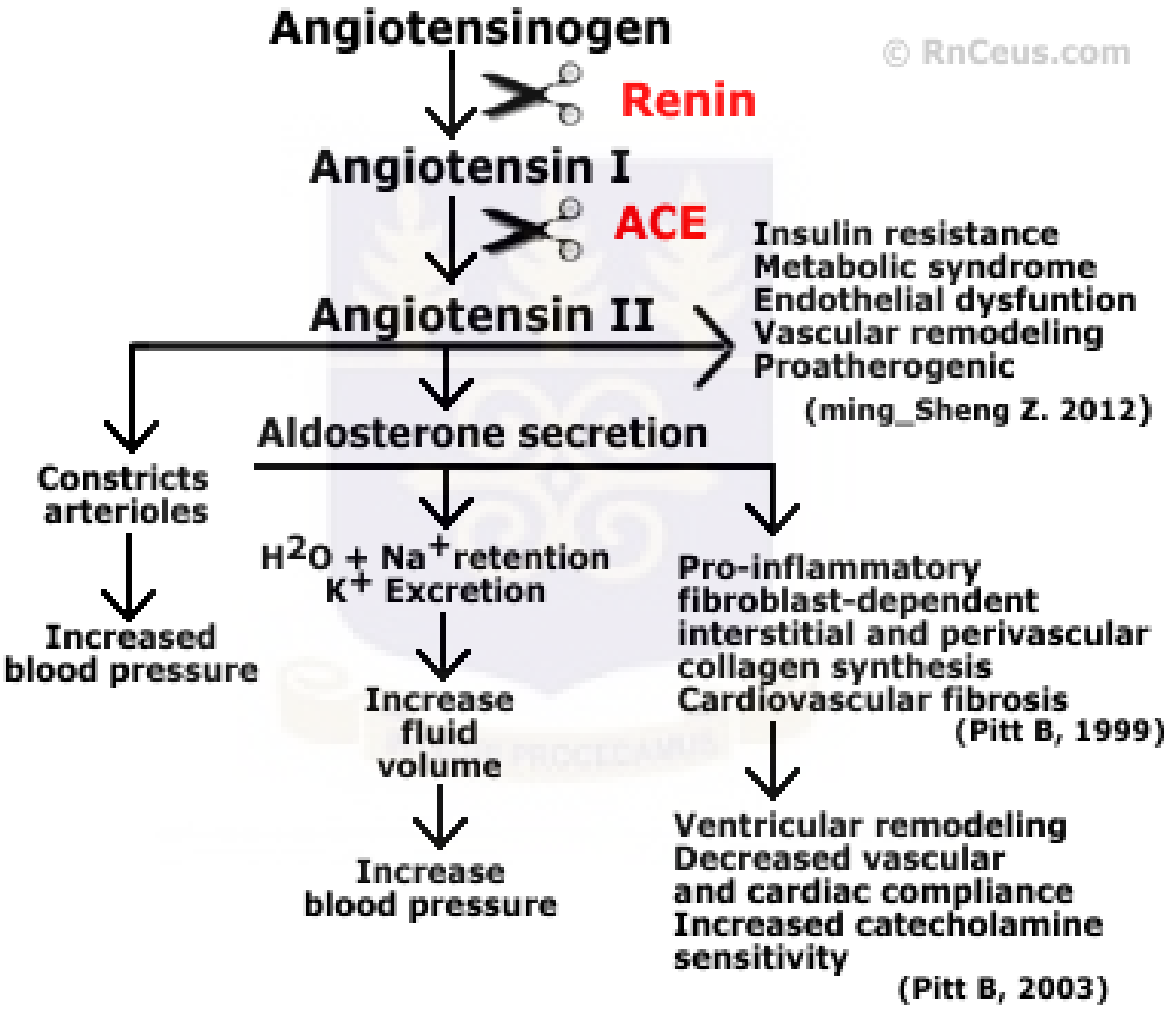


Figure 2.4: Renin-angiotensin system depicting the effect of angiotensin II
Source: RnCeus.com

2.3.3 Aetiology of DN and implicated signalling pathways

The exact cause of diabetic nephropathy is unknown, nonetheless, it is well established that poor metabolic control of glucose in blood is critical in the aetiology of DN. Hyperglycaemia leads to glomeruli hyperfiltration, renal injury or damage, advanced glycation products, and activation of cytokines (Fig. 2.5).

Hyperglycaemia leads to the activation of several biological mediators or enzymes (e.g. AGEs, PKC, PP) that triggers downstream cascade activities resulting in the overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS) that have a damaging effect on endothelial cells (Fig. 2.6).

Nitrogen species and oxygen species play a pivotal role in the pathology of diabetes and diabetic complications (Negi *et al*., 2011). It's also evident that increase in blood glucose brings about both metabolic and haemodynamic changes in biological tissues (Fig. 2.6). This is so because elevated blood glucose produces free radicals from autoxidation of glucose and glycosylation of proteins (Al-Faris *et al*., 2010). Increased levels of redox imbalance lead to a decrease in antioxidant activity resulting to high oxidative stress that can cause insulin resistance, endothelial cell dysfunction, and alterations in pancreatic β cells. This eventually leads to the development and progression of diabetic microvascular and macrovascular complications (Bandeira *et al*., 2013).

A study has demonstrated that ROS and RNS induce multiple cellular downstream cascade activities (Fig. 2.7) that ultimately lead to the transcription of stress-related genes which promote the development of diabetic complications (Ayepola *et al*., 2013). For

example, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), a nuclear transcription factor, becomes activated when ROS are in excess. Activation of NF-κB results in the transcription of pro-inflammatory proteins that intensifies the conditions of the disease. Pro-inflammatory chemokines and cytokines like macrophage chemotactic protein (MCP-1), tumor necrosis factor (TNF-α), and interleukins (IL-1β and 6) have been implicated in the progression of diabetes nephropathy complications (Ayepola *et al*., 2013).

When there is insulin resistance, the pancreas is forced to increase its insulin output, which put stress on the β cells, ultimately resulting in β-cell enervation. Hyperglycaemia and high levels of saturated fatty acids create an inflammatory medium, resulting in the activation of the innate immune system, which causes the release of inflammatory mediators such as, interleukin (IL)–1β and tumour necrosis factor (TNF)–α. These inflammatory mediators promote systemic insulin resistance and β-cell damage (autoimmune insulitis). Hyperglycaemia and high serum levels of free fatty acids and IL-1 lead to glucotoxicity, lipotoxicity, and IL-1 toxicity, resulting in apoptotic β-cell death (Deshpande *et al*., 2013).

Hyperglycaemia increases the expression of transforming growth factor-β (TGF-β) in the glomeruli and of matrix proteins, specifically stimulated by this cytokine. TGF-β and vascular endothelial growth factor (VEGF) may contribute to the cellular hypertrophy and enhanced collagen synthesis. This may induce the vascular changes observed in persons with diabetic nephropathy (Chiarelli *et al*., 2009; Rask-Madsen & King, 2010). Hyperglycaemia activates a number of signalling pathways (Fig. 3.3), which may

contribute to renal disease and other vascular complications of diabetes (Ziyadeh, 2004).

Other etiologic factors include hypertension, genetic predisposition and smoking. Most significant, however, is the presence of hypertension, not only before and after the onset of microalbuminuria but probably also as another familial marker of risk, since patients with diabetes and a positive family history of hypertension are at higher risk of nephropathy (Lieb *et al*., 2013) (Table 1.1).

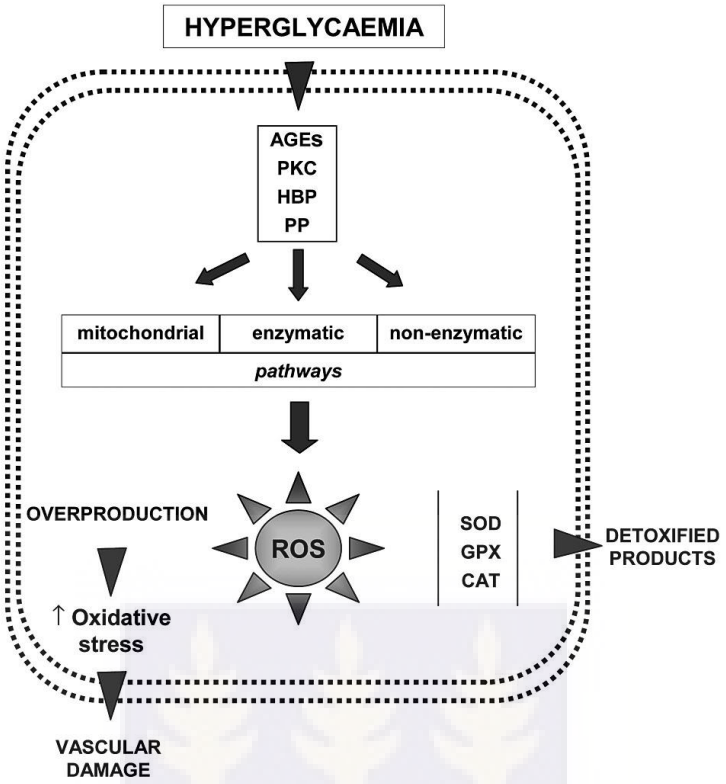


Figure 2.5: Hyperglycaemia-induced activation of secondary messengers increases ROS leading to vascular damage

Source: http://diabetes.diabetesjournals.org

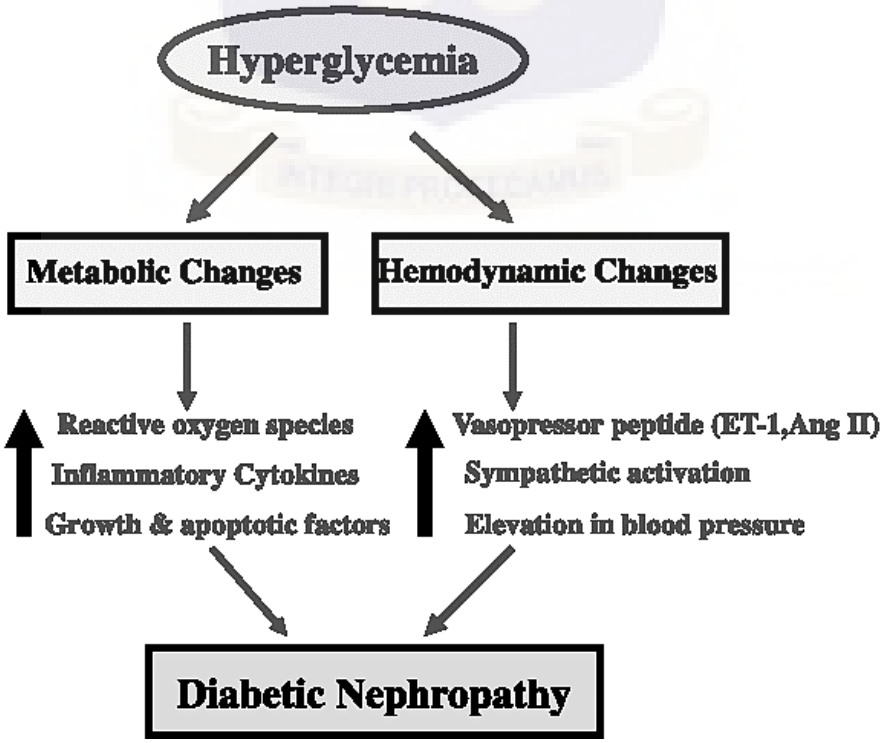


Figure 2.6: Hyperglycemia-induced metabolic and hemodynamic changes leading to nerve dysfunction

Source: http://diabetes.diabetesjournals.org

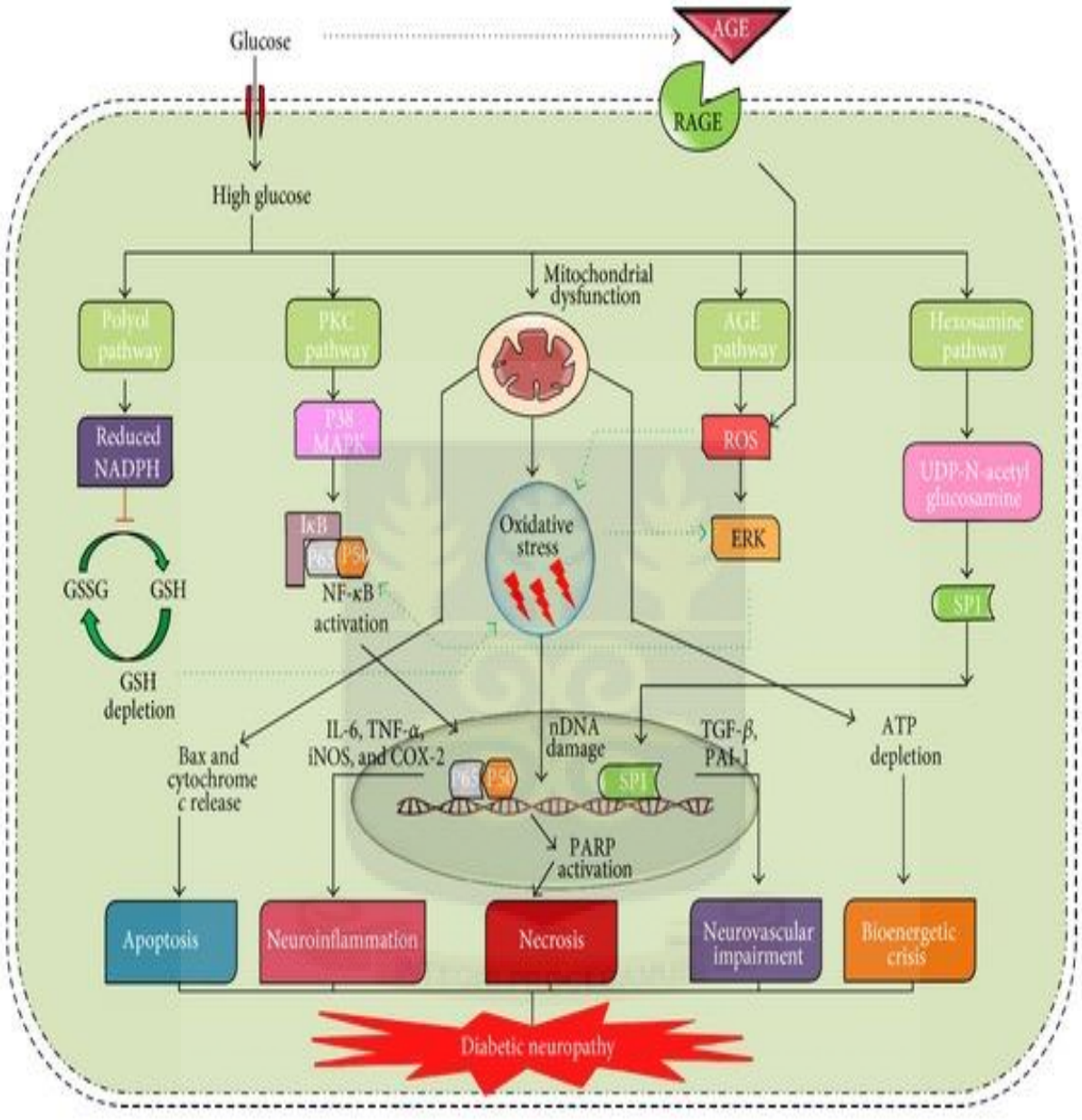


Figure 2.7: Hyperglycaemia-induced over activation of protein kinase c (PKC) leads to downstream signalling activities.

Source: www.ncbi.nlm.nih.gov/pmc/articles/PMC2877591

Table 1.1: Risk Factors for Diabetes-Associated Microvascular and Macrovascular Complications

Risk Factor	Retinopathy	Neuropathy	Nephropathy	Cardiovascular Disease	Cerebrovascular Disease	Peripheral Vascular Disease
Hyperglycemia	Yes	Yes	Yes	Yes	Yes	Yes
Hyperinsulinemia					Yes	
Age	Yes	Yes	Yes	Yes		
Tobacco use	Yes	Yes	Yes	Yes	Yes	
Insulin treatment	Yes					
Dyslipidemia	Yes	Yes	Yes	Yes		
Pregnancy	Yes					
Renal disease	Yes					
Elevated homocysteine level	Yes					
High-fat diet	Yes					
Chronic diabetes mellitus		Yes				Yes
Hypertension		Yes		Yes	Yes	Yes
Obesity				Yes		Yes
Atrial fibrillation					Yes	
Heart failure					Yes	
Proteinuria			Yes		Yes	
Microalbuminuria		Yes	Yes		Yes	
Hyperuricemia					Yes	
Blood inflammatory molecules					Yes	
Elevated blood fibrinogen level						Yes
Physical inactivity				Yes		Yes
Elevated height		Yes				
Ketoacidosis		Yes				
Carotid artery stenosis					Yes	

Source: http://static.pubmed.gov

2.3.4 Classification of DN

The degree of proteinuria / albuminuria correlates with the magnitude of renal damage in experimental models, and its reduction helps to preserve renal function (Remuzzi & Bertani, 1998). Diabetic nephropathy is mainly classified according to the extent of

albuminuria in urine output in addition to the glomerular filtration rate (GFR; that is, creatinine clearance [CCr]). Pathologically, DN can be classified based on proteinuria / albuminuria and glomerular lesion, and considered an indicator of glomerular disease severity (Campese *et al*., 2005). The proposed effects of proteinuria on the kidney include increased severity of glomerulosclerosis, tubule-interstitial inflammation, and subsequent fibrosis, thereby contributing to progressive renal function loss. These facts led to the establishment of a "proteinuria hypothesis" that consists of three postulates: higher levels of proteinuria predict adverse clinical outcomes; reduction of proteinuria correlates with slowing of renal progression; and proteinuria is a surrogate end point and target of clinical interventions (Williams, 2005).

According to Haneda *et al*. (2015), DN can be classified into five stages namely; Stage l (prenephropathy) - having normoalbuminuria of < 30 mg/g Cr with GFR > 29 mL/min/1.75m^2; stage II (incipient nephropathy) – microalbuminuria between 30 – 299 mg/g Cr with GFR > 29mL/min/1.75m^2; stage III (overt nephropathy) – macroalbuminuria ≥ 300 mg/g Cr with GFR > 29 mL/min/1.75m^2; stage IV (kidney failure) – persistent proteinuria/albumin with GFR less than 30 mL/min/1.75m^2; & stage V (dialysis) – normo/micro/macroalbuminuria with GFR ≥ 30 mL/min/1.75m^2.

Tervaert *et al*. (2010) classified DN based on glomerular lesions (Fig. 2.8). In this classification, four main descriptions and criteria were used, specifically, (I) having mild or nonspecific changes on light microscopy and conformed GBM thickening proven by electron microscopy: GBM > 395 nm (female), GBM > 430 nm (male); (IIa) mild

mesangial expansion in > 25% of the observed mesangium; area of mesangial proliferation < area of capillary cavity; (IIb) severe mesangial expansion in >25% of the observed mesangium; (III) At least one convincing nodular sclerosis (Kimmelstiel-Wilson lesion); (IV) Advanced diabetic glomerulo-sclerosis in >50% of glomeruli. The Tervaert classification best reflect the course of progressive DN.

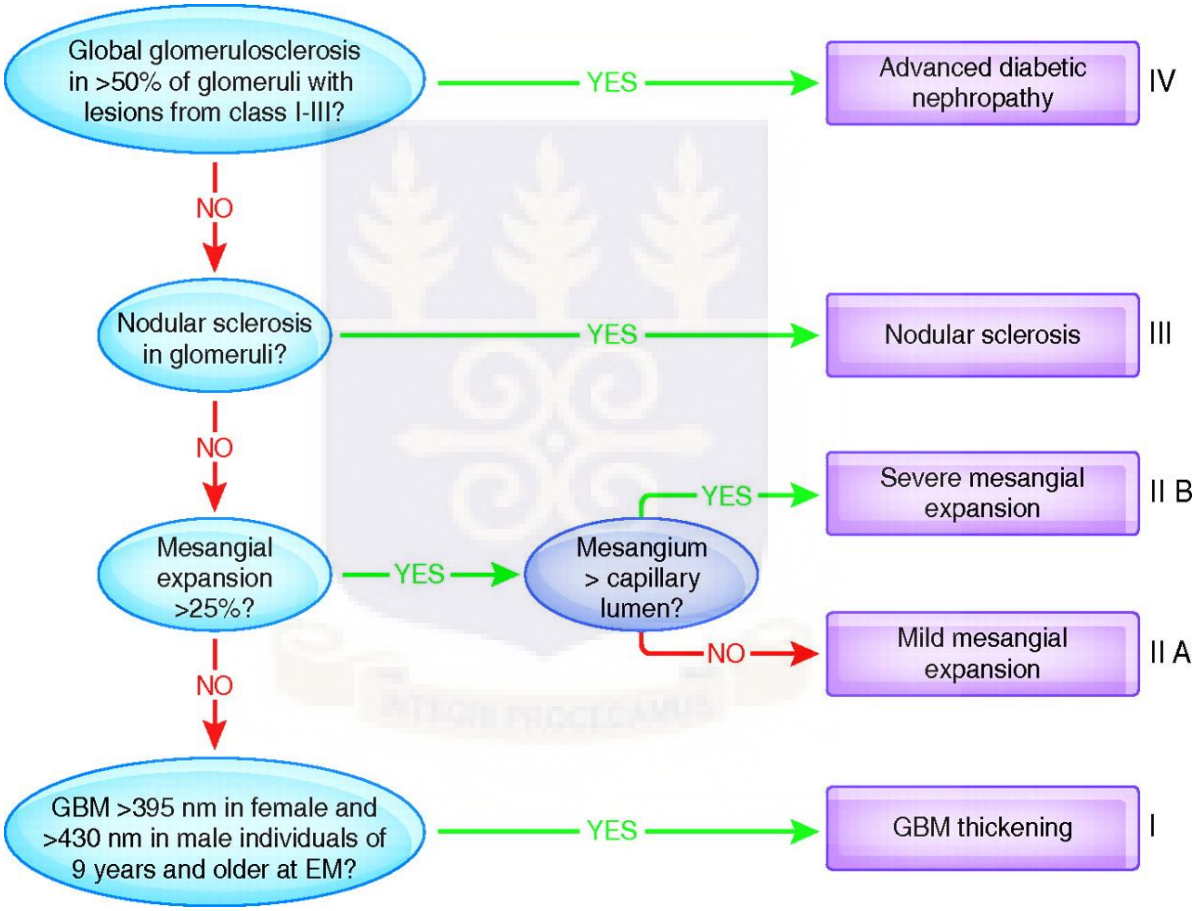


Figure 2.8: Sequence of glomerular changes

Source: https://jasn.asnjournals.org

Diabetic nephropathy is clinically diagnosed by the use of light microscopy. According to Dabla (2010), diabetic nephropathy kidney tissues on microscopic examination will

show excessive accumulation of extracellular matrix with thickening of glomerular and tubular basement membranes, expansion of the mesangial matrix leading to a decrease in the surface area of the glomerular capillaries. Occasionally, immunofluorescence and electron microscopy may be used in advanced laboratories to authenticate earlier findings from light microscopy. Light microscopy may show an increase in the solid spaces of the tuft, most frequently observed as coarse branching of solid (positive periodic-acid Schiff reaction) material (diffuse diabetic glomerulopathy). Large acellular accumulations also may be observed within these areas. These are circular on section and are known as the Kimmelstiel-Wilson lesions/nodules.

Patients with unexplained proteinuria, fatigue, retinopathy, pedal oedema, and passing of foamy urine are likely to be considered nephropathic patients. Type-2 diabetic mellitus patients with long standing of high blood glucose typically have diabetic nephropathy (Vecihi *et al*., 2018).

2.3.5 Strategies in the Management of DN

Several published data suggest that early treatment prevents or delays the onset of diabetic nephropathy (Collins *et al*., 2012; Satirapoj & Adler, 2014; Nazar, 2014). This has consistently been shown in both type 1 and type 2 diabetes mellitus (Bjornstad *et al*., 2014; Nazar, 2014; Eboh & Chowdhury, 2015). In recent years, there has been tremendous works in understanding the pathophysiology, prevention, and treatment of DN. Some approaches to managing DN are; proper glycaemic control (chemotherapy), rigorous control of blood pressure, immunotherapy, hormone therapy, antioxidant

therapy, dialysis, kidney transplantation, among others (Molitch, 1997). In some cases, combination therapies may be employed.

2.3.5.1 Strict blood pressure control

According to the United Kingdom Prospective Diabetes UKPD (2003), strict blood pressure control is vital in the prevention and progression of DN in T2DM patients. Adler *et al*. (2003), also reported that decrease in systolic blood pressure by 10 mmHg reduces the risk of development of diabetic complications by 12 %. Furthermore, Ferrario, (2006) indicated that, decrease in systolic blood pressure values below 120 mmHg helps in retarding diabetic complications. According to the current guidelines on arterial hypertension treatment, blood pressure goal in patients with DM should be less than 130/80 mm Hg (European Society of Hypertension, 2007). Antihypertensive therapy may be started even when blood pressure values are normal.

2.3.5.2 Inhibition of the Renin-Agiotensin System (RAS)

Alterations in the renin-angiotensin system (RAS) has demonstrated promising results in the prevention of DN progression in patients (Santos *et al*., 2019). Angiotensin II is the most effective factor of the RAS, which is directly associated with blood pressure regulation, body fluid volume, and vascular response to injury and inflammation (McGrowder *et al*., 2003). The wrong activation of this system increases the blood pressure and has anti-inflammatory, prothrombotic, and proatherogenic effects, which in the long run lead to irreversible damage of the kidney (Hilgers & Mann, 2002).

Nephroprotective effect of Angiotensin Converting Enzyme Inhibitor (ACE-I) and Angiotensin Receptor Blocker (ARB) has been confirmed in a meta-analysis showing that ACE-I and ARB decrease albuminuria in patients with DM more effectively than antihypertensive medications whose mechanism of action excludes RAS (Casas *et al*., 2005). Early treatment with ACE-I (captopril) may avert microalbuminuria, which is the early indication of glomerular damage and marker of cardiovascular risk in patients with DM. Aldosterone receptor antagonists and renin inhibitors also decrease albuminuria in patients with DM (Estacio, 2009). However, large randomized trial is needed to determine their possible advantage over ACE-I and ARB either as monotherapy or combined therapy. McGrowder *et al*. (2003) demonstrated that captopril has no significant reduction in post-prandial blood glucose levels in animal models.

2.3.5.3 Intensive blood glucose control using chemotherapy

Good glycaemic control has been shown in several studies to decrease the risk of microvascular disease in both type 1 and type 2 diabetes (Di Landro *et al*., 1998; Molitch, 1997). It is evident that a good glycaemic control, as in bringing blood glucose to normal without causing significant hypoglycaemia plays a critical role in stabilizing and slowing nephropathy even with established renal damage (Giaccari *et al*., 2009). Glycaemic control also stabilizes or decreases the elimination of proteins in patients with pronounced proteinuria. (Fioretto *et al*., 2006).

Currently, the main drugs used in the treatment and management of DN include xenobiotics such as, insulin secretagogues (sulphonylurea), insulin sensitizers (biguanides), alpha glucosidae inhibitors (Acarbose), and sodium glucose co-transporter-2 inhibitors (SGLT-

2). These agents either increase peripheral insulin levels (promote glucose transport to tissues) or inhibit hepatic and intestinal glucose synthesis (decrease blood glucose levels).

Sulphonylureas (glibenclamide) is a first-line therapy which is used in the management of type-2 diabetes and its complications (Simonson *et al*., 1984). It increases peripheral insulin levels by blocking the ATP-sensitive potassium (K) channels on pancreatic beta cells, which leads to depolarization of the cells and insulin secretion. This drug enhances the sensitivity of beta-cell to glucose and binds to the transmembrane sulphonylureas receptor (SUR-1) to mediate the closing of the K-sensitive ATP channels on the cell membrane. This consequently decreases cellular efflux of K leading to membrane depolarisation. Calcium influx mediated by opening of the voltage-dependent Ca^{2+} - channels, promote the release of pre-formed insulin granules which lie just adjacent to the plasma membrane (Fig.2.9) (Huri *et al*., 2015). Hypoglycaemia can occur with the use of glibenclamide because, it increases the release of insulin even at low glucose concentration (Rydberg *et al*., 1994). It's well absorbed after oral administration and reach peak plasma concentrations within 2-4 hours.

The alpha-glucosidase inhibitors (Acarbose), inhibits glycoside hydrolases needed to digest carbohydrates. Inhibition of these enzyme systems reduces the rate of digestion of complex carbohydrates (Caspary, 992). Moreover, SGLT-2 inhibits proteins located in the renal tubules of the kidneys which are responsible for reabsorbing glucose back into the blood. The renal significance of these drugs comes from their ability to restore a dysregulated Tubuloglomerular feedback (TGF). Restoration of this mechanism results in reduced glomerular filtration and glomerulomegaly (Nelson & Tuttle, 2007).

Unfortunately, these xenobiotics which are currently the most frequently prescribed oral anti diabetic drugs for the management of diabetic nephropathy worldwide, seems to be associated with increased mortality and cardiovascular risk (Hong *et al*., 2013; Azimova *et al*., 2014).

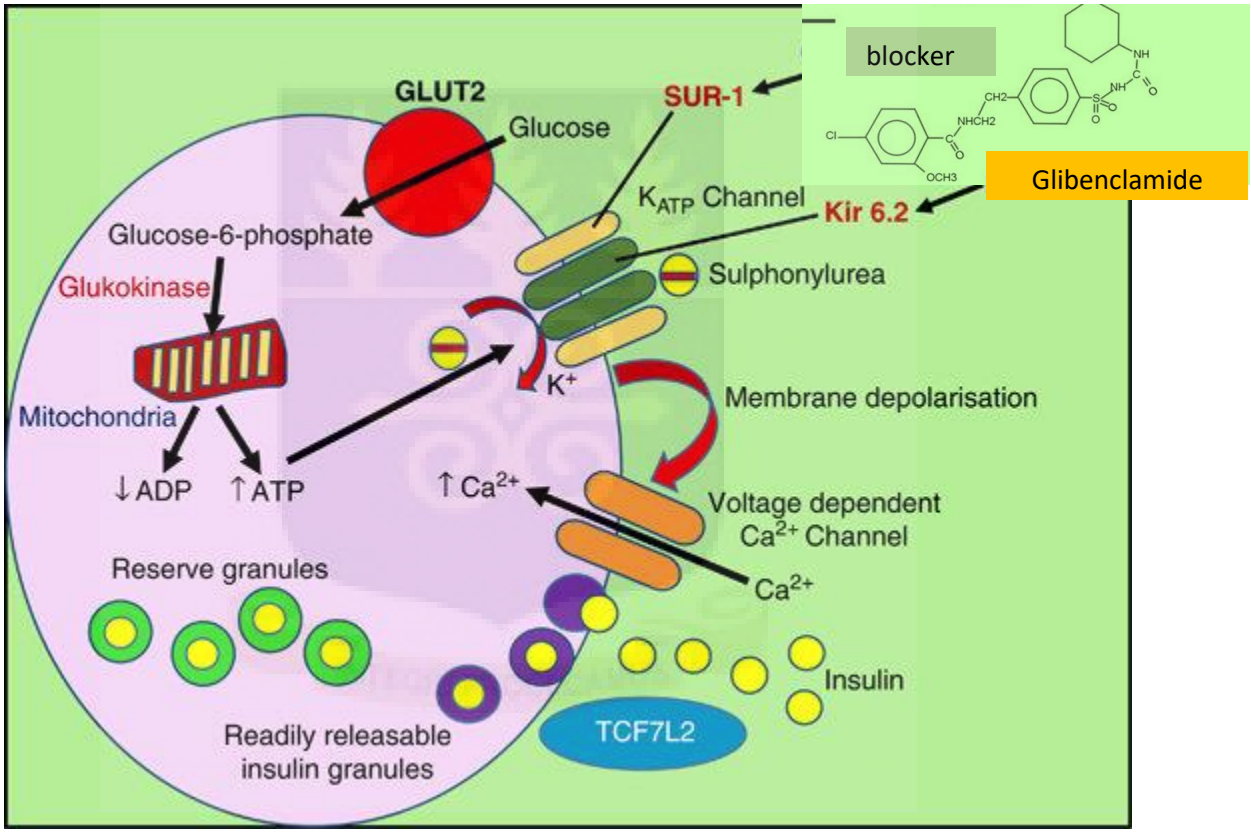


Figure 2.9: Sulphonylurea receptor ***Source: scarabgenomics.com***

Mechanism of insulin secretion from pancreatic β-cells as a result of binding of sulphonylureas to the SUR-1 receptor

2.3.5.4 Antioxidant therapy

Dietary supplements with antioxidant properties are proving essential tools in the management of oxidant stress-related microvascular complications (Traverso *et al*., 2013). Antioxidants like alpha-lipoic acid and resveratrol are known to be effective in reducing diabetic complications (Mozafari *et al*., 2015). Oxidative stress has been projected as a significant mechanism in progression of renal disease. There is emerging evidence to support that reactive oxygen species cause impaired activity of the transcription factor called nuclear-1-factor related factor-2 (Nrf-2) (Ruiz *et al*., 2013). Bardoxolone methyl (a synthetic triterpenoid derivative), is a potent activator of Nrf-2 that was shown to reduce oxidative stress in rat models (Dinkova-Kostova *et al*., 2005; Yates *et al*., 2007). Unfortunately, bardoxolone methyl was prematurely terminated after 9 months due to higher rate of cardiovascular deaths.

In recent times, another oxidative stress pathway mediated by activation of apoptotic signal-regulating kinase-1 (ASK-1) has received attention. ASK-1 pathway activation results in downstream activation of terminal kinases, leading to the production of inflammatory chemokines (Adhikary *et al*., 2004). Another novel dietary agent (GS-4997) was shown to reduce inflammation in rat models by inhibiting ASK-1 pathway. This suggest that, dietary supplements rich in antioxidant may be a useful agent in the management of DN.

2.4 Glutathione-enhancer dietary supplements

2.4.1 Overview of Glutathione (GSH)

Rey-Paihade (1888) discovered glutathione (GSH) from sources of yeast and many animal tissues (beef, liver, fish skeletal muscle, lamb small intestine, and sheep brain) and in fresh egg white. Rey-Paihade named GSH as *philothion* meaning sulphur-loving in Greek. In 1921, F. Gowland Hopkins, the "Father of Biochemistry" of Cambridge, England, renamed the substance *"glutathione."* GSH plays several roles in a multitude of cellular processes, including cell differentiation, proliferation, and apoptosis. GSH is involved in the etiology and progression of many human diseases including diabetes nephropathy. While GSH deficiency leads to an increased vulnerability to oxidative stress implicated in disease progression (Salim, 2017).

Glutathione is a ubiquitous thiol-containing intracellular low molecular weight tripeptide (γ-glutamyl-cysteinyl-glycine) present in high concentration in all mammalian cells (McGill, 2015). GSH, among other roles, due to its chemical properties and relative abundance in cells makes it well-suited to function as an antioxidant. It occurs in reduced (GSH) and in oxidized form (glutathione disulfide [GSSG]) and is the most important non-enzymatic endogenous antioxidant that can be regenerated by glutathione reductase with the consumption of NADPH (Gul *et al*., 2000). This helps to maintain optimum levels of reduced GSH (Kohen & Nyska, 2002; Halliwell, 2006). The endogenous ratio of GSH to GSSG is considered an indicator of redox homeostasis within cells. McGill (2015) categorically states that GSH combined with the protective effects of reversible protein S-glutathionylation, the scavenging property of GSH effectively saves other molecules in the

cell from the same fate. Because of this, the ratio of GSSG to GSH in biological samples is often used as an indicator of oxidation stress and injury in toxicological studies (Jones, 2002; Valko *et al*., 2007, McGill, 2015).

The increased levels of blood glucose in diabetes produce superoxide anions and hydroxyl radicals in the presence of transition metal ions which cause oxidative damage to cell membranes. The oxidation reduction state of cellular thiols plays a central role in antioxidant defense and in the regulation of a large number of signal transduction pathways and metabolic functions (Griffith, 1999). The tripeptide glutathione (GSH), that is, L-g-glutamyl-Lcysteinyl-glycine (MW 307), represents the major low-molecular-mass thiol compound participating in cellular redox reactions and thio-ether formation. Under oxidative stress, GSH is oxidized to glutathione disulphide (GSSG) and further to other products such as sulphonates. Glutathione-cysteinyl disulphides can also be formed on proteins and such bound glutathione makes up a considerable amount of the cellular glutathione pool. The redox reactions are catalyzed by GSH peroxidases (GSH-Px) and GSSG reductases (GSSG-Rd), whereas a major class of enzymes involved in thioether formation is given by the glutathione transferases (GST). Interestingly, GSH-Px activity has been extended to new functions, such as, peroxynitrite reduction, protection against apoptosis and sperm maturation, and at present glutathione is considered as the most important antioxidant in vivo. Besides its antioxidant activity, glutathione has many physiological functions including detoxification of xenobiotics, modulation of redox-regulated signal transduction, storage and transport of cysteine, regulation of cell proliferation, synthesis of deoxyribonucleotide, regulation of immune response, and

regulation of leukotriene and prostaglandin metabolism. GSH is able to increase the activation of cytotoxic T cells in vivo. The normal functioning of T lymphocytes is dependent upon cellular supplies of cysteine. The cells acquire the amino acid largely by uptake of GSH by macrophages and lymphocytes. Impaired immune responses are associated with a reduction in the glutathione concentration of immune tissue (Droge *et al*., 1991).

2.4.2 The function of glutathione as an antioxidant

Antioxidants are present in the human body (endogenous) as a normal defense mechanism of the cell or obtained exogenously from diet and supplements. Examples include enzymatic antioxidants like catalase (CAT), superoxide dismutase (SOD), glutathione S-transferase (GST), glutathione peroxidase (GSH-Px), and non-enzymatic antioxidants like reduced glutathione (GSH), lipoic acid, carotenoids, uric acid, flavonoids, and vitamins A, C, and E. These antioxidants function by either reducing the harmful effects of free radicals by preventing their formation; scavenging and inactivating them or boost the natural defense systems by inducing the activities of antioxidant enzymes and regenerating other proteins involved in antioxidant pathways (Oyenihi *et al*., 2015). Antioxidants have a variety of actions by which they exert their biochemical effects and reduces nerve dysfunction in diabetes by acting directly against oxidative damage. Oxidative damage has long been implicated in disease pathogenensis (Hussain *et al*., 2003), suggesting that antioxidant treatment may provide protection from diseases.

Physiologically, Reactive oxygen species (ROS) are formed by aerobic cells (Droge, 2002) and their production escalates under circumstances of cell injury (Valko *et al*., 2007). ROS facilitate essential intracellular signalling pathways and are vital for cell survival. Conversely, an excess of ROS formation brings about oxidative damage to cells and apoptosis. To prevent their reversible cell damage, the increase of ROS induces an adaptive response, consisting of a compensatory upregulation of antioxidant systems, aimed to restore the redox homeostasis (Landriscina *et al*., 2009).

GSH participates not only in antioxidant defense systems, but also in many biological metabolic processes (Sies, 1999). One main function of GSH is the detoxification of xenobiotics and some endogenous compounds in the human body. These substances are electrophiles and form conjugates with GSH enzymatically, in reactions catalyzed by GSH-S-transferases (GST) (Meister, 1988). More so, GSH function to maintain the intracellular redox balance (Droge, 2002) and performs an antioxidant function (Fig.2.10).

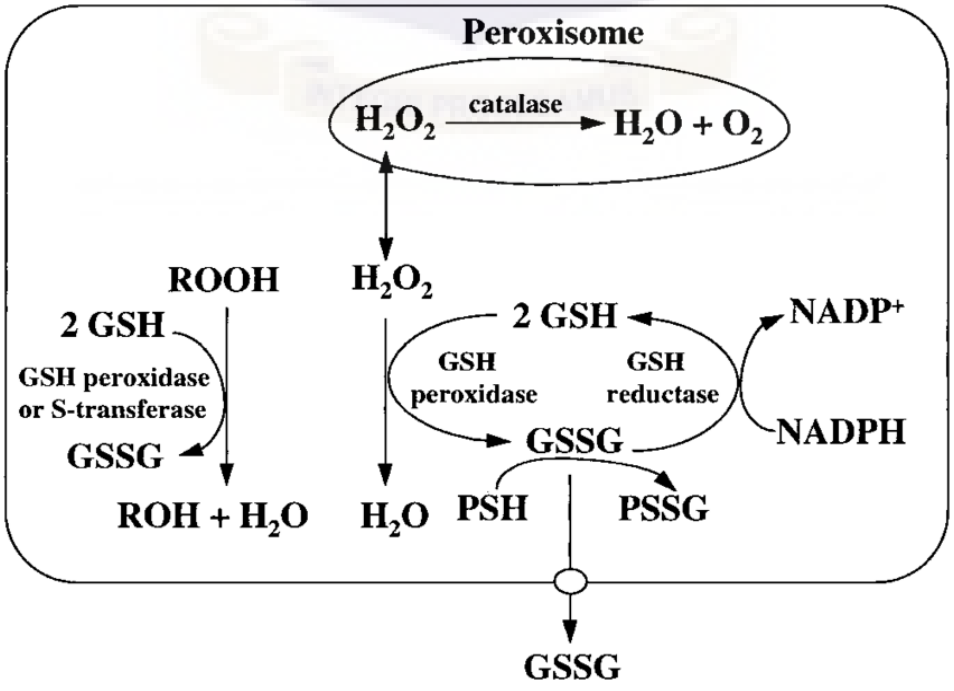


Figure 2.10: Antioxidant function of GSH. ***Source: https://www.researchgate.net***

The hydrogen peroxide (H_2O_2), produced during the aerobic metabolism, is metabolized in the cytoplasm by both glutathione peroxidase (GSH-Px) and catalase in peroxisomes. To prevent oxidative damage, the GSSG is reduced to GSH by GSSG reductase using NADPH as energy source to reconvert GSSG to 2GSH (Lu, 2009). Other peroxides (e.g. organic ones) can be reduced by GSH-Px and GSH-transferase (GST).

2.4.3 The role of GSH in diabetes nephropathy development and progression

Depletion of GSH has been correlated with lower immune function and increased susceptibility to infection due to the liver's reduced ability to detoxify (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3048347). As the generation of free radicals exceeds the body's ability to neutralize and eliminate waste products, oxidative stress builds up in the body. The primary function of glutathione is to lessen this oxidative stress. GSH is used as a cofactor by (1) multiple peroxidase enzymes, to detoxify peroxides generated from oxygen radical attack on the kidneys, pancreas, and other associated organs; (2) transhydrogenases, to reduce oxidized centers on DNA, proteins, and other biomolecules involved in the pathogenesis of diabetic nephropathy; and (3) glutathione S-transferases (GST) to conjugate GSH with endogenous substances (e.g. estrogens), exogenous electrophiles (e.g. arene oxides, unsaturated carbonyls, organic halides), and diverse xenobiotics. Low GST activity may increase risk for diabetic nephropathy and other diseases.

Gamma-glutamyl cysteine synthetase (GCS) is a glutamate-cysteine ligase which is involved in synthesizing glutathione, specifically catalyzing the first reaction of combining glutamate and cysteine to form a dipeptide known as γ-glutamylcysteine (Griffith *et al.*,

1999). This enzyme is a target of certain pharmacological interventions assessing the actions of glutathione in a cell, as it can be inhibited with Buthionine SulfOximine (BSO) resulting in a depletion of active GSH (Reliene *et al.*, 2006) and enhancing its actions can enhance glutathione activity and its antioxidant effects (Das *et al*., 2006). A deficiency of GCS results in both a depletion in glutathione as well as a cellular glutathione-S-transferase activity (Beutler *et al*., 1986). ROS, RNS and RSS that result from the respirative cycle of oxidative phosphorylation may attack biological macromolecules like cellular DNA, giving rise to single and double strand breaks that may eventually cause cell ageing, diabetic nephropathy, cardiovascular diseases, mutagenic changes and cancerous tumor growth. When natural defenses of the organism (of enzymatic, non-enzymatic or dietary origin) are overwhelmed by an excessive generation of ROS/RNS, a situation of oxidative stress occurs, in which cellular and extracellular macromolecules (proteins, lipids and nucleic acids) can suffer oxidative damage, causing tissue injury in organs like the kidneys and liver. It has been established that the consumption of foods rich in natural antioxidants (e.g. dietary supplements) provides an efficient way of combating tissue injuries, undesired transformations and prevent health risks (Halliwell & Gutteridge, 2007). GSH status is in fact correlated with diseases progression and its levels associated with cellular proliferation (Carretero, *et al*., 1999).

Although a close causal link between GSH and T2DM has been established; and the role of GSH dietary food supplementation in the management of glucose homeostasis has been reported severally; however, whether increasing GSH levels in blood have scavenging effects on free radicals and having nephroprotective functions in a T2DM model to control glucose homeostasis is not clearly defined. Thus, here the aims of this research is to

measure markers related to glucose metabolism such as glutathione levels, insulin sensitivity, fasting insulin, arteriole blood pressure, lipid profile, and malondialdehyde.

2.4.4 Mechanisms of action of glutathione

In both normal and malicious cells, increased GSH level is linked with a proliferative response and is essential for cell cycle progression (Messina & Lawrence, 1989; Lu & Ge, 1992). The molecular mechanism of how GSH modulates cell proliferation remains largely speculative. Antioxidants slow down the oxidation rates of foods by a combination of scavenging free radicals, chelating prooxidative metals, quenching singlet oxygen and photosensitizers, and inactivating lipoxygenase (Huang *et al*., 2005; Schlesier *et al*., 2002). They scavenge free radicals of foods by donating hydrogen to them, and they produce relatively stable antioxidant radicals with low standard reduction potential (Lee, 2013). The higher stability of antioxidant radicals than that of food radicals is due to resonance delocalization throughout the phenolic ring structure (Choe & Min, 2005). The activity of an antioxidant is determined by: (a) Its reactivity as a hydrogen or electron-donating agent (which relates to its reduction potential), (b) The fate of the resulting antioxidant-derived radical, which is governed by its ability to stabilize and delocalize the unpaired electron, (c) Its reactivity with other antioxidants and (d) The transition metal-chelating potential (Shahidi, 2000). GSH availability down-regulates the pro-inflammatory potential of leukotrienes and other eicosanoids.

2.4.5 Therapeutic Potential of GSH Dietary Supplements

GSH-based supplements might represent a promising therapeutic strategy for overcoming disease progression and cell proliferations (Traverso *et al*., 2013). For this reasons, chemically modified analogues of GSH have been generated in order to mimic glutathione's various pharmacological effects. Some of these analogues chemically synthesised includes N-acetyl cysteine (Mucomyst) and a monoester GSH (YM737). These GSH-analogues target S-glutathionylation and directly protect tissues against the damaging effects of ROS/NS due to the ability of these analogues to act on a wide range of signalling pathways (Wondrak, 2009).

2.4.6 Cellgevity®

Cellgevity, an oral source of glutathione antioxidant in the form of dietary supplement is made up of riboceine (a cysteine based molecule), curcumin, resveratrol, aloe extract, milk thistle, quercetin, broccoli seed extract, alpha lipoic acid, grape seed extract, vitamin C, seleno methionine, cordyceps, and piperine. These numerous constituents of Cellgevity have been reported to have a variety of role in the scavenging of free radicals as well as having the potential to protect intracellular cells (Nagasawa, 2015; N'guessan *et al*., 2018; Awodele *et al*., 2018). For example, lipoic acid acts as an antioxidant substance that protects against cell damage. Lipoic acid has been researched for its effect on insulin sensitivity, glucose metabolism and diabetic neuropathy (Jacob *et al*., 1996). Lipoic acid is a critical co-factor for mitochondrial dehydrogenase reactions and has been found to increase glucose transport in muscle cells (Hamilton *et al*., 2007). Jacob *et al*. (1999) demonstrated that, a placebo-controlled explorative study in T2DM patients significantly increased insulin-mediated glucose uptake, presumably by modulating insulin sensitivity.

A multiplicity of antioxidants as the case of Cellgevity is beneficial in biological systems because, different antioxidants tend to locate preferential areas of tissues and cells (Chaudhari, 2008). For example, vitamin E is only effective in lipid-containing areas, whereas glutathione is functional in aqueous areas (cytoplasm) (Doba *et al*., 1985). The constituents of Cellgevity are of very high antioxidant properties which interrupt in one way or the other the generation of oxidative stress. Most of the components (resveratrol, α-lipoic acid, curcumin, quercetin) play vital role in the binding of heavy metals like iron thus preventing Fenton and Haber Weiss reactions. Moreover, selenomethionine and cysteine are essential component of the enzyme glutathione peroxidase and thioreductase, which uses glutathione to neutralize hydrogen peroxide (Ganther, 1999; Lu *et al*., 2009; Turanov *et al*., 2010).

Cellgevity like most other dietary supplements are globally acceptable adjunct therapies for NCDs, emphasising on their natural modes of healing coupled with less side effects (Cheung *et al*., 2007; Afolayan & Wintola, 2014). Several studies have shown the use of dietary supplements for the managements of NCD (Afolayan & Wintola, 2014; Siddharthan *et al*., 2015; Forouhi *et al*., 2018). However, not all dietary antioxidant supplements have protective effects (Poljšak & Fink, 2014), thus this research seeks to investigate the antidiabetic and nephroprotective potentials of Cellgevity in STZ-NA induced type-2 diabetes mellitus rat models.

2.5 Literature review on some of the methods used in this study

Some of the methods employed during experimentation include induction of type-2 diabetes mellitus in rats, induction of diabetic nephropathy, assessment of inflammatory markers (haematological assay), and renal function markers (serum biochemistry assay).

2.5.1 Animal Models of Diabetes Mellitus (DM)

Animal models of diabetes mellitus are considered as very useful tools for understanding the pathophysiology and the clinical aspects of the disease and are always used as the first step for investigating a prospective new therapy (Etuk, 2010). Animal models have been used extensively in diabetes research and earlier researchers used pancreatectomised dogs, cats, pigs, primates, to confirm the primary role of the pancreas in glucose homeostasis. However, in recent times animal experimentation is contentious and subject to legal and ethical restrictions. Various models use small animals like rats and mice, and not the relatively larger ones though results from studies using larger animals are more appropriate if conclusions will be extrapolated to humans (Lukacinova *et al*., 2013). The smaller animals are relatively easier to handle, affordable, and require less space.

DM can be induced completely in less than 72 hours after STZ intraperitoneal injection. It is modelled in both non-obese and obese animal models using a variety of methods (genetic, surgical and chemical), and each of these have its own characteristics. The mostly used one is the chemical method as it is cheaper and easier to maintain (Lukacinova *et al*., 2013). These animal models are used for various purposes including pharmacological studies, testing, of genetics and understanding disease mechanisms. The choice of organism to be used in a study depends on the type and purpose of study. Gender, strains, and species differences can also be take into consideration when selecting an animal model for the study of diabetes (Franconi *et al*., 2008).

2.5.2 Chemical properties of diabetogenic agents

Chemical compounds such as alloxan, streptozotocin, vacor, dithizone, and 8-hydroxyquinolone, can be used to induce diabetes in experimental animals (rats, mice). Alloxan and streptozotocin (STZ) are most prominent diabetogenic chemicals and have been extensively used in studies published between 1996 and 2006 (STZ, 69 % and Alloxan, 31 %) (Etuk, 2010). The reason being that both have similar structure to glucose (Bansal *et al*., 1980), so glucose can compete with alloxan and STZ, thus, fasting animals tend to be more susceptible. Both are cytotoxic glucose analogues, relatively unstable chemical compounds, and for the latter reason, the solutions should ideally be made immediately prior to injection. STZ is relatively more stable at normal body temperature and pH when compared to alloxan. The type of induction of diabetes is very much useful in the testing of a drugs or agents that decreases blood glucose level by a non- β-cell dependent mechanism (Jederstrom *et al*., 2005; Sheshala *et al*., 2009). Major disadvantage with the use of alloxan and STZ is that, it has toxic effect on organs such as liver, kidney, lung, intestines, testis and brain. Thus, this should be considered when drugs are being tested in these models (Lee *et al*., 2010).

2.5.3 Mechanism of action of streptozotocin (STZ)

Streptozotocin (STZ) (2-deoxy-2-({[methyl(nitroso)amino]carbonyl} amino)-β-D-glucopyranose) is a synthetic chemical compound isolated from the fungi *Streptomyces achromogenes* and *Streptomyces griseus* that exhibits broad spectrum antibacterial properties (Wei *et al*., 2003; Etuk, 2010). STZ is transported into B-cells via low affinity GLUT-2 transporter in the plasma membrane and causes demage (alkylation) of the DNA

(Szkudelski, 2001; Tjalve *et al*., 1976; Karunanayake *et al*., 1976) and consequently leading to increased activity of poly-ADP-ribose polymerase (PARP-1) to repair DNA (King, 2012). Hyperactivity of this enzyme brings about depletion of intracellular nicotinamide adenine dinucleotide (NAD^+) and adenosine triphosphate (ATP), and render the insulin-secreting cells to undergo necrosis (Fig. 2.11) (Tahara *et al*., 2008). The dose of STZ (40–60 mg/kg body weight) may lead to hyperglycaemia without the development of severe ketoacidosis even if insulin is not administered (Ganda *et al*., 1976), but higher doses (75 mg/kg body weight) can result in spontaneous ketosis and death in few days if insulin is not administered (Wei *et al*., 2003). To avoid this from happening, STZ-induced diabetic rats are provided 5% to 10% sucrose solution for 12 hours to prevent sudden death from drug-induced hypoglycaemic shock (Kiran *et al*., 2012).

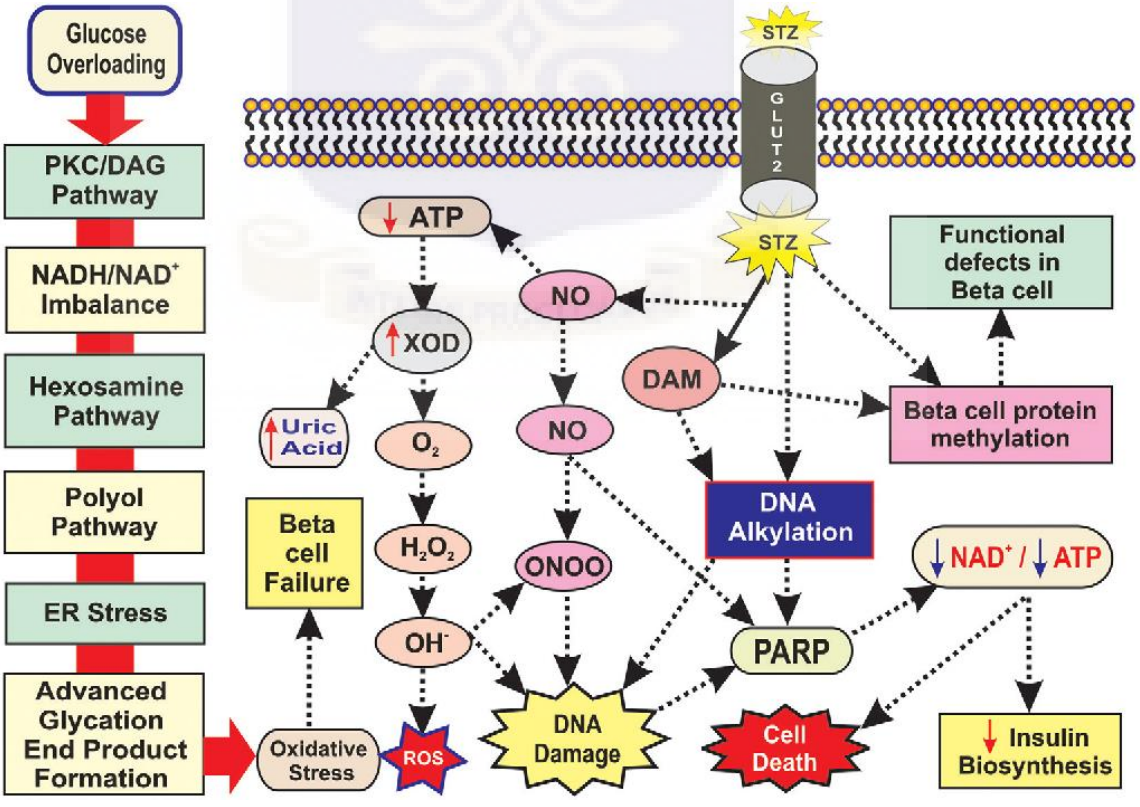


Figure 2.11: The mechanism of action of streptozotocin

Source: https://image.sementicscholar.org

2.5.4 Experimental type 2 diabetes mellitus

Type 2 diabetes mellitus have been successfully induced in rats and mice models with single intraperitoneal injection (*i.p.*) of nicotinamide (NA) fifteen (15) minutes prior to the administration (*i.p.*) of low dose of STZ (35-60 mg/kg body weight) (Masiello *et al*., 1998; Etuk, 2010). Other studies administered NA fifteen or five minutes first before injecting STZ into experimental models (Kamble & Bodhankar, 2013). The NA preserves appreciable number of β-cells and this is attributed to the inhibition of PARP-1 by NA. It is reported that NA prevents the depletion of intracellular nicotinamide adenine dinucleotide (NAD^+) and thereby increases its concentrations (Tahara *et al*., 2008). Yakhchalian *et al*., (2018) reported that there is high level of hyperglycaemia in diabetic rats due to the extensive β-cells necrosis and degeneration caused by streptozotocin exposure. Several studies indicated that some haematological parameters (erythrocytes [RBC], lymphocytes [WBC], neutrophils [N], haemoglobin [HGB], mean corpuscular volume [MCV], mean corpuscular haemoglobin [MCH], mean corpuscular haemoglobin concentration [MCHC]) are significantly decreased in diabetic rat models (Mahmoud, 2013; Cho *et al*., 2008; Yeom *et al*., 2016; Yakhchalian *et al*., 2018).

2.6 Diabetic Nephropathy in Rat Models

Diabetic complications (neuropathy, nephropathy, cerebrovascular diseases) in both man and animals (rats and mice) are gradual developmental processes. It is this property of diabetic complications that allows experimental rat models to be investigated (Wei *et al*., 2003). Studies have shown that diabetic nephropathy occurred in rats' models four to eight weeks post STZ-NA administration (Pari & Srinivasan, 2010; Kiran *et al*., 2012). Kamble

and Bodhankar (2013) reported that serum creatinine, urea and uric acid levels in STZ-NA diabetic rats significantly increased by the fourth week. In previous studies using diabetic models, it was demonstrated that hyperglycaemia leads to hyper-aggregation and low deformability of erythrocytes by changing haemoglobin and membrane proteins of erythrocyte, and serum proteins (fibrinogen and globulins) (Tomaiuolo, 2014). Such hemorheological changes are implicated in the progression of retinal failure in diabetic retinopathy and renal failure in diabetic nephropathy (Cho *et al*., 2008; Tomaiuolo, 2014).

2.7 Haematological assay

Haematology deals with the study of blood and its constituents (red blood cells [erythrocytes], white blood cells [leucocytes], platelets [thrombocytes], etcetera). Blood act as a pathological predicator of an individual's diseased conditions (Doyle, 2006). The examination of blood provides the opportunity to clinically investigate the presence of metabolites and other constituents in the body of animals and it plays a pivotal role in the diagnosis of many diseases as well as investigation of the extent of damage to blood (Togun *et al*., 2007; Doyle, 2006). Changes in haematological parameters are of value in assessing the responses of animals to various physiological and disease conditions (Schalm *et al*., 1975; Yadav *et al*., 2002; Khan & zafar, 2005).

Haematologic changes (abnormalities and alterations) have been documented in patients with diabetes mellitus as well as in streptozotocin-induced diabetes mellitus models (Jones *et al*., 1981; Adedapo *et al*., 2016). Most studies showed that diabetic models have significantly high levels of white blood cell (WBC) (Schmidt *et al*., 1999; Vozarova *et al*., 2002; Moradi *et al*., 2012; Twig *et al*., 2013). On the contrary, red blood cell (RBC),

haemoglobin (HGB), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) are noted to be significantly reduced (Yakhchalian *et al*., 2018). However, platelet count (PLT) of the diabetic models are significantly increased in comparison to the non-diabetic models (Keskin *et al*., 2016). The normal reference range values for male Sprague-Dawley rats by Bioscience® (2017) for WBC, RBC, MCV, HGB, and PLT are 9.4±3.2 $x10^3/\mu L$, 6.9±0.3 x $10^6/\mu L$, 69.9±1.7 fL, 14.5±0.8g/dL, and 1358.0±123.8 $x10^3/\mu L$ respectively.

Chronic kidney failure is associated with increased values of PLT and WBC. A study by Shurtz-Swirski *et al*. (2004) documented that probable mechanism of raised WBC count is oxidative stress, tissue damage, and elevated ROS that occur in diabetes mellitus. Yeom *et al*., 2016 reported that RBC count, PCV, MCV as an RBC index are down rising and MCHC, red blood cell distribution width (RDW) are uprising as a new marker associated with higher mortality in health and disease. Low values of MCV, MCH, and HGB are linked with long lasting kidney failure and chronic kidney disorders. In diabetic patients, the glycaemic control does affect the MCHC and RDW. Good glycaemic control is associated with lower RDW and MCH value than in patients with poor control (Yeom *et al*., 2016).

2.8 Serum Biochemistry assay

In addition to the haematological parameters, it has been shown that diabetes is associated with abnormal serum creatinine and lipid profiles. Hyperlipidemia is a feature of drug-induced diabetes in animals (Kunjathoor *et al*., 1996), as well as poorly controlled diabetes in humans (The Diabetes Control and Complications Trial Research Group, 1993).

Adedapo *et al*. (2016) and Zhang *et al*. (2017) reported that there is increased levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine (CREA), triglycerides (TRIG), blood urea nitrogen (BUN) and a corresponding reduction in the levels of high density lipoprotein (HDL) in diabetic rat models. This is attributed to inflammatory component of diabetes mellitus and have been investigated and proved in many studies (Zafar *et al*., 2009). Pro-inflammatory cytokines can activate signalling cascade (PKC, p38 MAPK) which may cause increased oxidative stress in underlying organs and down-regulate antioxidant levels (West, 2000; Ozcan *et al*., 2004). These processes could lead to many alterations in serum proteins concentration especially serum creatinine and lipid profile (Hotamisligil, 2006; Zafar *et al*., 2009). Increased in serum creatinine may be a prognosticator for kidney related disorders and vascular complications.

CHAPTER THREE

MATERIALS AND METHOD

3.1. Animal Handling

Seventy male Sprague-Dawley (SD) rats with an average weight of 200±20 g were purchased from Department of Animal Experimentation, Centre for Plant Medicine Research (Mampong - Akuapim) and accommodated at the University of Ghana Medical School – Korlebu Animal House Unit under constant temperature (24±2°C), humidity (60% – 70%), and photoperiod (12 hours light / dark). All rats were acclimatized for 14 days before the start of the experiment. Rats were fed with standard rat chow (AGRIMAT, Kumasi) and tap water. Rat experiments were performed in compliance with the University of Ghana Animal Care Guideline. The study protocol was approved by the College of Health Sciences Ethical Committee of the University of Ghana Medical School (CHS-Et/M.10-P5.6/2018-2019).

3.2. Materials

The following materials were acquired for the experiment: nicotinamide (NA) was obtained from Sigma-Aldrich Chemical Co. (St. Louis, Mo, USA); Streptozotocin (STZ) and Citrate buffer were obtained from Teva Parenteral Medicine Inc. (Irvine, CA, USA). Glibenclamide (Daonil®, T320 M918 E921); Captopril (accord, PL20075/0309); Cellgevity (Max International, 83379501); Gold-Accu glucometer and glucose test strips (Shaghai Int.Corp. GmbH-Europe).

3.3. Experimental Design

The 70 male SD rats were assigned to ten randomized groups (n = 7 rats per group), including normal rats administered with 1 ml distilled water (N) per day as a placebo; normal rats administered with Cellgevity (N+Cell.MD); a diabetic untreated (DU) group; diabetic treated with Cellgevity low, medium, and high doses (D+Cell.LD; D+Cell.MD; D+Cell.HD); diabetic treated with glibenclamide (D+Glib); diabetic treated with captopril (D+Cap); diabetic treated with glibenclamide and captopril (D+Glib+Cap); and diabetic treated with glibenclamide and glutathione dietary supplement (D+Glib+Cell.HD). All diabetic rats were treated for 28 days.

3.4. Experimental Protocol

3.4.1. Induction of Type 2 Diabetes Mellitus using Nicotinamide and STZ

Two days (T-2) prior to STZ administration, the body weight (W-2) and blood glucose (BG-2) of the animals were measured and afterwards animals were subjected to 12 hour fasting. The body weight (W-1) as well as fasting blood glucose (BG-1)) were determined a day (T-1) before the administration of STZ.

Type 2 diabetes mellitus was induced on day (T_0) after weighing (W_0) and determining the blood glucose (BG_0) by injecting 56 male SD rats with single intraperitoneal (*i.p.*) administrations of NA (110 mg/kg b.wt) 15 minutes before single intraperitoneal administrations of a freshly dissolved STZ (60 mg/kg b.wt) in 0.1 M citrate buffer (pH 4.5) as described by Masiello *et al*., 1998 with a few modifications. The remaining 14 rats were injected with 0.9% normal saline. All rats were given 10% sucrose in their drinking water

for 12 hours after the STZ/NA administration to avoid hypoglycaemic related death. After the 12 hours, animals were provided access to tap water and *Ad libitum*. The animals became diabetic just after 24 hours of STZ/NA administration and as such, their body weight (W_{1-28}) and blood glucose (BG_{1-28}) were recorded for 28 days at intervals of T_1; T_3; T_6; T_9; T_{12}; $T_{15;}$ T_{22} and T_{29}) using an electronic scale and glucometer (Gold-Accu®). Blood samples were obtained from the tails of rats. Rats with blood glucose readings of at least 11.1 mmol/L (>200 mg/dL) were considered diabetic in this study.

3.4.2 Antidiabetic study

Rats were assigned to ten (10) groups (Fig 3.1) with seven rats in each group (i.e. n = 7) namely: group 1 – normal rats administered with 1 ml of distilled water (N); group 2 – normal rats administered with Cellgevity (N+Cell.MD) [80 mg/kg b.wt]; group 3: diabetic rats administered with 1 ml of distilled water (DU); group 4: diabetic rats treated with Cellgevity (D+Cell.LD) [Low dose, 40 mg/kg b.wt]; group 5: diabetic rats treated with Cellgevity (D+Cell.MD) [Medium dose, 80 mg/kg b.wt]; group 6: diabetic rats treated with Cellgevity (D+Cell.HD) [High dose, 160 mg/kg b.wt]; group 7: diabetic rats treated with glibenclamide (D+Glib) [15 mg/kg b.wt]; group 8: diabetic rats treated with captopril (D+Cap) [20 mg/kg b.wt]; group 9: diabetic rats treated with glibenclamide and captopril (D+Glib+Cap) [glibenclamide 15 mg/kg b.wt + captopril 20 mg/kg b.wt] and group 10: diabetic rats treated with glibenclamide (15 mg/kg b.wt) + Cellgevity (160 mg/kg b.wt) (D+Glib+Cap)with each group assigned to this treatment for a period 28 days (T_{1-29}). The antihyperglycaemic effect of these regimens on fasting blood glucose and body weight were determined within 24-hours (acute) and 28-days (subacute) period.

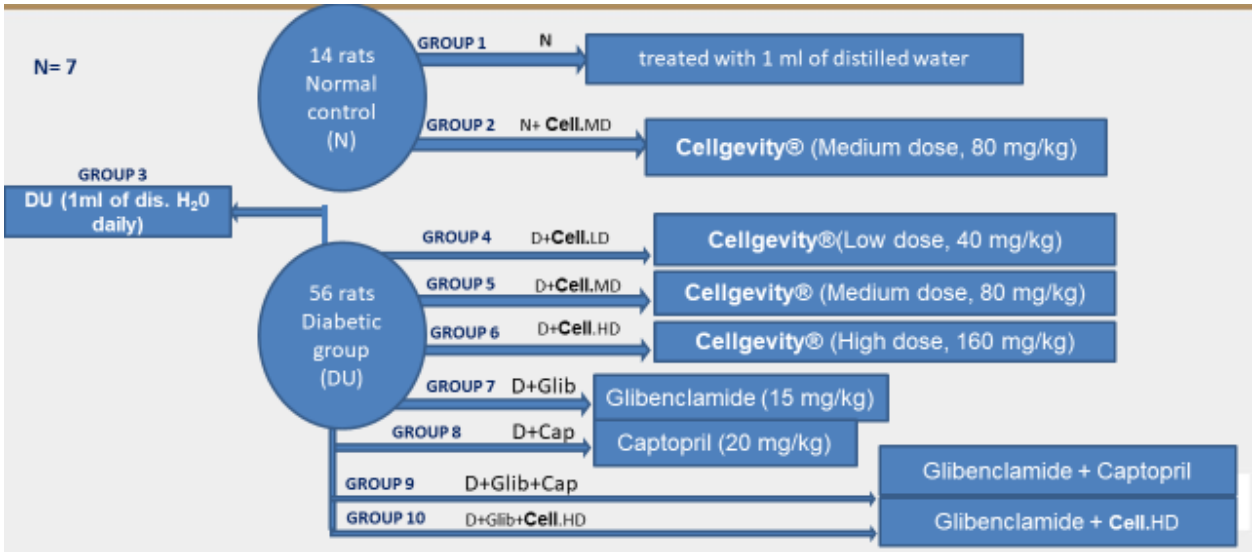


Figure 3.1: Schematic diagram of experimental groupings

3.4.2.1 Determination of hypoglycaemic activity (acute phase)

Fasting blood glucose was determined using blood from tail snip. FBG readings were taken at hourly-intervals of 1, 2, 4, 6, 12 and 24 from all rats in the respective groupings.

3.4.2.2 Determination of hypoglycaemic activity (subacute phase)

The test samples were administered orally for 28-days consecutively. FBG level of each rat was determined at 1st; 3rd; 6th; 9th; 12th; 15th, 22nd and 29th days after an hour administration of the test samples. Body weight of rats were also monitored on the same days. On the 29th day, all rats were sacrificed and their pancreas and kidneys removed for histological studies.

3.5. Preparation of solutions

3.5.1 Preparation of low, medium, and high dose of Cellgevity

Cellgevity is packaged in capsules with a capsule approximately weighing 400 mg. A suspension of Cellgevity weight per volume (w/v) was freshly prepared daily by carefully

separating the capsule body and cap, and transferring the granules into a clean mortar. The granules were triturated into fine powder and accurately weighed out (an equivalent dose administered to human) 40 mg, 80 mg, 160 mg of the powder into three separate labelled amber glass containers for the low, medium and high dose respectively using a chemical weighing balance. The weighed powders were then thoroughly dissolved in 10 ml of distilled water (vehicle) and vortexed. The suspension was shaken intermittently and administered using an oral gavage (graduated) with respect to the body weight (b.wt.) of the rat (Table 3.1). The volume to be administered to each rat was calculated as follows: $Animal\ weight\ (kg) x\ Animal\ dose\ (\frac{mg}{kg} b.wt)\ /\ Concentration\ of\ stock\ (mg/ml)$ (Appendix VI). The doses of Cellgevity® administered to rats were animal equivalents of what pertains in humans. The serving dose of Cellgevity® in human is 400 to 800 mg/kg bis die.

Table 3.1: Volume of treatment agents required for administration based on rats' body weight

Body weight (g)		***Dose (mg)***			***Volume per dose (ml)***
≤ *249*	**Cellgevity**	40 mg	**Dose (mg) of Glibenclamide 15 mg**	**Dose (mg) of Captopril 20 mg**	1 ml
≥250					**1.5 ml**
≤ *249*		80 mg			1 ml
≥250					**1.5 ml**
≤ *249*		160 mg			1 ml
≥250					**1.5 ml**

3.5.2 Preparation of glibenclamide and captopril

Tablets of glibenclamide and captopril were grounded using mortar and pestle and accurately weighed using a precision balance. Uniform mixtures (w/v) were obtained by dissolving in the vehicle (10 ml distilled water) and stirred continuously for 2 minutes. Solutions were administered to rats with emphasis on body weight using an oral gavage. The volume administered to rat models were calculated in a similar fashion as in 3.5.1.

3.5.3 Preparation of combined agents

All combined agents (glibenclamide and captopril; glibenclamide and high dose of Cellgevity) were prepared separately in a similar way as in 3.5.1 and administered to rats in two separate shots using the oral gavage.

3.6 Haematological evaluation

Twenty-eight (28) days after the induction, animals were sacrificed using di-ethyl ether (8 mg/kg b.wt). The blood samples were collected into EDTA containing tubes and were immediately used for determination of haematological parameters. The haematological parameters were determined using a Sysmex microlab analyser machine at the Madina Polyclinic Laboratory, Rawlings circle – Accra. The total red blood cell (RBC), white blood cell (WBC), haemoglobin (HGB), red blood cell indices (MCV, HCT, MCHC) among other variables were measured.

3.7 Biochemical analysis

Blood samples were collected into chemistry tubes and centrifuged at 3000 rpm for 20 minutes to obtain clear serum. The serums were separated and stored at – 80 ^{0}C until analysis. The following parameters were measured using the stored serum; urea (BUN), serum creatinine (CREA), total cholesterol (T.CHOL), triglycerides (TRIG), total protein (T.PROT), albumin (ALB), and high density lipoprotein (HDL).

3.8 Histopathology

The sacrificed rats were opened up and organs such as the liver, kidney, pancreas and heart harvested for histology. Sections of the kidney were fixed in 10% formalin, paraffin-embedded, cut into 5-micron sections and stained with haematoxylin and eosin. The stained slides were fixed with mountant, allowed to dry and viewed under the microscope (x400) by a pathologist who was blind to the experimental profiles.

3.9 Statistical analysis

Statistical analysis was performed using Graph-pad prism 7.0 statistical software. Statistical significance was assumed if p-value was less than 0.05.

Two sample t-test and paired t-test were used to analyse for significant difference between two groups. Analysis of variance (ANOVA) was used to compare the means among groups. A post-test analysis was done using Dunnett's multiple comparison test if a statiscally significant difference was determined with ANOVA.

CHAPTER FOUR

RESULTS

4.1 Healthy / Normal rats (N)

4.1.1 General characteristics and Behaviour

Rats in this group were orally administered with daily dose of the vehicle and fed with standard rat chow. Rats in this group were very active and consistently showed no signs of weakness. All rats were healthy, moved around frequently, and increased in weight with time. The percentage survival rate for the normal rats throughout the experiment was 100%.

Figure 4.1: *Healthy Sprague Dawley rats*

4.1.2 Body weight variations in normal rats administered with the vehicle ($T_0 - T_{29}$)

The average body weight of the normal control rats (N) at the beginning of the experiment (T_0) was 200 g. At the final day of the experiment (T_{29}), the body weight recorded was 231 g, representing 15.5% increase in mean body weight (Table 4) (Fig. 4.2).

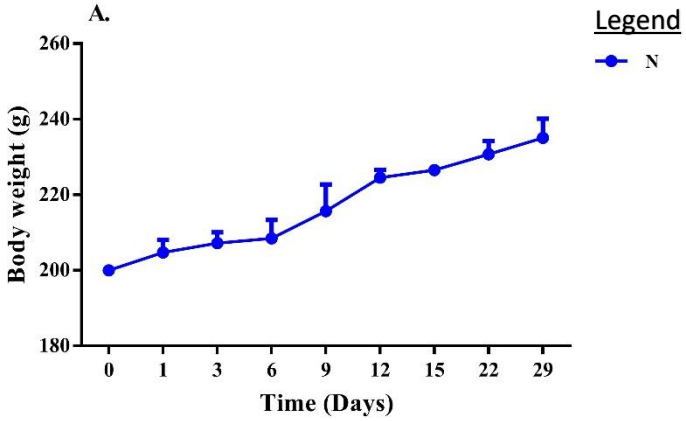


Figure 4.2: *Time course variations in body weight of normal control rats*

4.1.3 Alterations in mean fasting blood glucose levels in normal control rats administered with the vehicle for 28 days ($T_0 – T_{29}$)

The average fasting blood glucose (FBG) level at the beginning of the experiment (T_0) was 5.72 ± 0.21 mmol/L. At two weeks' interval (T_0 - T_{15}), an average of 1.4% increase in FBG value was recorded in the normal control rats. Correspondingly, the mean FBG value recorded at the end of the experiment (T_{29}) was 5.25 ± 0.35 mmol/L (Fig. 4.3). One-way ANOVA followed by Dunnett's multiple comparison tests showed that, the alterations within the mean FBG values at the various time intervals were not significantly different ($p > 0.05$).

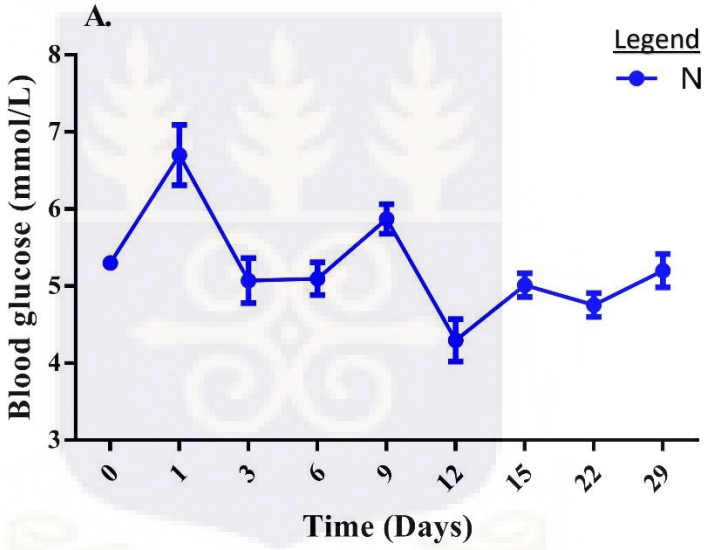


Figure 4.3: *Variations in mean FBG values of normal control rats for 28 days. Time course monitoring graph (values are represented as mean ± SEM)*

4.1.4 Haematological parameters

Haematological parameters such as WBC, PLT, RBC, HGB, MCV, MCH, and MCHC were measured in the normal control rats. The mean values for each haematologic parameter is as follows: WBC (7.13 ± 2.97 x 10^3/µL), RBC (7.76±0.95 x 10^6/µL), HGB (13.84±2.74 x 5.1 g/dL), PLT (773±228.13 x 10^3/µL), MCV (61.06±1.25 x fL), MCH (17.34±2.03 fmol/cell), and MCHC (28.36±3.31 g/dl).

4.1.5 Serum biochemistry

Serum levels of urea nitrogen (BUN), creatinine (CREA), high density lipoprotein (HDL), total cholesterol (T. CHOL), triglyceride (TRIG), total protein (T. PROT), and albumin (ALB) were measured in all sacrificed rats (Table 4.1).

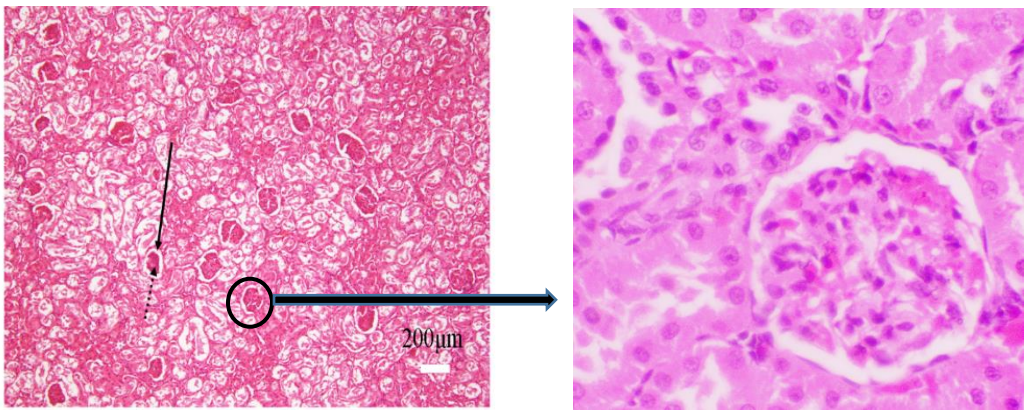
Table 4.1: Serum biochemistry parameters for the normal rats administered with the vehicle

UREA (mmol/L)	**8.67±0.19**
CREA (µmol/L)	**123.7±79.4**
T.CHOL (mmol/L)	**1.36±0.33**
TRIG (mmol/L)	**1.39±0.36**
HDL (mmol/L)	**0.87±0.18**
LDL (mmol/L)	**2.51±0.04**
T. PROT (g/L)	**64.43±2.38**
ALB (g/dL)	**98.25±1.04**

Values are represented as mean ± SEM

4.1.6 Kidney histology (H & E stain)

It was observed that (40X), the kidney histology for the normal rat were with no visible lesions, relatively large glomeruli tuft (renal corpuscle), non-ballooned bowman space (layer forming surrounding of renal corpuscle), less cell infiltrations (Fig.4.4). The normal rats administered with the vehicle exhibited typical kidney histological structures of rats.



Kidney Architecture (10X) *Histomorphometric (40X)*

Figure 4.4: Photomicrographs showing representative sections of kidney tissues (10X & 40X) in normal rats. Complete arrow(s) indicates bowman's space, broken arrow indicates glomerular tuft. N; Normal rats

4.2 Normal rats administered with medium dose of Cellgevity® (N+Cell.MD)

4.2.1 General characteristics and Behaviour

In order to investigate the effect of Cellgevity in normal rats, 80 mg/kg b.wt of Cellgevity (an equivalent dose consumed by humans) was administered orally to healthy / normal rats (n = 7) and all parameters as the case of the normal control rats (N) administered with the vehicle were measured. Rats in this group showed a heightened sense of physical activity and were very healthy throughout the experimental period. The survival rate in this group was 100%.

4.2.2 Body weight variations in normal rats administered with 80 mg/kg b.wt of Cellgevity for 28 days ($T_0 - T_{29}$)

Mean weight in this group was increased from 200 g (T_0) to 210.7 g (T_9) between the first and second week. There was a further increase in mean weight from 210.7 g (T_9) to 222 g

(T_{22}) at the end of the 3rd week. The final mean weight recorded at the end of the experiment for this group was 235 g representing 17.5% increase from the initial body weight. Time course assessment for within group ANOVA revealed a significant steady increase ($P<0.05$) in the mean weights for this group from the 3rd to 4th week. However, the overall mean weight differences for between group analyses as revealed by the graph at Fig. 4.5 indicated no significant weight differences ($P>0.05$) for this group as compared to the normal control rats.

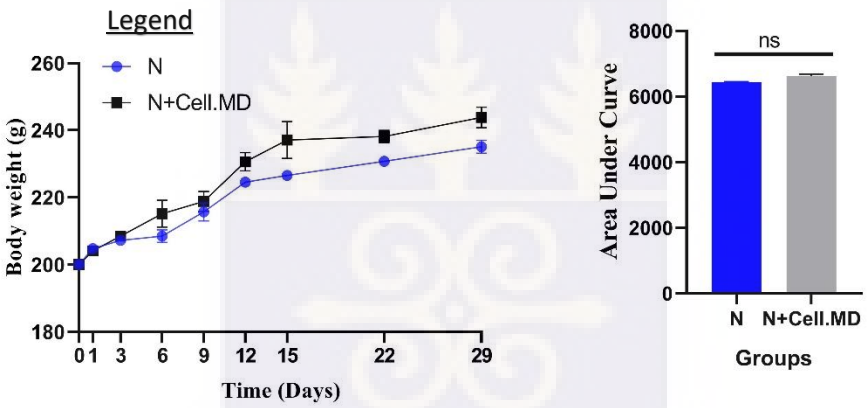


Figure 4.5: *Time course variations in body weight of normal rats administered with Cellgevity [80 mg/kg b.wt] within 28 days*

N; Normal rats administered with the vehicle, N+Cell.MD; Normal rat administered with Cellgevity [80 mg/kg b.wt]. Data (Fig. 4.5A) are presented as mean ± SEM, n=7 in a time series. Data (Fig. 4.5B) are presented as mean of Area under curve (from Fig. 4.5A) AUC ± SEM, n=7. ***ns*** *means non-significant*

4.2.3 Alterations in mean fasting blood glucose levels in normal rats administered with Cellgevity [80 mg/kg b.wt] for 28 days ($T_0 – T_{29}$)

The mean FBG value recorded at the beginning of the experiment (T_0) for this group was the same as that recorded for the normal rat group (N) (5.72 ± 0.21 mmol/L) (Fig. 4.6). It

increased from 5.72 ± 0.21 mmol/L (T_0) to 6.47 ± 0.38 mmol/L (T_1) and progressively declined to 4.96 ± 0.18 mmol/L at the end of week one (T_6). It increased slightly from T_6 to 5.78 ± 0.32 mmol/L (T_9) and stabilized with the mean FBG of the normal rat at T_{12}, T_{15}, and T_{22}. The average FBG level at T_{29} was 0.46% decreased as against the normal rat group (N) administered with the vehicle, but the decrease was not significant ($F_{8,8}$=1.635, p>0.05) (Fig.4.6). Variations in FBG were still within normoglycaemic state (that is, between 4.35 to 6.69 mmol/L).

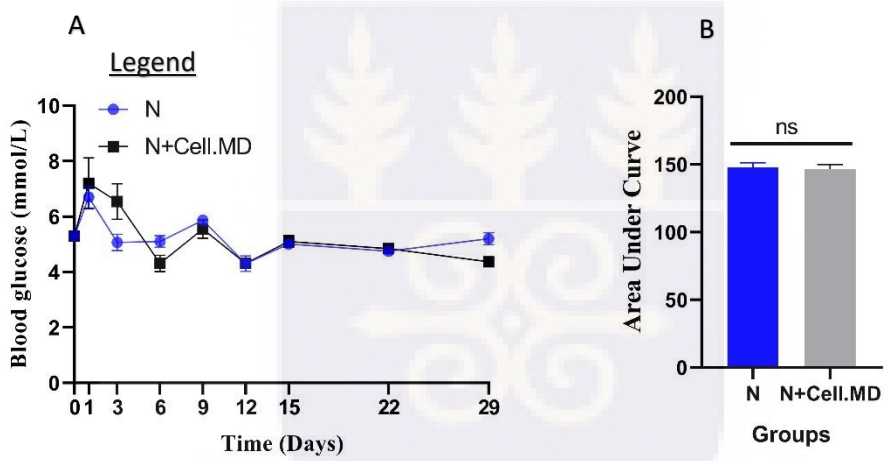


Figure 4.6: *Variations in mean FBG values of normal rats administered with Cellgevity [80 mg/kg b.wt] for 28 days. Time course monitoring graph (values are represented as mean ± SEM)*

4.2.4 Haematological parameters

Results of tests for haematological parameters are presented in Table 4.2. There were slight variations in the haematological parameters of this group as compared to the normal control rat (N). The average WBC value recorded in this group was 8.52±4.33 x10^3/µL, which was 0.25 x 10^3/µL greater in comparison with that of the normal control rat. The PLT measured in this group was 660 x 10^3/µL, representing 113 x 10^3/µL increase in PLT value as

compared to normal control rat (773 x 10^3/µL). A paired T-test indicated no significant difference between these alterations. Nonetheless, the RBC, HGB, MCV, MCH, and MCHC values were similar to that recorded in the normal control rat (Table 4.2).

Table 4.2: Effect of Cellgevity administration on haematological parameters of normal rats

Haematological parameters	N	N + Cell.MD
WBC (x10^3/µL)	7.13±2.97	8.52±4.33^{ns}
RBC (x10^6/µL)	7.76±0.95	8.01±1.66^{ns}
HGB (5.1 g/dL)	13.84±2.74	14.02±5.12^{ns}
PLT (x10^3/µL)	773±228	660±294^{ns}
MCV (fL)	61.06±1.25	59.88±1.44^{ns}
MCH (fmol/cell)	17.34±2.03	17.45±6.21^{ns}
MCHC (g/dL)	28.36±3.31	29.15±10.37^{ns}

Values represented as mean±SEM ***^{ns}p>0.05 vs the N***

4.2.5 Serum biochemistry

Results of tests for serum biochemistry are presented in Table 4.3. The BUN, TRIG, and T. CHOL values in this group were the same when compared to the normal rat group (N). The HDL, and T. PROT were slightly decreased (Table 4.3). However, CREA and ALB were markedly decreased as compared to the normal rat (N), though the decrease was not statistically significant (P>0.05) (Table 4.3).

Table 4.3: Effect of Cellgevity administration on serum biochemistry parameters of normal rats

Parameters	N	N+Cell.MD
UREA (mmol/L)	8.67±0.19	7.23±0.61^{ns}
CREA (µmol/L)	123.7±79.4	63.85±14.92^{ns}
T. CHOL (mmol/L)	1.36±0.33	1.52±0.12^{ns}
TRIG (mmol/L)	1.39±0.36	1.58±0.20^{ns}
HDL (mmol/L)	0.87±0.18	0.77±0.00^{ns}
LDL (mmol/L)	2.51±0.04	0.03±0.01^{ns}
T. PROT (g/L)	64.43±2.38	62.18±2.86^{ns}
ALB (g/dL)	98.25±1.04	82.93±1.23^{ns}

Values represented as mean±SEM $^{ns}p>0.05$ ***vs the N***

4.2.6 Kidney histology (H & E stain)

There were no visible lesions, large renal corpuscle with average bowman space and less cell infiltrations were observed. The kidney histological structures by the H & E staining technique depicted similar images as compared to that of the normal control rat (N) (Fig. 4.7). This group exhibited typical kidney histological structures of rats.

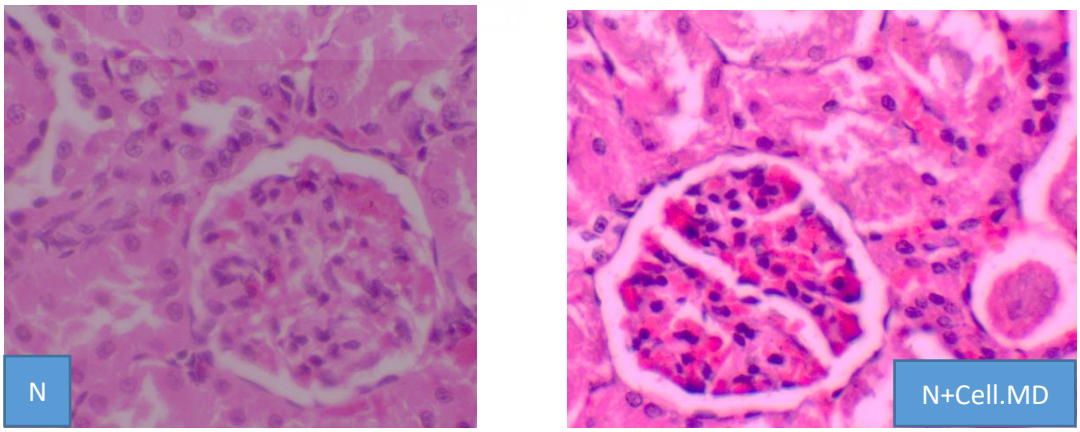


Figure 4.7: Photomicrographs showing representative sections of kidney tissues (40X) in normal rats (N) and normal rats administered with Cellgevity [N+Cell.MD]. Complete arrow(s) indicates bowman's space, broken arrows indicates glomerular tuft.

4.3 STZ-NA treated rats (Diabetic model [DU])

4.3.1 General Characteristics / Behaviour of Rats

Following STZ-NA injection, it was observed that, rats that were administered with the diabetogenic agent (diabetic untreated group and the treatment groups) became weak with high fasting blood glucose level (> 11.1 mmol/L) within 24-hours and moved around less frequently (rats appeared ill-looking). The normal control rats (N; N+Cell.MD) were more active and consistently showed no signs of weakness (rats were healthy, active and increased in weight with time). Diabetic rats administered with captopril were hyperactive. It was noted that water consumption by diabetic rats in all diabetic groups were higher (polydipsia) as compared to non-diabetic ones. Few diabetic rats presented with pedal oedema (Fig. 4.8).

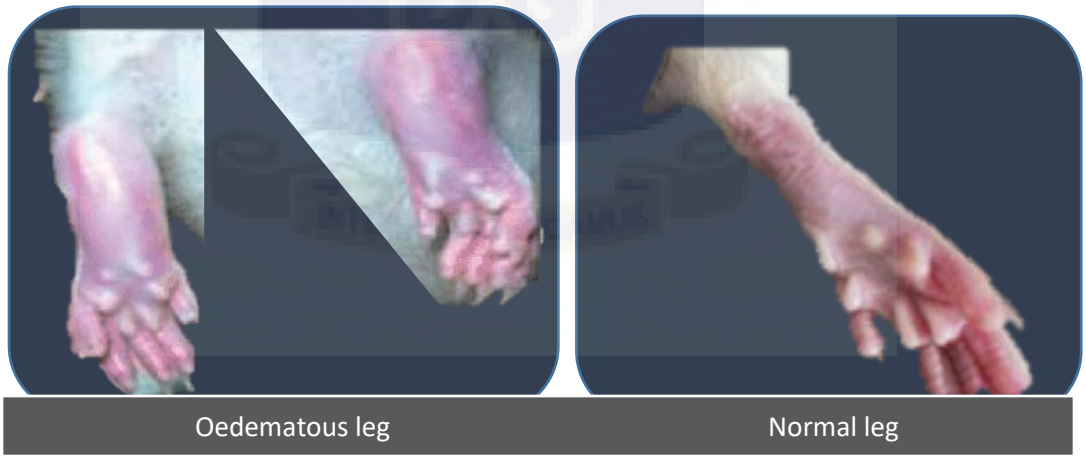


Figure 4.8: *Presentation of pedal oedema in STZ-NA diabetic rats*

4.3.2 Induction of Type-2 Diabetes Nephropathy (T2DN)

T2DN is marked by high FBG greater than 11.1 mmol/L. Approximately fifty-six rats (n = 56) were administered with STZ-NA at T_0. After 24-hours of STZ-NA injection, the average FBG value recorded for all rats that received the diabetogenic agent was 30.81 ± 1.96 mmol/L. The STZ-NA rats exhibited a significant increase in FBG value (Fig. 4.9).

4.3.3 Effect of STZ-NA on fasting blood glucose

All rats administered with STZ-NA recorded elevated FBG values (>30.0 mmol/L). These rats exhibited symptoms of hyperglycaemia with 486% increase in FBG (Fig.4.9A). There was 25.09 mmol/L increase in mean FBG value. The increase in FBG value was statistically significant ($F_{3,3}$=2.78, P<0.001) as compared to the FBG value of the normal rat group (N) (Fig.4.9B). The rats were very weak and were sacrificed within the first week (T_6) due to the destruction of the beta cells, making rats very hyperglycaemic and in a near-coma state.

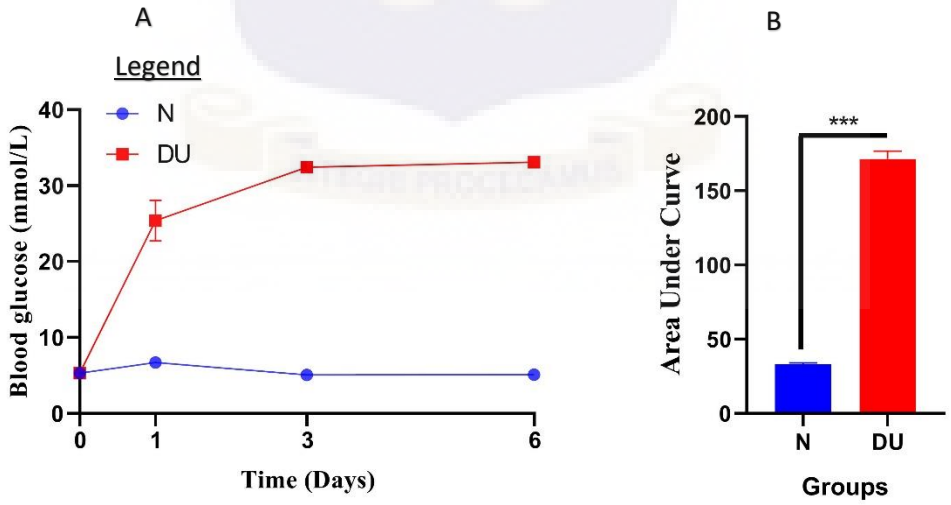


Figure 4.9: Time course variations in mean FBG values of diabetic rats (DU), N; Normal rats administered with the vehicle, DU; Diabetic rats treated with the vehicle. Data (Fig. 4.9A) are presented as mean ± SEM, n=7 in a time series. Data (Fig. 4.9B) are presented as mean of Area under curve (from Fig. 4.9A) AUC ± SEM, n=7. ***p<0.001 vs N, means significant difference

4.3.4 Effect of STZ-NA on body weight

All diabetic rats continuously decreased in body weight from 200 g (T_1) to 184 g(T_6). There was 16 g (8%) reduction in body weight within the 1st week. Diabetic rats depicted lower gain in body weight when compared to the normal rats (Fig.4.10B). The decrease in body weight was not significantly different ($F_{3,3}$=1.730, p>0.05) between the two groups (Fig. 4.10B).

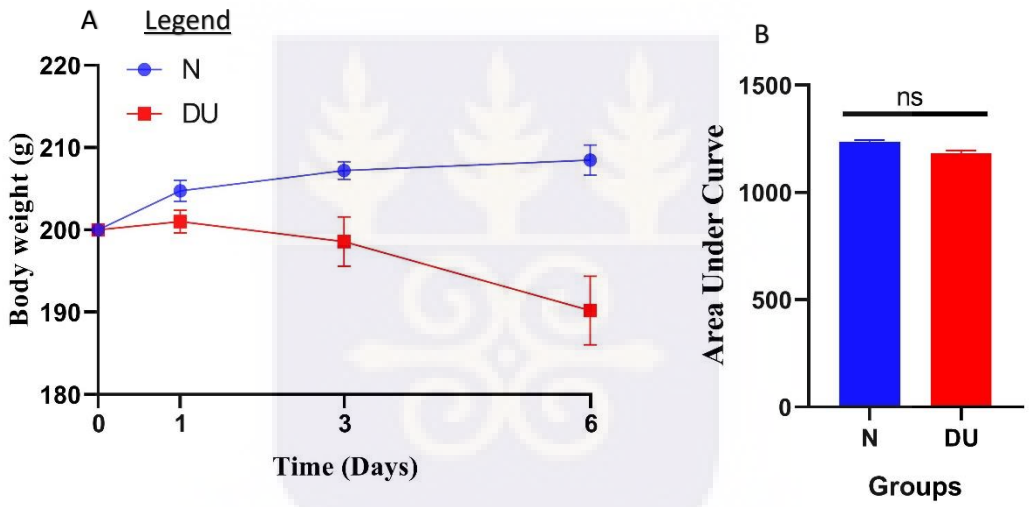


Figure 4.10: *Time course variations in mean body weight of diabetic rats (DU), N; Normal rats administered with the vehicle, DU; Diabetic rats treated with the vehicle. Data (Fig. 4.10A) are presented as mean ± SEM, n=7 in a time series. Data (Fig. 4.10B) are presented as mean of Area under curve (from Fig. 4.10A) AUC ± SEM, n=7. *p<0.05 vs N, means significant difference*

4.3.5 Effect of STZ-NA on haematological parameters

In T2DN, inflammation, stress, increased release of reactive oxygen species, and long-lasting kidney failure are known to be caused by elevated levels of inflammatory markers such as WBC and PLT.

There was 154.98% and 12.81% increase in WBC and PLT levels as compared with the healthy rat group (N) respectively (Table 4.4). Increase in WBC was significantly different ($p<0.05$) as compared to the normal control rats. Furthermore, red blood cell indices such as MCV and MCH was decreased indicating anaemic condition in diabetic rats. There were significant alterations in haematological parameters when compared to the normal control rats.

Table 4.4: Effect of STZ-NA injection on haematological parameters of diabetic rats

	PARAMETERS	N	DU	Effect of STZ-NA
Haematology	WBC ($x10^3/\mu L$)	7.13±2.97	$18.18±6.82^{****}$	↑
	RBC ($x10^6/\mu L$)	7.76±0.95	$9.23±0.20^{ns}$	↑
	HGB (5.1 g/dL)	13.84±2.74	$13.56±0.65^{ns}$	=
	PLT ($x10^3/\mu L$)	773±228	$872±175^{ns}$	↑
	MCV (fL)	61.06±1.25	$38.72±0.61^{***}$	↓
	MCH (fmol/cell)	17.34±2.03	$14.68±0.69^{ns}$	↓
	MCHC (g/dL)	28.36±3.31	$33.16±1.21^{ns}$	↑

Values represented as mean±SEM ***$^{***}p<0.05$, $^{****}p<0.0001$ $^{ns}p>0.05$ vs the N***

4.3.6 Effect of STZ-NA on serum biochemistry

Diabetic nephropathy is known to be linked with elevated BUN, TRIG, T. CHOL, and CREA, with a simultaneous reduction in T. PROT, HDL, and ALB. Increased levels of CREA and BUN are indicative of nephrotoxicity.

Most of the serum biochemistry parameters were out of range hence, were abnormal (Table 4.5). The BUN and CREA increased by 110% and 81.20% respectively in comparison to the healthy rat (N). Furthermore, T. PROT and ALB were 47.94% and 75.46% decreased

when compared to N. Alterations in BUN, CREA, T.PROT, and ALB were significantly different in comparison to N (Table 4.5).

Table 4.5: Effect of STZ-NA injection on serum biochemistry parameters of diabetic rats

	PARAMETERS	N	DU	Effect of STZ-NA
Serum Biochemistry	UREA (mmol/L)	8.67±0.19	18.4±1.05***	↑
	CREA (µmol/L)	123±79	224±8**	↑
	T. CHOL (mmol/L)	1.36±0.33	2.72±0.36ns	↑
	TRIG (mmol/L)	1.39±0.36	3.05±0.16ns	↑
	HDL (mmol/L)	0.87±0.18	0.26±0.19*	↓
	LDL (mmol/L)	2.51±0.04	Undetectablens	↓
	T. PROT (g/L)	64.43±2.38	33.54±0.04****	↓
	ALB (g/dL)	98.25±1.04	24.11±0.13****	↓

Values represented as mean±SEM ****p<0.05, **p<0.01 ***p<0.001, ****p<0.0001 nsp>0.05 vs the N***

4.3.7 Effect of STZ-NA on kidney histology

Kidney histological structures showed noticeable alterations. There were some levels of lesions in the kidney tissues. The bowman space (renal layer) was ballooned and the glomeruli tuft shrank. (Fig. 4.11).

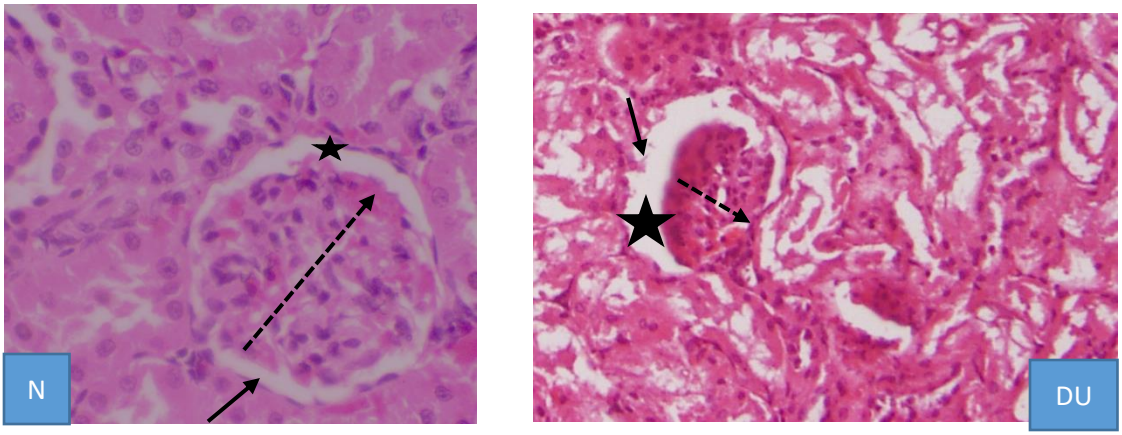


Figure 4.11: Photomicrographs showing representative sections of kidney tissues (40X) in normal rats (N) and normal rats administered with STZ-NA (DU). Complete arrow(s) indicates bowman's space, broken arrows indicate glomerular tuft, and star shows size of bowman's space.

4.4 Hypoglycaemic activity of Cellgevity and selected conventional drugs

Forty-nine (49) diabetic rats were grouped into seven with seven rats in each group. They were orally administered with the management agents (Cellgevity, Glibenclamide and Captopril) for 28-days. The hypoglycaemic effect of these management agents were assessed in two phases, namely the acute (24-hr) and subacute (28-days).

4.4.1 Acute hypoglycaemic effect of Cellgevity on FBG within 24-hour interval

The aqueous forms of Cellgevity were prepared and administered to diabetic rats at varying dose levels (low [40 mg/kg b.wt]; medium [80 mg/kg b.wt]; high [160 mg/kg b.wt]) to determine the acute effect of Cellgevity on FBG. Changes in the FBG levels in each treatment group were monitored during a 24-hour period (Fig. 4.12A-C). The low and medium doses of Cellgevity showed a slight reduction (11.26% and 14.01% respectively) in FBG levels within the 24-hour period. The groups D+Glib and D+Glib+Cap within the

24-hour period exhibited 18.21% and 25.71% decrease in FBG levels respectively. No remarkable decrease in FBG levels was observed for D+Cap, and D+Glib+Cell.HD. Both the Cellgevity and Conventional drugs did not have significant effect on FBG within the 24-hr period.

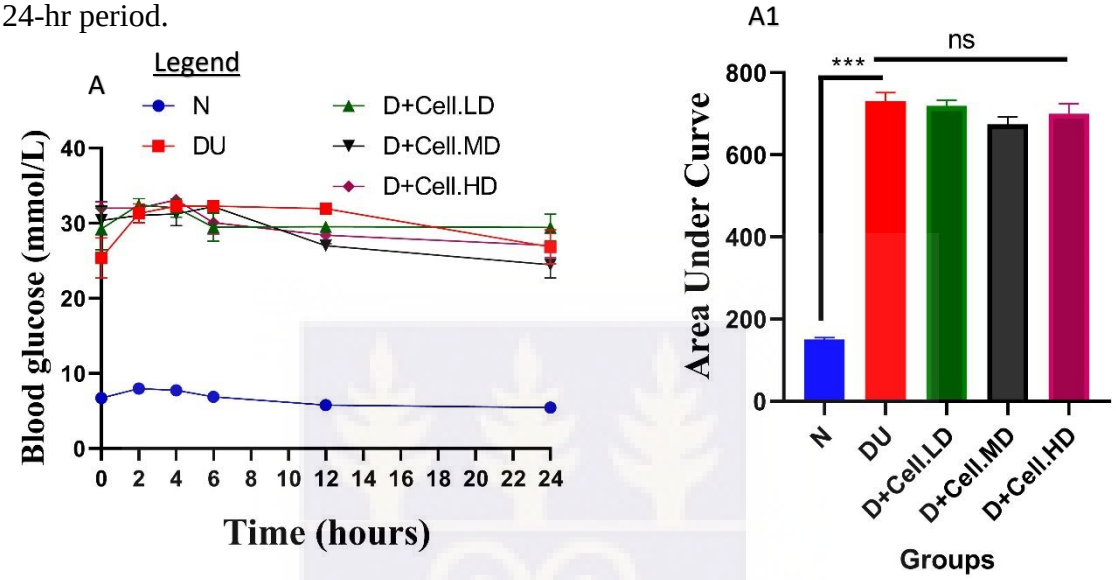


Figure 4.12 (A-A1): Acute effect of varying doses of Cellgevity on FBG levels. Time course monitoring of fasted blood glucose with hourly treatment for 24-hr

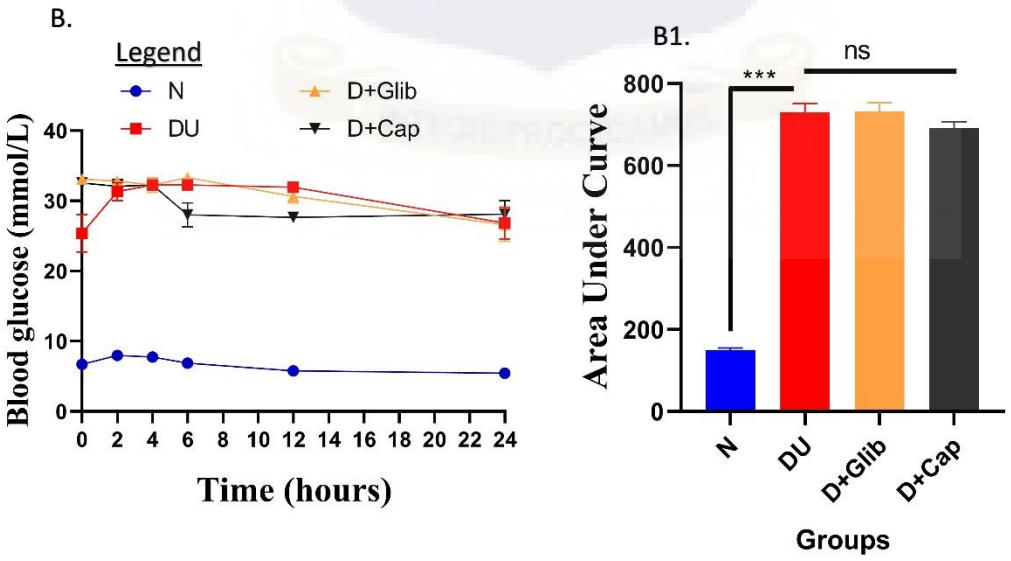


Figure 4.12 (B-B1): Acute effect of glibenclamide and captopril on FBG levels. Time course monitoring of fasted blood glucose with hourly treatment for 24-hr

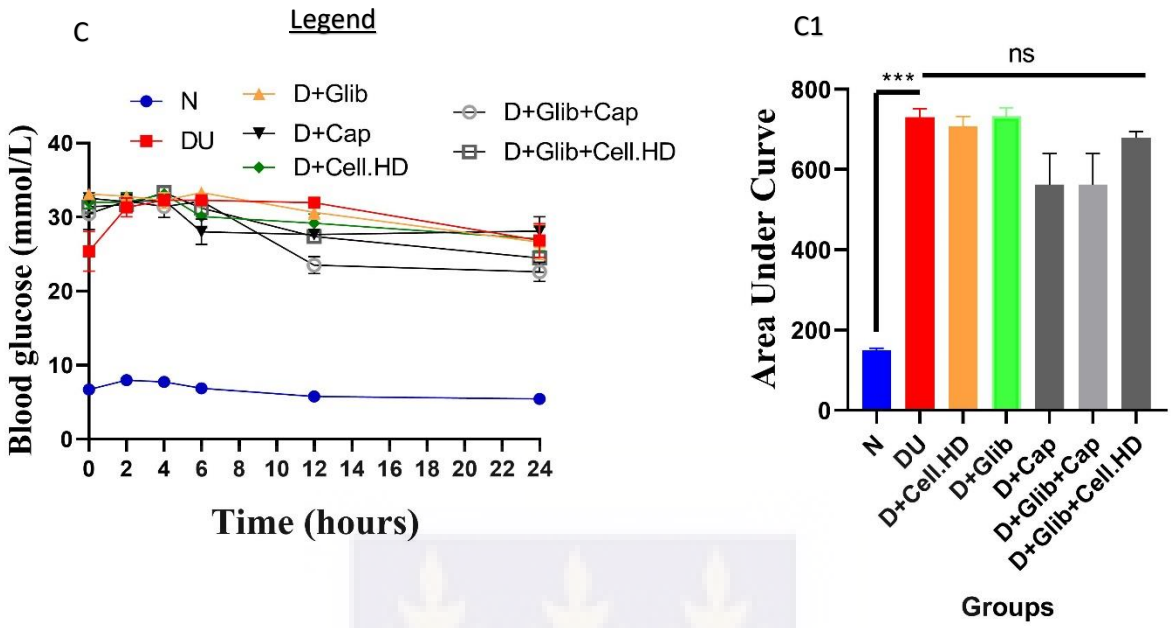


Figure 4.12 (C-C1): Acute effect of glib+cap and glib +Cell.HD on FBG levels. Time course monitoring of fasted blood glucose with hourly treatment for 24-hr

*N; Normal rats treated with 1ml distilled water, N+Cell.MD; Normal rat treated with medium dose of Cellgevity (80mg/kg b.wt), DU = Diabetic rat, D+Cell.LD; Diabetic rat treated with low dose of Cellgevity (40mg/kg b.wt), D+Cell.MD; Diabetic rat treated with medium dose of Cellgevity (80mg/kg b.wt), D+Cell.HD; Diabetic rat treated with high dose of Cellgevity (160mg/kg b.wt). Data (Fig. 4.12A-C) are presented as mean ± SEM, n=7 in a time series. Data (Fig. 4.12A1-C1) are presented as mean of Area under curve (from Fig. 4.12A-C) AUC ± SEM, n=7. Data were analyzed using One-way ANOVA followed by Dunnett's Multiple Comparison Tests: *** means the differences are significant with p<0.001 and ns means no significance difference, when compared to DU group.*

4.4.2 Subacute hypoglycaemic effect of varying doses of Cellgevity on FBG within 28-days period

In order to determine the subacute effects, the low, medium, and high doses of Cellgevity were administered for 28 days consecutively and its hypoglycaemic effect compared to that of glibenclamide and captopril. The FBG levels of each rat in each group was monitored

on 1st; 3rd; 6th; 9th; 12th; 15th; 22nd; 29th days after the administration of the test samples. The initial hypoglycaemic activity of the low, medium, and high doses were observed between the 1st to 6th days and continued successively to the 29th day (Fig. 4.13A-C).

The low (40 mg/kg b.wt) and medium (80 mg/kg b.wt) doses of Cellgevity significantly decreased FBG from 30.33 mmol/L to 17.33±3.69 mmol/L and 17.35±9.07 mmol/L respectively. The 17.33 mmol/L represent 44% decrease in FBG. The Glib and Glib+Cap reduced to 9.85±3.96 mmol/L and 9.98±5.89 mmol/L respectively. This also represents 68% decrease in FBG value. The following 3 groups; D+Cell.HD, D+Cap, and D+Glib+Cell.HD did not show noticeable decrease in FBG within the 28-days period (Fig.4.13A-C).

The observed effect with the low and medium doses of Cellgevity were more pronounced as compared to the high dose. However, the observed effect with the standard agent in the following two groups (D+Glib; D+Glib+Cap) was more pronounced (67.23%) than that of the low and medium doses of Cellgevity (44.68%).

Daily administration of all management agents irrespective of dosage at day six (T_6) tended to decrease the FBG values (Fig. 4.13A-C) when compared to DU. From the third week (T_{22}) onwards, the diabetic group treated with Cellgevity (low and medium doses) exhibited marked decrease in FBG levels (T_1-T_{29}) (Table 4.6, Fig. 4.13A), whereas the diabetic group (D+Cell.HD) treated with high dose of Cellgevity (160 mg/kg b.wt) did not show any noticeable reduction in FBG level. Similarly, the high dose of Cellgevity combined to the standard agent (glibenclamide) in group (D+Glib+Cell.HD) did not exhibit

reduction in FBG level (Fig. 4.13C). Significant decrease ($p<0.05$) in FBG were observed in the following three groups; D+Cell.LD, D+Glib, and D+Glib+Cap (Table 4.6).

A

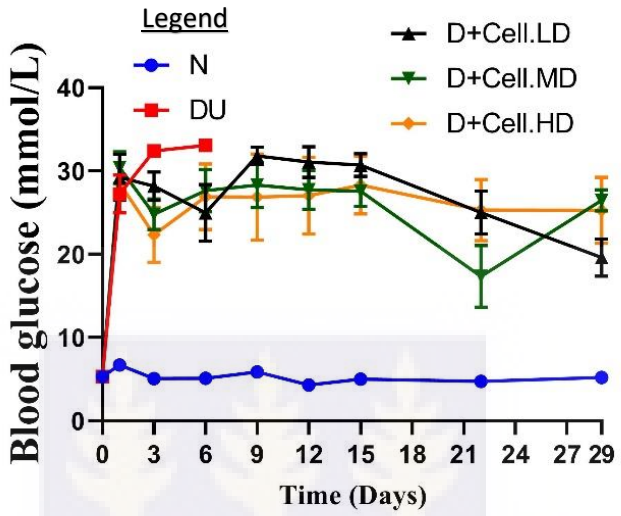


Figure 4.13(A): Subacute effect of varying doses of Cellgevity on FBG levels. Time course monitoring of fasted blood glucose.

B

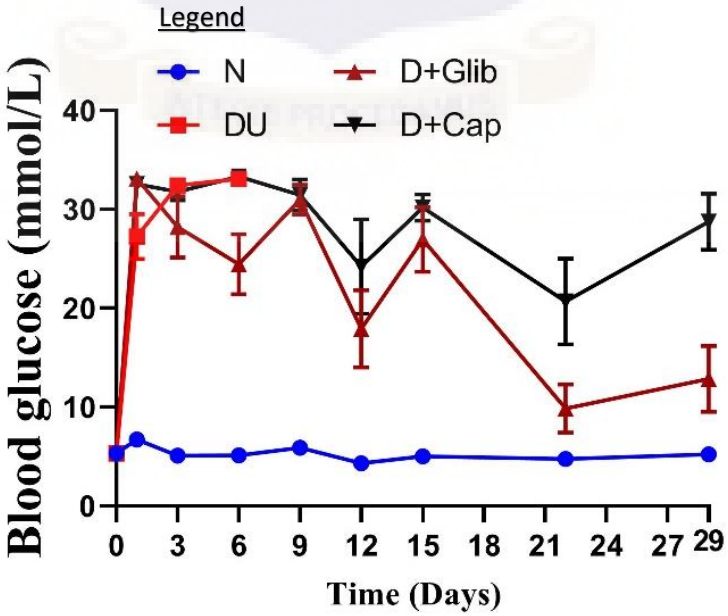


Figure 4.13(B): Subacute effect of glibenclamide & captopril on FBG levels. Time course monitoring of fasted blood glucose.

C

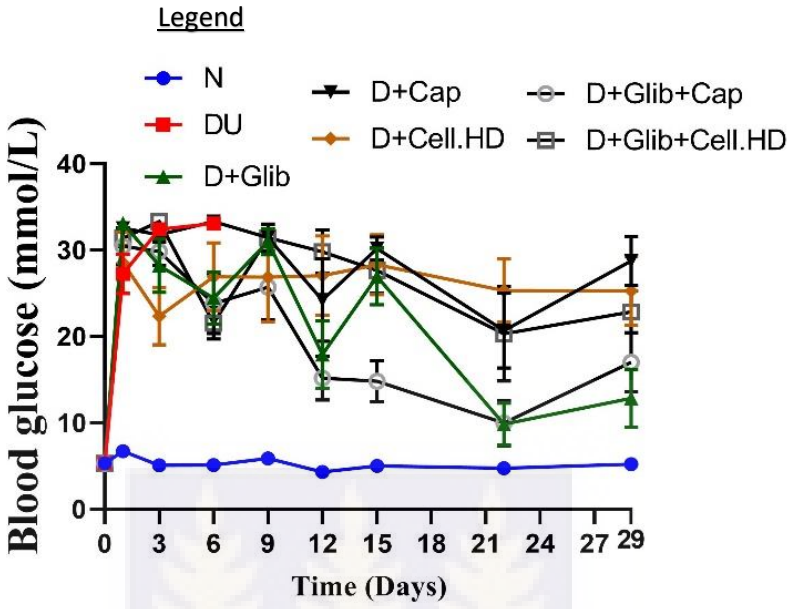


Figure 4.13(C): Subacute effect of different therapeutic agents on FBG levels. Time course monitoring of fasted blood glucose

N; Normal rats treated with 1ml distilled water, N+Cell.MD; Normal rat treated with medium dose of Cellgevity (80mg/kg), DU = Diabetic rat, D+Cell.LD; Diabetic rat treated with low dose of Cellgevity (40mg/kg), D+Cell.MD; Diabetic rat treated with medium dose of Cellgevity (80mg/kg), D+Cell.HD; Diabetic rat treated with high dose of Cellgevity (160mg/kg). Data (Fig. 4.13A-C) are presented as mean ± SEM, n=7 in a time series. Data were analyzed using One-way ANOVA followed by Dunnett's Multiple Comparison Tests.

Table 4.6: Effect of Cellgevity, Glibenclamide and Captopril on FBG

GROUPS	Mean FBG (T_1, 24-hr)	Mean FBG (T_{29}, 28-days)	Percentage change in FBG	p-value
D+Cell.LD	29.44±4.38	17.64± 5.24	↓ 40.08 % *	0.016
D+Cell.MD	24.49±1.59	19.97±7.23	↓ 18.46 % ns	0.208
D+Cell.HD	*27.03±3.59*	28.66±5.10	↑ 6.03 % ns	0.707
D+Glib	26.63±5.83	12.85±7.47	↓ 51.75 % *	0.016
D+Cap	28.10±4.76	28.76±5.68	↑ 0.0066 %	0.730
D+Glib+Cap	27.62±2.92	17.26±4.61	↓ 37.50 % *	0.019
D+Glib+Cell.HD	24.5±4.67	22.87±3.42	↓ 6.65 % ns	0.611

Values represent means ± S.E.M. for 7 rats per group; ***ns****: non-significant; * P<0.05, means significant*

N; Normal rats, N+Cell.MD; Normal rat administered with medium dose of Cellgevity (80mg/kg b.wt) DU = Diabetic untreated rat, D+Cell.LD; Diabetic rat treated with low dose of Cellgevity (40mg/kg b.wt), D+Cell.MD; Diabetic rat treated with medium dose of Cellgevity (80mg/kg), D+Cell.HD; Diabetic rat treated with high dose of Cellgevity (160mg/kg b.wt), D+Glib; Diabetic rat treated with glibenclamide, D+Cap; Diabetic rat treated with captopril, D+Glib+Cap; Diabetic rat treated with glibenclamide plus captopril, D+Glib+Cell.HD; Diabetic rat treated with glibenclamide plus high dose of Cellgevity. P-values were obtained using paired t-test to assess for level of significance between T_1 and T_{29} values.

4.4.3 Subacute effect of Cellgevity on body weight within 28-days period

The normal rat groups (N and N+Cell.MD) continuously recorded a significant increase ($p < 0.05$) in mean body weight till the end of experiment (T_{29}) while the diabetic groups continuously decreased in body weight (Table 4.7). The mean weight gain for the normal rats was 11 g (5%) whereas the mean weight reduction in body weight among the diabetic group was 28 g (17.59%). However, it was observed that diabetic rats administered with Glib and Cellgevity [40-80 mg/kg b.wt] reversely increased in weight at the end of the experiment. Diabetic rats on captopril (20 mg/kg b.wt) and high dose of Cellgevity (160 mg/kg b.wt) constantly reduced in weight till T_{29}.

4.4.4 Effect of Cellgevity on haematological parameters

Haematological alterations in diabetic groups (treated and untreated) were observed (Table 4.8A-B). The DU group had 154.98% increase in WBC in comparison with N. Nonetheless, the diabetic rats on Cellgevity (low, medium and high) doses had 25.39% decrease in WBC when compared to DU. Similarly, there was 64.25% reduction in WBC in rats administered with Glib when compared to DU. RBC and HGB levels in diabetic rats (DU) decreased (anaemia) in comparison to normal rat (N).

4.4.5 Effect of Cellgevity on serum biochemistry

There was significant increase in serum albumin (176.52%) and total proteins (79.13%) in diabetic rats administered with low and medium doses of Cellgevity respectively in

comparison with DU. Serum albumin levels in D + Glib and D + Glib + Cap increased significantly by 109.30% and 210.83% respectively compared to DU and significantly increased total protein by 27.10% and 61.87% respectively as compared with the DU.

Administration of Cellgevity low dose resulted in 68.78% and 55.87% reduction in serum creatinine and urea respectively. Similarly, the groups that were administered with D+Glib and D+Glib+Cap had a significant reduction in serum creatinine by 56.61% and 58.69% respectively in comparison with DU. The results showed that BUN and CREA were noticeable elevated in the diabetic rats (DU) as compared to the normal rat group (N) (Table 4.8A-B). BUN and CREA levels were significantly reduced in groups treated with Cellgevity (low and medium doses) and glibenclamide.

HDL were significantly elevated in rats administered with low, medium, and high doses of Cellgevity in comparison to DU (Table 4.8A). Similarly, the normal rat (N) had a higher HDL as against the DU. Groups treated with monotherapy of glibenclamide and captopril as well as their combination forms recorded a very low HDL when compared to DU (Table 4.8B).

Table 4.7: Subacute effect of Cellgevity on body weight

Parameters	*N*	*N+Cell.MD*	*DU*	*D+Cell.LD*	*D+Cell.MD*	*D+Cell.HD*	*D+Glib*	*D+Cap*	*D+Glib+ Cap*	*D+Glib+ Cell.HD*
n	**7**	**7**	**7**	**7**	**7**	**7**	**7**	**7**	**7**	**7**
Initial body weight (g)	**223**	**215**	**235**	**201**	**201**	**211**	**209**	**207**	**215**	**204**
T_0	**± 16**	**± 8**	**± 13**	**± 13**	**± 10**	**± 5**	**± 10**	**± 6**	**± 9**	**± 13**
Final body weight (g)	**232**	**228**	**184**	**168**	**168**	**184**	**187**	**164**	**187**	**172**
T_6 *–DU;* T_{28} *- others*	**±17****	**± 11^{ns}**	**± 13*****	**± 8*****	**± 6*****	**± 13***	**± 4***	**± 7*****	**± 9****	**± 14*****
Change (+/-)	**+9**	**+13**	**-51**	**-33**	**-32**	**-27**	**-23**	**-44**	**-28**	**-32**
% change in weight	**+4 %**	**+6 %**	**-22 %**	**-16 %**	**-17 %**	**-13 %**	**-11 %**	**-21 %**	**-13 %**	**-16 %**
p-value	**0.0080**	**0.1138**	**0.0005**	**0.0005**	**0.0003**	**0.0245**	**0.010**	**0.0005**	**0.0064**	**0.0002**

* P<0.05 ** P<0.01 ***P<0.001

Values represent means ± S.E.M. for 7 rats per group; **ns**: non-significant

Effects of Cellgevity, glibenclamide and captopril on body weight in STZ-NA induced type-2 diabetic nephropathy.

N; Normal rats, N+Cell.MD; Normal rat treated with medium dose of Cellgevity (80mg/kg b.wt) DU = Diabetic untreated rat, D+Cell.LD; Diabetic rat treated with low dose of Cellgevity (40mg/kg b. wt), D+Cell.MD; Diabetic rat treated with medium dose of Cellgevity (80mg/kg b.wt), D+Cell.HD; Diabetic rat treated with high dose of Cellgevity (160mg/kg b.wt), D+Glib; Diabetic rat treated with glibenclamide, D+Cap; Diabetic rat treated with captopril, D+Glib+Cap; Diabetic rat treated with glibenclamide plus captopril, D+Glib+Cell.HD; Diabetic rat treated with glibenclamide plus high dose of Cellgevity. P-values were obtained using paired t-test to assess for level of significance between initial and after values.

Table 4.8A: Effect of Cellgevity and Conventional drugs on haematological and serum biochemistry parameters after 28 days

PARAMETERS	N	DU	Effect of STZ-NA	D+Cell.LD	Effect of LD	D+Cell.MD	Effect of MD	D+Cell.HD	Effect of HD
WBC (x10^3/µL)	7.13±2.97	18.18±6.82	↑##	5.32±2.8	↓**	5.11±3.10	↓**	4.72±3.08	↓**
RBC (x10^6/µL)	7.56±0.95	9.23±0.20	↑#	6.82±1.55	↓	6.83±1.83	↓	7.54±0.22	↓
HGB (5.1 g/dL)	13.24±2.74	14.56±0.65	=	12.32±2.76	↓	12.54±2.94	↓	13.48±0.37	=
PLT (x10^3/µL)	773±228	872±175	↑ ns	757±221	↓	704±98	↓	689±177	↓
MCV (fL)	61.06±1.25	38.72±0.61	↓##	65.44±3.90	↑**	66.34±5.35	↑**	61.73±2.25	↑**
MCH (fmol/cell)	17.34±2.03	14.68±0.69	↓##	18.1±0.47	↑**	18.6±1.15	↑**	17.85±0.34	↑**
MCHC (g/dL)	28.36±3.31	33.16±1.21	↑	27.7±1.13	↓	28.07±0.72	↓	28.09±0.62	↓
UREA (mmol/L)	8.67±0.19	18.4±1.05	↑##	7.62±2.26	↓***	8.71±1.98	↓**	13.57±1.83	↓ns
CREA (µmol/L)	123±79	224±8	↑##	79.5±12.87	↓***	48.45±15.77	↓***	102.3±19.80	↓***
T.CHOL (mmol/L)	1.36±0.33	2.72±0.36	↑#	1.92±0.35	↓*	1.74±0.09	↓*	2.73±0.72	=
TRIG (mmol/L)	1.39±0.36	3.05±0.16	↑#	2.73±0.81	=	2.51±0.04	=	3.84±1.71	=
HDL (mmol/L)	0.87±0.18	0.26±0.19	↓#	0.93±0.13	↑*	1.13±0.08	↑*	1.23±0.13	↑**
LDL (mmol/L)	2.51±0.04	Undetectable	↓	Undetectable	=	Undetectable	=	Undetectable	=
T. PROT (g/L)	64.43±2.38	33.54±0.04	↓##	60.08±1.23	↑**	58.74±0.34	↑**	66.21±1.04	↑**
ALB (g/dL)	98.25±1.04	24.11±0.13	↓##	66.67±2.98	↑**	74.13±1.42	↑**	114.34±4.61	↑**

#P<0.05 vs N, * P<0.05 vs DU **P<0.01 means significant difference

ns means not significant

Values represented as mean±SEM

Table 4.8B: Effect of Cellgevity and Conventional drugs on haematological and serum biochemistry parameters after 28 days

PARAMETERS	N	DU	Effect of STZ-NA	D+Glib	Effect of Glib	D+Cap	Effect of Cap	D+Glib+Cap	Effect	D+Glib+Cell.HD	Effect
WBC (x10^3/µL)	7.13±2.97	18.18±6.82	↑##	6.5±2.77	↓**	5.74±3.66	↓**	9.71±4.51	↓*	9.4±4.38	↓*
RBC (x10^6/µL)	7.56±0.95	9.23±0.20	↑	7.29±0.67	↓	6.17±1.50	↓	7.27±1.43	↓	5.55±1.79	↓
HGB (5.1 g/dL)	13.24±2.74	14.56±0.65	=	13.57±0.87	↓	11.52±2.53	↓	13.3±2.72	↓	10.57±3.87	↓
PLT (x10^3/µL)	773±228	872±175	↑ns	533±237	↓	769±196	↓	650±267	↓	857±40	=
MCV (fL)	61.06±1.25	38.72±0.61	↓##	64.8±4.57	↑**	67.56±4.82	↑**	62.79±2.52	↑**	48.17±0.90	↑**
MCH (fmol/cell)	17.34±2.03	14.68±0.69	↓##	18.63±0.64	↑*	18.78±0.63	↑*	18.26±0.40	↑	27.33±1.23	↑**
MCHC (g/dL)	28.36±3.31	33.16±1.21	↑	28.88±1.40	↓	27.86±1.11	↓	29.09±0.91	↓	27.33±1.51	↓
UREA (mmol/L)	8.67±0.19	18.4±1.05	↑##	12.58±2.27	↓***	14.87±1.19	↓ns	10.27±0.81	↓***	13.43±4.80	↓**
CREA (µmol/L)	123±79	224±8	↑##	97±3	↓***	101.3±24.18	↓***	92.6±21.78	↓***	41.9±17.82	↓***
T.CHOL (mmol/L)	1.36±0.33	2.72±0.36	↑#	2.09±0.14	=	1.36±0.39	↓*	1.74±0.07	↓*	2.25±0.13	↓*
TRIG (mmol/L)	1.39±0.36	3.05±0.16	↑#	4.06±1.91	=	1.43±0.57	↓*	2.59±0.83	↓*	7.1±4.41	↑*
HDL (mmol/L)	0.87±0.18	0.26±0.19	↓#	0.02±0.01	↓	Undetectable	↓	Undetectable	↓	0.01±0.01	↓
LDL (mmol/L)	2.51±0.04	0	↓	0.23±0.74	=	0.71±0.13	↑	0.56±0.31	↑	0	=
T. PROT (g/L)	64.43±2.38	33.54±0.04	↓##	42.63±0.62	↑*	45.08±2.11	↑*	54.29±2.57	↑**	57.24±1.13	↑**
ALB (g/dL)	98.25±1.04	24.11±0.13	↓##	50.46±0.58	↑**	22.54±0.06	↓ns	74.94±1.22	↑***	88.01±1.01	↑***

Values represented as mean±SEM

#P<0.05 vs N, * P<0.05 vs DU **P<0.01 means significant difference, **ns** means not significant

4.5 Nephroprotective activities of Cellgevity

4.5.1 Kidney weight

The average weights of kidneys harvested from the different groups were measured. The average kidney weight of DU was 61.25% increase compared to N. The DU group had the highest mean kidney weight followed by D+Cap, D+Glib+Cap, and D+Glib+Cell.HD (Fig. 4.14). The mean kidney weight of the normal rat groups (N and N+Cell.MD) were similar to groups treated with glibenclamide, and Cellgevity (low, medium, and high doses). There was no significant difference between mean kidney weights of the normal rat (N) and that treated with Cellgevity (low, medium and high) doses. Also, the diabetic rats treated with the conventional drugs exhibited similar results (Fig. 4.14).

The confidence intervals (95% CI) using the mean kidney weights for the different groups were compared (Table 4.9).

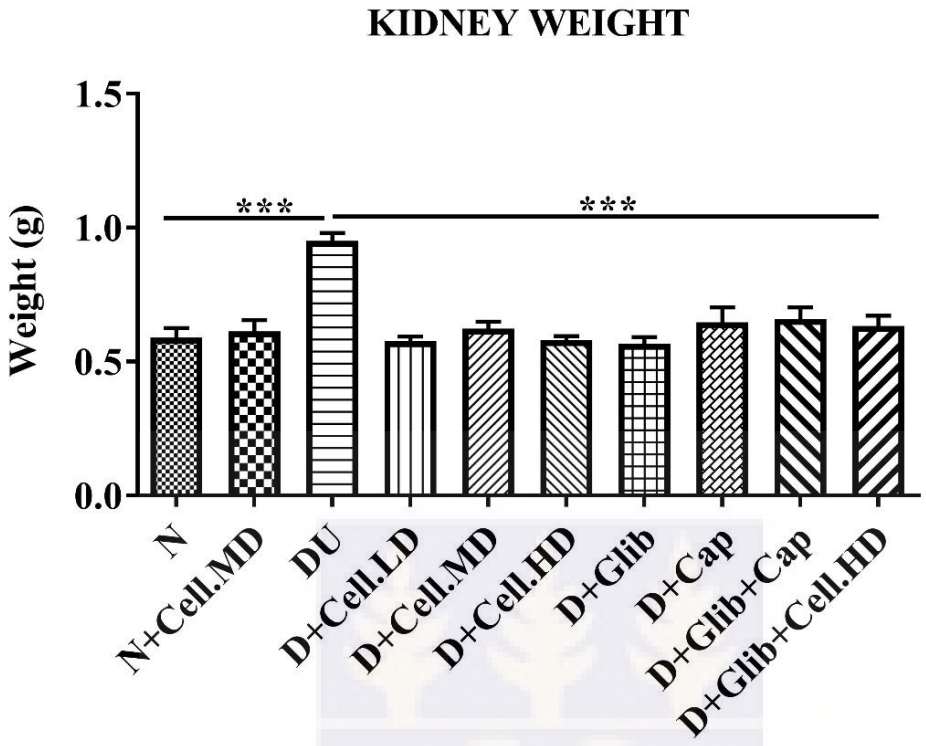


Figure 4.14: Effect of various treatments agents on Kidney weight in type 2 diabetic rats.

*N; Normal rats, N+Cell.MD ; Non diabetic rat treated with Medium dose of Cellgevity (80mg/kg b.wt) DU = Diabetic rat, D+Cell.LD; Diabetic rat treated with low dose of Cellgevity (40mg/kg b.wt), D+Cell.MD; Diabetic rat treated with medium dose of Cellgevity (80mg/kg b.wt), D+Cell.HD; Diabetic rat treated with high dose of Cellgevity (160mg/kg b.wt), D+Glib; Diabetic rat treated with glibenclamide, D+Cap; Diabetic rat treated with captopril, D+Glib+Cap; Diabetic rat treated with glibenclamide plus captopril, D+Glib+Cell.HD; Diabetic rat treated with glibenclamide plus high dose of Cellgevity. Data are presented as mean ± SEM, n=7. Data were analyzed using One-way ANOVA followed by Dunnett's Multiple Comparison Tests: *** means the differences are significant with $p<0.001$, as compared to DU group.*

Table 4.9: Mean kidney weight (g) of experimental groups post STZ-NA injection at T_{29}

N	DU	D+LGDS	D+MGDS	D+HGDS	DG	DC	DGC	DG+HGDS	95% CI
0.591 ± 0.035	0.954 ± 0.030	0.577 ± 0.017	0.623 ± 0.026	0.581 ± 0.014	0.567 ± 0.025	0.647 ± 0.056	0.659 ± 0.044	0.633 ± 0.039	DU:N (0.2193 to 0.5007)*** DU:D+Cell.LD (0.221 to 0.525)*** DU:D+Cell.MD (0.1864 to 0.4679)*** DU:D+Cell.HD (0.2236 to 0.5147)*** DU:D+Glib (0.2378 to 0.5289)*** DU:D+Cap (0.151 to 0.455)*** DU:D+Glib+Cap (0.15 to 0.4314)*** DU:D+Glib+Cell.HD (0.141 to 0.492)*** N:DU (-0.5007 to -0.2193)*** N:N+Cell.MD (-0.152 to 0.1049) ns N:D+Cell.LD (-0.128 to 0.1537) ns N:D+Cell.MD (-0.1613 to 0.0956) ns N:D+Cell.HD (-0.1245 to 0.1429) ns N:D+Glib (-0.1104 to 0.157) ns N:D+Glib+Cap (-0.1977 to 0.05918) ns N:D+Glib+Cell.HD (-0.2092 to 0.1225) ns

N; Normal rats, N+Cell.MD ; Non diabetic rat treated with Medium dose of Cellgevity (80mg/kg) DU = Diabetic rat, D+Cell.LD; Diabetic rat treated with low dose of Cellgevity (40mg/kg), D+Cell.MD; Diabetic rat treated with medium dose of Cellgevity (80mg/kg), D+Cell.HD; Diabetic rat treated with high dose of Cellgevity (160mg/kg), D+Glib; Diabetic rat treated with glibenclamide, D+Cap; Diabetic rat treated with captopril, D+Glib+Cap; Diabetic rat treated with glibenclamide plus captopril, D+Glib+Cell.HD; Diabetic rat treated with glibenclamide plus high dose of Cellgevity. Data were analyzed using One-way ANOVA followed by Dunnett's Multiple Comparison Tests

4.5.2 Kidney Morphometric

4.5.2.1 Glomerulus tuft and Bowman's space

The morphometric variables at low power (10X) showed an architecture of the glomerulus tuft (G.T) and bowman's space in the ten different groups. The kidney microstructures of the diabetic rat (DU) revealed changes as compared to the normal rat (N). In comparison with N, it was observed that the G.T in the DU group were reduced in size and the bowman's space was ballooned at high power (40X) (Fig.4.15). There was marked differences in G.T and bowman's space in groups receiving the different concentrations of Cellgevity in comparison to DU. Similar trends were observed for groups receiving the standard regimens (D+Glib, D+Cap and D+Glib+Cap) as compared to DU (Fig.4.15).

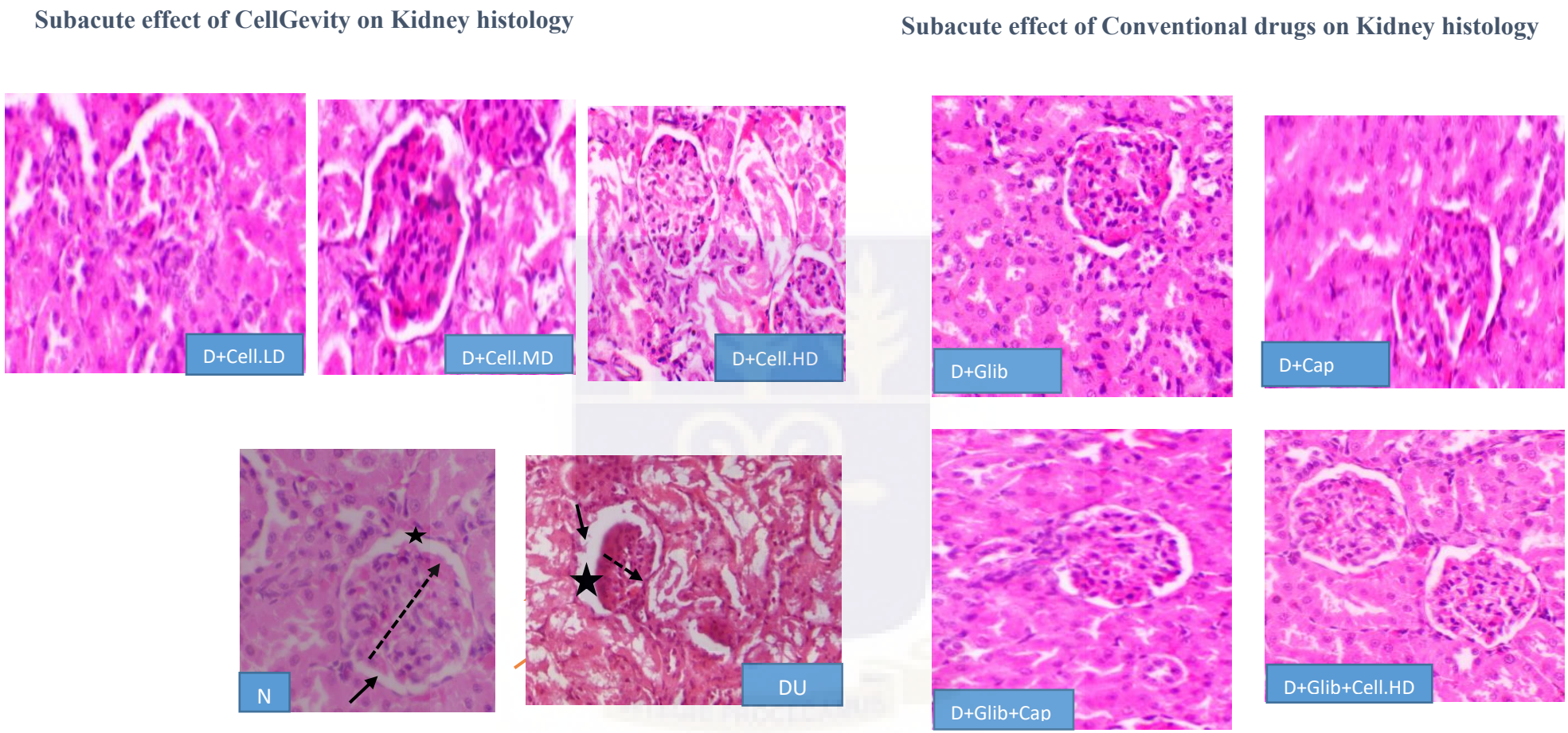


Figure 4.15: Photomicrographs showing representative sections of kidney tissues (40X). Complete arrow(s) indicates bowman's space, broken arrows indicate glomerular tuft, and stars shows level of damage

CHAPTER FIVE

DISCUSSION

5.1 Introduction

Type-2 diabetes mellitus (T2DM) is known as a complex metabolic disorder pronounced by lack of blood glucose concentration homeostasis and abnormal metabolic pattern of carbohydrates and lipids (Szablewski, 2011; Han *et al*., 2016). Persistent hyperglycaemia during T2DM can cause many chemical alterations such as nonenzymatic glycosylation of proteins in the body (Szablewski, 2011) or generating Reactive Oxygen Species (ROS). These chemical mechanisms in the disease aetiology are responsible for most of the complications associated to patients living with the disorder.

Diabetic nephropathy caused as a result of injury to the kidney is mostly linked with increased oxidative stress, reactive oxygen species (ROS), and alterations in haematological and serum biochemistry parameters. These agents affect the levels of endogenous antioxidants like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPH-Px). Dietary supplements are good sources of exogenous antioxidants which might be considered important remedies to attenuate pathological alterations in oxidative stress–related chronic kidney diseases (CKD) through reduction of lipid peroxidation and enhancement of levels of endogenous antioxidants (Palipoch, 2013).

This study was done to investigate the hypoglycaemic and renoprotective potential of a glutathione dietary supplement (Cellgevity) against STZ/NA-induced type 2 diabetic nephropathy in experimental rat models. It was expected that the dietary supplement rich

in antioxidant would serve as an intervention that favours depuration of ROS to attenuate or prevent the oxidative stress, thereby mitigating subsequent renal damage.

5.2 General behaviour /characteristics of rats

All rats before STZ-NA injection were healthy and very active. After STZ-NA injection, rats exhibited some level of behavioural changes including drinking more water (polydipsia), and urinating frequently (polyuria). Few rats in the diabetic groups presented with pedal oedema indicating symptoms of nephritis (acute kidney inflammation). This was attributed to high levels of renal function markers like urea and creatinine. Mortality in the diabetic untreated group was 100% higher in comparison to the healthy / normal rats. The diabetic untreated group (DU) exhibited 100% mortality rate just within six days post STZ-NA injection. Animals in this group were very weak and due to this, they were sacrificed on the 6th day. This was contrary to other diabetic research works which used similar diabetogenic agents (120 mg/kg b.wt NA; 55-65 mg/kg b.wt STZ) *sensu* Ayodele *et al*. (2018) and Mozafari *et al*. (2015). The high mortality rate in this group could be attributed to the destruction of the beta cells, making rats hyperglycaemic and in a near-coma state.

5.3 Effect of Cellgevity®, glibenclamide and captopril on body weight

The body weights of rats were measured as a means of determining the growth index in this experiment. The normal group recorded an increased in body weight throughout the experiment. The diabetic groups administered with the various agents consecutively

decreased in body weights up to the twenty-second (22^{nd}) day and began to increase in weight till the twenty-ninth (29^{th}) day. The slight upturn in body weight at the end of the experiment could be ascribed to better glycaemic control, less oxidative stress, and preservation of pancreatic beta cells by the various agents. Though there was a significant decrease in weight for the groups that were administered with standard drugs (D+Glib only and D+Glib+Cap) and the group that were given the test drug Cellgevity®, this decrease was estimated to be 40% for the test groups which was better as compared to the negative control group (DU). Hence Cellgevity® may have some antidiabetic properties for which's mechanism of action has to be studied. The weight gain was higher in groups receiving low and medium doses of Cellgevity (D+Cell.LD and D+Cell.MD). A possible explanation may be due to the fact that Cellgevity contains high level of antioxidant / polyphenols which is known to mitigate oxidative stress in the body (Nagasawa, 2015; N'guessan *et al*., 2018; Awodele *et al*., 2018).

The diabetic untreated group (DU) significantly ($p < 0.05$) reduced in body weight post STZ-NA injection. A study by Ayodele *et al*. (2018) indicated a significant reduction in body weight among the diabetic untreated group using at least 60 mg/kg b.wt STZ with 120 mg/kg b.wt NA. More so, research shows that reduction in insulin action as the case of T2DM leads to weight loss (Zheng *et al*., 2012; Adedapo *et al*., 2016). Increased levels of blood glucose are linked with reduced insulin levels in the peripheral system.

5.3 Effect of Cellgevity, glibenclamide and captopril on fasting blood glucose

Before the induction of diabetes in the various groups, there was no significant difference among all the groups and the average recorded FBG (5.35 mmol/L). On the 6^{th} day (T_6),

there was a 486% increase in FBG when compared to the normal rat group (N). Low dose of Cellgevity caused 40.08% decrease in FBG within the 28-days period. Similarly, the groups that received Glib and a combination of Glib and Cap had 51.75% and 37.50% decrease in FBG levels respectively within the 28 days.

The FBG levels at various time intervals were higher in the diabetic untreated rats. An unusual pattern in the FBG levels of diabetic rats treated with the various agents was observed with time. The FBG increased steeply initially until two weeks (T_{12}) post STZ/NA effect and then decreased sharply until the third week (T_{22}). The FBG levels further decline at the 4th week (T_{29}) following daily administration of the treatment agents in the following groups (D+Cell.LD; D+Cell.MD; D+Glib; D+Glib+Cap). The groups treated with captopril (D+Cap), high dose of Cellgevity (D+Cell.HD), and its combination (D+Glib+Cell.HD) had an elevated blood glucose level at T_{29}. Similar trends have been reported by several researches (Fonseca, 2009; Matheka *et al*., 2012; Adam *et al*., 2016).

Cellgevity low and medium doses showed a marked decrease on FBG within the experimental period. However, there were no dose-specific patterns in FBG related to the administration of Cellgevity. The low dose of Cellgevity compared with both the medium and high doses exhibited a significant decrease ($p<0.05$) on FBG. Furthermore, the Cellgevity (low dose) had a similar activity on FBG in comparison with glibenclamide (a known antidiabetic, hypoglycaemic, and antihyperglycaemic agent). Hence, Cellgevity (low dose) may have hypoglycaemic and antidiabetic effect in management of T2DM.

5.4 Effect of Cellgevity, glibenclamide and captopril on Serum biochemistry and haematological parameters

5.4.1 Renal function markers (BUN and CREA)

In DN, the clinical symptoms include elevated serum creatinine (CREA), blood urea nitrogen (BUN), with a corresponding decline in glomerular filtration rate (GFR) (Bjornstad *et al*., 2015; Karar *et al*., 2015). Blood assays for CREA has been commonly used as a measure of the occurrence and advancement of kidney related diseases. According to research by Macauley (2015), it is expected that with kidney disease and subsequent loss of nephrons, the level of CREA will be significantly elevated. On the contrary, with improving kidney function, the level of CREA will therefore exhibit a downward drift. Testing for blood levels of BUN is the least complicated way to monitor kidney function. BUN is produced when protein breaks down and is primarily excreted by the kidneys. When there is kidney abnormalities, BUN is poorly excreted and so reversely accumulate in the blood that consequently result in an increase in blood levels of urea (Molitoris, 2007).

In this study, administration of low dose Cellgevity resulted in a 68.78% and 55.87% decrease in CREA and UREA respectively when compared to DU. It may be inferred that, Cellgevity had some nephroprotective effect which was evident in the results. The groups that received conventional drugs (Glib and/ or Cap) had 45.59% and 57.4% decrease in CREA and UREA respectively in comparison with DU.

Serum UREA and CREA levels increased in the diabetic untreated (DU) group. This was congruent to that described by Latchoumycandane (2014). In his work, he attributed the sudden loss of kidney function to poor glomerular filtration rate leading to the increased levels of the serum UREA and CREA. It is an undisputable fact that, these parameters do not increase in the blood until at least half of the kidney nephrons are destroyed (DeRossi *et al*., 2008). The normal and diabetic groups receiving different agents of treatment had a lower serum UREA and CREA levels as compared to the DU group.

The glutathione dietary supplement caused a diminution in serum UREA and CREA levels depicting that there was a synergistic action of the dietary supplement polyphenols which conferred nephroprotection.

5.4.2 Serum protein and albumin

The total serum protein and albumin for the diabetic untreated group (DU) was very low as compared to the normal and the diabetic treated with the varying doses of dietary supplement (D+Cell.LD; D+Cell.MD; D+Cell.HD). Moreover, the total protein for the groups administered with the standard regimens where higher in comparison to the DU group. However, the groups dubbed DG and DC recorded lower values of serum albumin. This result is similar to some research works done earlier (Karagul *et al*., 2000; Kafa, 2006; Bhonsle *et al*., 2012).

Albumin is known to be the most abundant proteins in blood and can be glycated in type-2 diabetic mellitus models (Bhonsle *et al*., 2012). In this study the serum albumin increased significantly ($p < 0.001$) in the normal and diabetic treated with varying doses of

glutathione dietary supplement. The albumin levels of groups (D+Glib and D+Cap) were however not-significant when compared to the DU group. These results are consistent with reports by the following research works (Yuksek *et al*., 2013; Mehmetoglu., 2002).

5.4.3 Inflammatory markers

It was observed that the white blood cells (WBC) and platelets (PLT) counts in the normal and diabetic treated with various agents were significantly reduced ($p < 0.01$) when compared to the diabetic untreated group (DU). The mechanism of WBC and PLT increase in diabetics is unknown. However, it is known that certain activated pathways like TNF-α, TGF1, NF-kB may contribute to this phenomenon (Yakhchalian *et al*., 2018; Mahmoud, 2013; Hoffman *et al*., 1998; Kanter *et al*., 2007).

5.4.4 Red blood cell indices

The total number of mean corpuscular volume (MCV) and mean concentration of haemoglobin (MCH) were significantly lower to that of the normal and diabetic groups treated. These trends observed suggest inflammatory conditions, anaemia, tissue damage, and chronic kidney failure. This is in congruent to well-documented research work by Weiss *et al*. (2005) and Keskin *et al*. (2016). Moreover, the MCV and MCH values in diabetic untreated group showed significant decrease in accordance with prior haematological studies reporting anaemia as a pathophysiological complication of diabetes mellitus (Akindele *et al*., 2012).

5.5 Kidney morphometry

5.5.1 Kidney weight

The result from this study proved that rats in the diabetic untreated group (DU) had the highest mean kidney weight when compared to the other groups. Meier *et al*., (2007) reported that protein kinase C (PKC) activation induced renal glomerular hypertrophy. PKC induces expression of transforming growth factor beta-1 (TGF-β1), fibronectin and type IV collagen in glomeruli as well as connective tissue growth factor thereby increasing the amount of diacyl glycerol (DAG) and increasing organ weight.

5.5.2 Glumeruli tuft and Bowman's space

Nephrotoxicity in diabetic untreated rats was histologically marked by damaged glomeruli tuft, severe ballooning of Bowman's space, and hypertrophy. This is similar to earlier studies (Wang *et al*., 2015; Zhang *et al*., 2016). These conditions were ameliorated in diabetic rats treated with the various regimens suggesting nephroprotective effect of dietary supplement, captopril and glibenclamide.

5.6 Summary of key findings

Type 2 diabetes mellitus (T2DM) and diabetic nephropathy (DN) were established as indicated by the following findings:

1. Mean fasting blood glucose of diabetic groups (DU, D+Cell.LD, D+Cell.MD, D+Cell.HD, D+Glib, D+Cap, & D+Glib+Cell.HD) at twenty-four hours (24-h) post STZ/NA injection were significantly increased as compared to the mean FBG of the

non-diabetic group (N). The mean FBG of the diabetic groups were significantly greater than the mean FBG recorded at baseline (two days [T_{-2}] and one day [T_{-1}] prior to STZ/NA injection). Consecutively, the mean body weight decreased among the groups administered with STZ/NA but the normal groups slightly increased in weight.

2. Fasting blood glucose, platelets, white blood cells, serum creatinine and urea were significantly increased in the diabetic untreated group as compared to the normal groups. More so, HDL, MCV, total protein and albumin were significantly reduced in the diabetic untreated (DU) group.

3. Nephrotoxicity was confirmed in rats administered with STZ/NA. This was evident in elevated levels of kidney function markers like blood urea, serum creatinine in DU group. The relatively minimal levels of serum urea and creatinine in groups treated with various agents affirmed that regular management of T2DM attenuated kidney injury physiologically.

4. Glycaemic control and nephroprotection by Cellgevity (low dose) in type-2 diabetic mellitus rats were comparable to those of conventional drugs (glibenclamide and captopril) as it had significant effect on FBG, creatinine, urea, leucocytes, platelets, total protein and albumin.

5. The mean kidney weights of the diabetic untreated (DU) group at the end of the experiment was significantly greater than the normal (N) and diabetic treated with different management agents (D+Cell.LD, D+Cell.MD, D+Cell.HD, D+Glib, D+Cap, & D+Glib+Cell.HD).

6. The glomeruli tuft and bowman space in the kidneys harvested from the diabetic untreated group showed noticeable alterations when compared to the diabetic treated with the test samples (Cellgevity, glibenclamide, and captopril).

7. Cellgevity (low dose) may be beneficial in the management of T2DM and help prevent its microvascular complications (that is, diabetic nephropathy).

5.7 Conclusion

This study has demonstrated nephrotoxicity caused by STZ/NA injection after six weeks of experimental period. It was however revealed that regular usage of Cellgevity® may be helpful in the management of hyperglycaemia and retard diabetic-nerve induced damages on kidney structure and function. Though Cellgevity® had good effect on FBG, the conventional drug glibenclamide had better activity on FBG.

Also, the histological analysis demonstrated that, diabetic groups treated with Cellgevity® and glibenclamide had smaller bowman's space when compared to diabetic untreated group. Administration of Cellgevity® in higher doses adjuvant to antidiabetic drugs may be detrimental to health.

This research confirmed that Cellgevity® has effect on blood glucose, however high doses of it may be harmful as a result of free-radical mechanism and provides proof that low doses of Cellgevity® protects the kidney and ameliorates the severity of injury caused by STZ/NA toxicity. However, extensive studies must be conducted to affirm its clinical application.

5.8 Limitations of the study

1. Levels of ROS and glutathione peroxidase activities were not measured in kidney tissue (or blood) to implicate the antioxidant property of the dietary supplements in T2DM-induced DN due to lack of fund.
2. Electron microscopy study of the glomerulus basement was not done due to time and financial constraints.

5.9 Recommendation

1. Female rats should be included in future studies to identify any sexual differences the therapeutic of dietary supplement may have on T2DM
2. Morphometric analysis of glomerular basement membrane should be considered at the Electron Microscopy (EM) level to assess its damage.
3. The levels of reactive oxygen species (ROS) and glutathione peroxidase (GSH-Px) activities should be measured in kidney tissue (or blood) to implicate the antioxidant property of the dietary supplements in T2DM-induced DN.

REFERENCES

ADA, (2017). Lifestyle Management. *Diabetes Care.* 1: 1247-1248. https://doi.org/10.2337/dc17-S007

Adam, S. H., Giribabu, N., Kassim, N., Kumar, K. E., Brahmayya, M., Arya, A., & Salleh, N. (2016). Protective effect of aqueous seed extract of Vitis Vinifera against oxidative stress, inflammation and apoptosis in the pancreas of adult male rats with diabetes mellitus. *Biomedicine and Pharmacotherapy.* 81:439–452. https://doi.org/10.1016/04.032

Adedapo A.A., Ogunmiluyi I.O., Adeoye A.T., Ofuegbe S.O., & Emikpe B.O. (2016). Evaluation of the medicinal potential of the methanol leaf extract of Chromolaena odorata in some laboratory animals. *Journal of Medicinal Plants Studies.* 4(3): 29-37

Adeshara K.A., Diwan A.G., & Tupe R.S. (2016). Diabetes and complications: cellular signaling pathways, current understanding and targeted therapies. *Current Drug Targets*. 17:1309–1328

Adhikary, L., Chow, F., Nikolic-Paterson, D. J., Stambe, C., Dowling, J., Atkins, R. C., & Tesch, G. H. (2004). Abnormal p38 mitogen-activated protein kinase signalling in human and experimental diabetic nephropathy. *Diabetologia*, *47*(7), 1210-1222.

Adler A.I, Stevens RJ, Manley, SE, Bilous, RW, Cull, CA, Holman, RR, and Group, U (2003). Development and progression of nephropathy in type 2 diabetes: the United Kingdom Prospective Diabetes Study (UKPDS 64). *Kidney International. 63*(1), 225-232.

Afolayan, A. J., & Wintola, O. A. (2014). Dietary supplements in the management of hypertension and diabetes - a review. *African journal of traditional, complementary, and alternative medicines. 11*(3), 248–258.

Akindele O, Babatunde A, Chinedu F, Samuel O, Oluwasola C, Oluseyi A. (2012). Rat model of food induced non-obese-type 2 diabetes mellitus; comparative pathophysology and histopathology. *International Journal of Physiology, Pathophysiology and Pharmacology. 2012;4(1):51-8.*

Al-Faris NA, Al-sawadi AD, Alokail MS (2010). Effect of samh seeds supplementation (Mesembryanthemum forsskalei Hochst) on liver enzymes and lipid profiles of

streptozotocin (STZ)-induced diabetic Wistar rats. *Saudi Journal of Biological Sciences.* 17(1):23-28.

American Diabetes Association, (2012). Diagnosis and classification of diabetes mellitus. *Diabetes care. 36:* S67–S74. doi:10.2337/dc13-S067

Anderson S., Rennke H., Brenner B. (1986). Control of glomerular hypertension limits glomerular injury in rats with reduced renal mass. *Journal of Clinical Invest76:612–619, 198631.*

Anderson S., Rennke H., Brenner B. (1993). Therapeutic advantage of con-verting enzyme inhibitors in arresting progressive renal disease associated with systemic hypertension in the rat. *Journal of Clinical Invest. 77:1993–2000, 198632.*

Arévalo-Lorido J. C, Carretero-Gómez J, García-Sánchez F, MaciáBotejara E, Ramiro-Lozano J. M. (2016). Secondary hyperparathyroidism prevalence and profile, between diabetic and non-diabetic patients with stage 3 to 4 chronic kidney disease attended in internal medicine wards MiPTH study. *Diabetes Metabolic Syndrome. 10:16-21.*

Aronoff, S. L., Berkowitz, K., Shreiner, B. & Want, L. (2004). Glucose metabolism and regulation: beyond insulin and glucagon. *Diabetes Spectrum*, 17(3), 183-190

Aros C., Remuzzi G. (2002). The renin-angiotensin system in progression, remission and regression of chronic nephropathies. *Journal Hypertension.* 20:[Suppl 3]:S45–S53

Awodele, O., Badru, W. A., Busari, A. A., Kale, O. E., Ajayi, T. B., Udeh, R. O., & Emeka, P. M. (2018). Toxicological evaluation of therapeutic and supra-therapeutic doses of Cellgevity® on reproductive function and biochemical indices in Wistar rats. BMC *Pharmacology and Toxicology*. 19(1): 1–16. https://doi.org/10.1186/s40360-018-0253-y

Ayepola, O. R., Chegou, N. N., Brooks, N. L., & Oguntibeju, O. O. (2013). Kolaviron, a Garcinia biflavonoid complex ameliorates hyperglycemia-mediated hepatic injury in rats via suppression of inflammatory responses. *BMC complementary and alternative medicine*, *13*(1), 363.

Ayodele O.M., Titilola A. S., Daniel A. A. (2018). Magnesium upregulates insulin receptor and glucose transporter-4 in streptozotocin-nicotinamide-induced type-2 diabetic rats. *Endocrine Regulations*, 52: 6–16, doi:10.2478/enr

Azimova, K., San Juan, Z., & Mukherjee, D. (2014). Cardiovascular safety profile of currently available diabetic drugs. *Ochsner Journal*, *14*(4), 616-632.

Badal, S. S., & Danesh, F. R. (2015). Diabetic nephropathy: emerging biomarkers for risk assessment. *Diabetes*. *64*(9), 3063-3065.

Bandeira SDM, da Fonseca LJS, Guedes GDS, Rabelo LA, Goulart MOF, Vasconcelos SML (2013). Oxidative stress as an underlying contributor in the development of chronic complications in diabetes mellitus. *International Journal of Molecular Sciences.* 14(2):3265- 3284.

Bansal, R.; Ahmad, N. & Kidwai, J. R. (1980). Alloxan-glucose interaction: effect on incorporation of 14C-leucine into pancreatic islets of rat. ***"Acta diabetologica Latina''.*** 17(2):135-43

Baradaran A. (2012). Lipoprotein (a), type 2 diabetes and nephropathy; the mystery continues. *Journal of Nephropathology. 1: 126-129, 2012*

Bastaki, S. (2005). Diabetes mellitus and its treatment. *International Journal of Diabetes and Metabolism.* 13 (3):111-134

Batuman, V., & Wedeen, R. P. (2014). The persistence of chronic lead nephropathy. *American Journal of Kidney Dis*, *64*(1), 1-3.

Batuman, V., Landy, E., Maesaka, J. K., & Wedeen, R. P. (1983). Contribution of lead to hypertension with renal impairment. *New England Journal of Medicine*, *309*(1), 17-21.

Beutler E, Gelbart T, Pegelow C. (1986). Erythrocyte glutathione synthetase deficiency leads not only to glutathione but also to glutathione-S-transferase deficiency. *Journal of Clinical Investigation*

Bhonsle, H. S., Korwar, A. M., Kote, S. S., Golegaonkar, S. B., Chougale, A. D., Shaik, M. L., ... & Kulkarni, M. J. (2012). Low plasma albumin levels are associated with increased plasma protein glycation and HbA1c in diabetes. *Journal of proteome research*, *11*(2), 1391-1396.

Bjornholm M., & Zierath J.R. (2005). Insulin signal transduction in human skeletal muscle: identifying the defects in type II diabetes. *Biochemical Society Transactions*. 33:354–357.

Bjornstad P, Cherney DZ, Snell-Bergeon JK, Pyle L, Rewers M, Johnson RJ, (2015). Rapid GFR decline is associated with renal hyperfiltration and impaired GFR in adults with Type 1 diabetes. *Nephrology, Dialysis, Transplantation* 30(10):1706–11.

Boateng, P. A. (2007). Diabetes Alert. *Daily Guide Newspaper*. Retrieved 30th November, 2018

Brahm K.T., Kanti B.P., Abidi A. B., & Syed I.R. (2013). "Markers of Oxidative Stress during Diabetes Mellitus," *Journal of Biomarkers*. Article ID 378790, 8 pages, 2013. https://doi.org/10.1155/2013/378790.

Bronzato, S., & Durante, A. (2018). Dietary supplements and cardiovascular diseases. *International journal of preventive medicine*, *9*.

Brownlee M., (2001). Biochemistry and molecular cell biology of diabetic complications. *Nature*. 414:813–820

Brownlee, M. (2005). The pathophysiology of diabetic complications: A unifying mechanism. *Diabetes*, 54(6), 1615-1625

Cade W. T. (2008). Diabetes-related microvascular and macrovascular diseases in the physical therapy setting. *Physical therapy*, *88*(11), 1322–1335. doi:10.2522/ptj.20080008

Campese, V. M., Nadim, M. K., & Epstein, M. (2005). Are 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors renoprotective?. *Journal of the American Society of Nephrology*, *16*(3 suppl 1), S11-S17.

Carretero, J., Obrador, E., Anasagasti, M. J., Martin, J. J., Vidal Vanaclocha, F., and Estrela, J. M., (1999). "Growth-associated changes in glutathione content correlate with liver metastatic activity of B16 melanoma cells," *Clinical and Experimental Metastasis. 17: 567–574*

Caspary, W. F. (1992). Physiology and pathophysiology of intestinal absorption.

Chang, C. L., Lin, Y., Bartolome, A. P., Chen, Y. C., Chiu, S. C., & Yang, W. C. (2013). Herbal therapies for type 2 diabetes mellitus: chemistry, biology, and potential application of selected plants and compounds. *Evidence-based complementary and alternative medicine. 2013*, 378657. doi:10.1155/2013/378657

Charron MJ, Katz EB, Olson AL. (1990). GLUT4 gene regulation and manipulation. *Journal Biological Chemistry. 274, 3253–3256*

Charron, M. J., & Kahn, B. B. (1990). Divergent molecular mechanisms for insulin-resistant glucose transport in muscle and adipose cells in vivo. *Journal of Biological Chemistry*. *265*(14), 7994-8000.

Chaudhari, R. (2008). The use of dietary antioxidants indrinks. *Food & Beverage Asia, June/July issue,* 28-30.

Chawla, A., Chawla, R., & Jaggi, S. (2016). Microvasular and macrovascular complications in diabetes mellitus: Distinct or continuum? *Indian Journal of Endocrinology and Metabolism*, *20*(4), 546–551. http://doi.org/10.4103/2230-8210.183480

Chen, L., Magliano, D.J. & Zimmet, P.Z. (2011). The worldwide epidemiology of type 2 diabetes mellitus — present and future perspectives. *Nature Reviews Endocrinology*, 8; 228–236

Cheung CK, Wyman JF, Halcon LL. (2007). Use of complementary and alternative therapies in community-dwelling older adults. *Journal of Alternative Complementary Medicine. 13(9):997–1006.*

Chiarelli, F., Cipollone, F., Mohn, A., Marini, M., Iezzi, A., Fazia, M., ... & Di Gioacchino, M. (2002). Circulating monocyte chemoattractant protein-1 and early development of nephropathy in type 1 diabetes. *Diabetes Care*, *25*(10), 1829-1834.

Cho, I. C., Park, H. B., Yoo, C. K., Lee, G. J., Lim, H. T., Lee, J. B., ... & Jeon, J. T. (2011). QTL analysis of white blood cell, platelet and red blood cell-related traits in an F2 intercross between Landrace and Korean native pigs. *Animal genetics*, *42*(6), 621-626.

Chung PM1, Cappel RE, Gilbert HF. (1991). Inhibition of glutathione disulfide reductase by glutathione. *Arch Biochemistry, Biophysiology* .

Coca, S. G., Singanamala, S., & Parikh, C. R. (2012). Chronic kidney disease after acute kidney injury: a systematic review and meta-analysis. *Kidney international*, *81*(5), 442-448.

Collins-McNeil, J., Edwards, C. L., Batch, B. C., Benbow, D., McDougald, C. S., & Sharpe, D. (2012). A culturally targeted self-management program for African Americans with type 2 diabetes mellitus. *CJNR (Canadian Journal of Nursing Research)*, *44*(4), 126-141.

Coresh, J., Astor, B. C., Greene, T., Eknoyan, G., & Levey, A. S. (2003). Prevalence of chronic kidney disease and decreased kidney function in the adult US population: Third National Health and Nutrition Examination Survey. *American journal of kidney diseases*, *41*(1), 1-12.

Crowley, S. T., Brownlee, M., Edelstein, D., Satriano, J. A., Mori, T., Singhal, P. C., & Schlondorff, D. O. (1991). Effects of nonenzymatic glycosylation of mesangial matrix on proliferation of mesangial cells. *Diabetes*, *40*(5), 540-547.

Dabla, P.K. (2010). Renal function in diabetic nephropathy. *World Journal of Diabetes, 1(2), 48-56*

Das G.C.1. (2006). Enhanced gamma-glutamylcysteine synthetase activity decreases drug-induced oxidative stress levels and cytotoxicity. *Molecular Carcinogensis*

DeFronzo R.A. (1988). The triumvirate: beta cell, muscle, liver – a conclusion responsible for

DeRossi, S., Cohen, D., Greenberg, M.S., Glick, M., & Ship, J.A. (2008). Renal disease (Eds) Burket's oral medicine (11th edition) BC Decker, Hamilton. Pp. 364-383

Deshpande A.D., Harris-Hayes M., & Schootman M., (2008). Epidemiology of diabetes and diabetes-related complications. *Physical Therapy*. 88(11):1254-64. doi:10.2522/ptj.20080020

Deshpande, S. D., Putta, S., Wang, M., Lai, J. Y., Bitzer, M., Nelson, R. G., ... & Natarajan, R. (2013). Transforming growth factor-β–induced cross talk between p53 and a microRNA in the pathogenesis of diabetic nephropathy. *Diabetes*, *62*(9), 3151-3162.

Di Landro, D., Catalano, C., Lambertini, D., Bordin, V., Fabbian, F., Naso, A., & Romagnoli, G. F. (1998). The effect of metabolic control on development and progression of diabetic nephropathy. *Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association-European Renal Association*, *13*(suppl_8), 35-43.

Dickinson DA1, Forman HJ. (2002). Cellular glutathione and thiols metabolism. *Biochemistry Pharmacol.*

Dinkova-Kostova, A. T., Liby, K. T., Stephenson, K. K., Holtzclaw, W. D., Gao, X., Suh, N., ... & Sporn, M. B. (2005). Extremely potent triterpenoid inducers of the phase 2 response:

correlations of protection against oxidant and inflammatory stress. *Proceedings of the National Academy of Sciences*, *102*(12), 4584-4589.

Doyle, D. (2006). William Hewson (1739–74): The father of haematology. *British journal of haematology*, *133*(4), 375-381.

Droge W, Eck HP, Gmunder H, Mihm S. (1991). Modulation of lymphocyte functions and immune responses by cysteine and cysteine derivatives. *American Journal of Medicine. 91: 140S–144S*

Dröge W. Aging-related changes in the thiol/disulfide redox state: implications for the use of thiol antioxidants. Journal of *Experimental Gerontology*. 2002;37:1333–45

Dronavalli, S., Duka, I., & Bakris, G. L., (2008). The pathogenesis of diabetic nephropathy. *Nature clinical Practice. Endocrinology and Metabolism*, 4(8), 444-452

Dugbartey, GJ (2017). Diabetic nephropathy: A potential savior with 'rotten-egg' smell. Journal of *Pharmacological Reports, 69*(2), 331-339.

Eboh, C., & Chowdhury, T. A. (2015). Management of diabetic renal disease. *Annals of translational medicine*, *3*(11).

Etuk, E. U. (2010). Animals models for studying diabetes mellitus. *Agriculture and Biology Journal of North America, 1 (2), 130 -134*

Fonseca V. A. (2009). Defining and characterizing the progression of type 2 diabetes. *Diabetes care*.

Forouhi N.G., Misra A., Mohan V., Taylor R., & Yancy W. (2018). Dietary and nutritional approaches for prevention and management of type 2 diabetes. *British Medical Journal*. 361:k2234

Franconi, F., Seghieri, G., Canu, S., Straface, E., Campesi, I., & Malorni, W. (2008). Are the available experimental models of type 2 diabetes appropriate for a gender perspective?. *Pharmacological Research*, *57*(1), 6-18.

Freedman, B. I., Yu, H., Spray, B. J., Rich, S. S., Rothschild, C. B., & Bowden, D. W. (1997). Genetic linkage analysis of growth factor loci and end-stage renal disease in African Americans. *Kidney international*, *51*(3), 819-825.

Gerich, J. E. (1993). Control of glycaemia. *Bailliere's clinical endocrinology and metabolism*, *7*(3), 551-586.

Giaccari, A., Sorice, G., & Muscogiuri, G. (2009). Glucose toxicity: The leading actor in the pathogenesis and clinical history of type 2 diabetes - mechanisms and potentials for treatment. Nutrition, Metabolism and Cardiovascular Diseases, 19(5), 365–377. https://doi.org/10.1016/j.numecd.2009.03.018

Griffith O.W. (1999). Biologic and pharmacologic regulation of mammalian glutathione synthesis. *Free Radical Biology, Medicine. 27: 922–935*

Griffith O.W., Mulcahy R.T. (1999). The enzymes of glutathione synthesis: gamma-glutamylcysteine synthetase. *Adv Enzymol Relat Areas Mol Biol.*

Grodsky, G.M., et. al., 1963: Effects of carbohydrates on secretion of insulin from isolated rat pancreas, *Am. J. Physiol-Legacy Content 205 (1963) 638 – 644.*

Gross, C. L., Innace, J. K., Hovatter, R. C., Meier, H. L., & Smith, W. J. (1993). Biochemical manipulation of intracellular glutathione levels influences cytotoxicity to isolated human lymphocytes by sulfur mustard. Cell Biology and Toxicology, 9(3), 259–267. https://doi.org/10.1007/BF00755604

Gross, J.L., de Azevedo, M. J., Silveiro, S. P., Canani, L. H., Caramori, M. L. & Zelmanovitz, T. (2005). Diabetic nephropathy: diagnosis, prevention, and treatment. *Diabetes Care*, 28(1), 164-76

Gul M, Kutay FZ, Temocin S, Hanninen O. (2000). Cellular and clinical implications of glutathione. *Indian J Exp Biol 38:625–634.*

Gunzler, D., Anthony, J.B., Robert, L.T., Alicia, O.B., Gregory, B. Russell, Abdus S.,… Sudha K.I., (2013). Diabetic nephropathy in a sibling and albuminuria predict early GFR decline: a prospective cohort study. *BMC Nephrology*. https://doi.org/10.1186/1471-2369-14-124

Ha, H., Hwang, I. A., Park, J. H., & Lee, H. B. (2008). Role of reactive oxygen species in the pathogenesis of diabetic nephropathy. Diabetes Research and Clinical Practice, 82(SUPPL. 1), 42–45. https://doi.org/10.1016/j.diabres.2008.09.017

Halat, M. K., & Dennehy, C. (2003). Botanicals and Dietary Supplements in Diabetic Peripheral Neuropathy. *The Journal of the American Board of Family Practice / American Board of Family Practice.* 16. 47-57. 10.3122/jabfm.16.1.47.

Halliwell B. (2006). Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. *Plant Physiology. 141:312–322.*

Halsad, C.H. (2003). Dietary supplements and functional foods: 2 sides of a coin. *The American Journal of Clinical Nutrition*. 77:1001-1007.

Han, H. S., Kang, G., Kim, J. S., Choi, B. H., & Koo, S. H. (2016). Regulation of glucose metabolism from a liver-centric perspective. *Experimental & molecular medicine*, *48*(3), e218. doi:10.1038/emm.2015.122

Haneda, M., Utsunomiya, K., Koya, D., Babazono, T., Moriya, T., Makino, H., Shide, K. (2015). A new Classification of Diabetic Nephropathy 2014: A report from Joint Committee on Diabetic Nephropathy. *Journal of Diabetes Investigation*, *6*(2), 242–246. https://doi.org/10.1111/jdi.12319

Harjutsalo, V., & Groop, P. H. (2014). Epidemiology and risk factors for diabetic kidney disease. *Advances in chronic kidney disease*, *21*(3), 260-266.

Hasslacher, C. H., Ritz, E., Wahl, P., & Michael, C. (1989). Similar risks of nephropathy in patients with type I or type II diabetes mellitus. *Nephrology Dialysis Transplantation*, *4*(10), 859-863.

Hayden M, Tyag S. (2002). Islet Redox Stress: The Manifold Toxicities of Insulin Resistance, Metabolic Syndrome and Amylin Derived Islet Amyloid in Type 2 Diabetes Mellitus. *Journal Pancreas.* 3(4): 86-108

Hayes J.D., Flanagan J.U., Jowsey I.R. (2005). Glutathione transferases. *Annual Review Pharmacology Toxicology.*

Hill, N. R., Fatoba, S. T., Oke, J. L., Hirst, J. A., O'Callaghan, C. A., Lasserson, D. S., & Hobbs, F. R. (2016). Global prevalence of chronic kidney disease–a systematic review and meta-analysis. *PloS one*, *11*(7), e0158765.

Hink U, Li H, Mollnau H, Oelze M, Matheis E, Hartmann M, Skatchkov M, Thaiss F., (2001). Mechanisms underlying endothelial dysfunction in diabetes mellitus. *Circulation Research.* 2;88 (2):E14-22. doi: 10.1161/01.RES.88.2.e14

Hong J, Zhang Y, Lai S, *et al*. 2013. Effects of metformin versus glipizide on cardiovascular outcomes in patients with type 2 diabetes and coronary artery disease. *Diabetes Care* 36: 1304-1311

Honka, H., Koffert, J., Kauhanen, S., Kudomi, N., Hurme, S., Mari, A., ... & Nuutila, P. (2018). Liver blood dynamics after bariatric surgery: the effects of mixed-meal test and incretin infusions. *Endocrine connections*, *7*(7), 888-896.

http://www.modernghana.com/news/146353/1/diabetes-alert.html, retrieved on 20th March, 2018.

https://www.ghanaweb.com/GhanaHomePage/health/4-million-suffer-from-Diabetes-National-Diabetes-Association-487177, retrieved on 30th August, 2018

Huang CS1, Moore WR, Meister A. 1988: On the active site thiol of gamma-glutamylcysteine synthetase: relationships to catalysis, inhibition, and regulation. *Proc Natl Acad Sci U S A.*

Huenchuguala S1, *et al*. 2014: Glutathione transferase mu 2 protects glioblastoma cells against aminochrome toxicity by preventing autphagy and lysosome dysfunction. Autophagy.

Jay D, Hitomi H, Griendling KK. Oxidative stress and diabetic cardiovascular complications. *Free Radical Biology & Medicine - Journal*. 2006;40:183–92

Jederström, G., Nordin, A., Sjöholm, I., & Andersson, A. (2005). Blood glucose-lowering activity of a hyaluronan–insulin complex after oral administration to rats with diabetes. *Diabetes technology & therapeutics*, *7*(6), 948-957.

Kafa, B. (2006). Liver enzymes and electrophoretic changes in serum proteins in streptozotocin induced experimental diabetic rats. *Master Science Thesis), Adnan Menderes University, Institute of Medical Sciences*.

Kamble, H. V., & Bodhankar, S. L. (2013). Trigonelline and sitagliptin attenuates nicotinamide-streptozotocin induced diabetic nephropathy in Wistar rats. *Int J Pharm Pharm Sci*, *5*(4), 583-589.

Karagül, H., Altintas, A., Fidanci, U. R., & Sel, T. (2000). *Clinical biochemistry* (pp. 161–164). Medisan Inc. Ankara. 315-316.

Karar, T., Alniwaider, R. A. R., Fattah, M. A., Al Tamimi, W., Alanazi, A., & Qureshi, S. (2015). Assessment of microalbuminuria and albumin creatinine ratio in patients with type 2 diabetes mellitus. *Journal of natural science, biology, and medicine*, *6*(Suppl 1), S89.

Keaney, Jr. J.F., & Loscalzo, J. (1999). Diabetes, oxidative stress and platelet activation. *Circulation*, 99: 189-191.

Khan, T. A., & Zafar, F. (2005). Haematological Study in Response to Varying Doses of Estrogen in Broiler Chicken. International Journal of Poultry Science, 4(10), 748-751.

King, A.J.F. (2012). The use of animal models in diabetes research. *British Journal of Pharmacology*, 166(3), 877-94

Kiran, G., Nandini, C. D., Ramesh, H. P. & Salimath, P. V. (2012). Progression of early phase diabetic nephropathy in streptozotocin-induced diabetic rats: evaluation of various kidney-related parameters. *Indian Journal of Experimental Biology*, 50(2), 133-40

Kohen R, & Nyska A. (2002). Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicological Pathol*ogy 30:620–650.

Kourkouta, L., Iliadis, C., Frantzana, E., Monios, A., Dimitriadou, A. & Papathanasiou, I. (2016). Health and Dietary Supplements. *International Journal of Engineering and Applied Sciences*. 3. 18-20.

Kunjathoor V.V., Wilson, D.L., & LeBoeuf, R.G. (1996). Increased atherosclerosis in STZ-induced diabetic mice. *Journal of clinical Ivest.* 97: 1767-1773

Kurutas E. B. (2016). The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutrition journal*, *15*(1), 71. doi:10.1186/s12937-016-0186-5

Lamos, E.L., Stein, S.A. & Davis, S.N. 2013. Sulfonylureas and meglitinides: Historical and contemporary issues. *Panminerva Medica* **55**: 239–251.

Landriscina, M., Maddalena, F., Laudiero, G., & Esposito, F. (2009). Adaptation to Oxidative Stress, Chemoresistance, and Cell Survival. *Antioxidants & redox signaling*. 11. 2701-16. 10.1089/ars.2009.2692.

Lang C.A., Naryshkin S., Schneider D.L., Mills B.J., Lindeman R.D. (1992). Low blood glutathione levels in healthy aging adults. *Journal of Laboratory Clinical Medicine;120:720–5.*

Lang CA. 1986: Research strategies for the study of nutrition and aging. *In: Chen LH, editor. Nutritional Aspects of Aging. Vol. 1. Boca Raton, FL: CRC Press Inc; pp. 3–18.*

Le Bras, M., Clement, M.V., Pervaiz, S., & Brenner, C. (2005). Reactive oxygen species and the mitochondrial signaling pathway of cell death. *Histology and histopathology*. 20. 205-19. 10.14670/HH-20.205.

Lee, J. H., Yang, S. H., Oh, J. M., & Lee, M. G. (2010). Pharmacokinetics of drugs in rats with diabetes mellitus induced by alloxan or streptozocin: comparison with those in patients with type I diabetes mellitus. *Journal of Pharmacy and Pharmacology*, *62*(1), 1-23.

Lee, K.M., Yang, S.J., Kim, Y.D., Choi, Y.D., Park, C.S. (2013). Disruption of the cereblon gene enhances hepatic AMPK activity and prevents high-fat diet-induced obesity and insulin resistance in mice. *Diabetes.* 62: 1855-1864

Lenzen S., Drinkgern J., Tiedge M. (1999). Hyperglycaemia causes oxidative stress in pancreatic beta-cells of GK rats, a model of type 2 diabetes. *Diabetes*. 48: 927-32

Lieb, W. *et al*. (2013). Genetic predisposition to higher blood pressure increases coronary artery disease risk. *Hypertension.* 61, 995–1001, https://doi.org/10.1161/ hypertensionaha.1

Loeffler, I., & Wolf, G. (2019). Mechanisms of Interstitial Fibrosis in Diabetic Nephropathy. In *Diabetic Nephropathy* (pp. 227-251). Springer, Cham.

Lowy, C. & Williams, E.D. (1967). The pancreas and diabetes mellitus. *Postgraduate Medical Journal*, 43; 51-60. doi:10.1136/pgmj.43.495.51

Lu J., Berndt C., & Holmgren A. (2009). Metabolism of selenium compounds catalyzed by the mammalian selenoprotein thioredoxin reductase. *Biochimistry Biophysics Acta.; 1790:1513–1519.*

Lu S. C., & Ge, J. L. (1992). "Loss of suppression of GSH synthesis at low cell density in primary cultures of rat hepatocytes," *American Journal of Physiology.* 263:1181– 1189

Lukačínová, A., Hubková, B., Rácz, O., & Ništiar, F. (2013). Animal models for study of diabetes mellitus. *Diabetes mellitus-insights and perspectives*, 229-54.

Macfarlane, W. M., et. al., (1997). The p38/Reactivating Kinase Mitogen-activated Protein Kinase Cascade Mediates the Activation of the Transcription Factor Insulin Upstream Factor 1 and Insulin Gene Transcription by High Glucose in Pancreatic β-Cells, *Journal Boilogical Chemistry.* 272: 20936 – 20944.

Mahmoud, A. M. (2013). Hematological alterations in diabetic rats - Role of adipocytokines and effect of citrus flavonoids. EXCLI Journal, 12, 647–657.

Masiello P, Broca C, Gross R, Roye M, Manteghetti M, Hillaire-Buys D, Novelli M, Ribes G. (1998). Experimental NIDDM: development of a new model in adult rats administered streptozotocin and nicotinamide. *Diabetes.* 47: 224– 229

Masiello P. (2006). Animal models of type 2 diabetes with reduced pancreatic beta-cell mass. *International Journal Biochemistry Cell Biology.* 38: 873–893

Matheka, D. M., Alkizim, F. O., Kiama, T. N., & Bukachi, F. (2012). Glucose-lowering effects of Momordica charantia (Karela) extract in diabetic rats. *African Journal of Pharmacology and Therapeutics*, *1*(2).

Mehmetoglu I. Clinical Biochemistry Manual; Konya: Inci Offset; 2002. p. 124-129.

Meier, M., Menne, J., Park, J. K., & Haller, H. (2007). Nailing down PKC isoform specificity in diabetic nephropathy—two's company, three's a crowd. *Nephrology, Dialysis, Transplant.* **22**(9): 2421-2425

Meister A. (1988). Glutathione metabolism and its selective modification. *Journal of Biological Chemistry.* 263:17205–08.

Meister A. (1991). Glutathione deficiency produced by inhibition of its synthesis, and its reversal; applications in research and therapy. *Pharmacology Therapy.* Meister A. 1994: Glutathione-ascorbic acid antioxidant system in animals. *Journal of Biological Chemistry. 269: 9397-9400.*

Meister, A., 1995: "Glutathione metabolism," *Methods in Enzymology*, vol. 251, pp. 3–7

Memisogullari R, Taysi S, Bakan E, Capoglu I. (2003). Antioxidant status and lipid peroxidation in type II diabetes mellitus. *Cell Biochemistry Function. 21:291–296*

Messina, J. P., & Lawrence, D. A. (1989). "Cell cycle progression of glutathione depleted human peripheral blood mononuclear cells is inhibited at S phase," *Journal of Immunology*, 143:1974–1981

Molitch M.E., DeFronzo R.A., Franz M.J. (2004). Nephropathy in diabetes. *Diabetes Care*. 27(Suppl 1): S79–83

Molitoris, B. A., Levin, A., Warnock, D. G., Joannidis, M., Mehta, R. L., Kellum, J. A., ... & Shah, S. (2007). Improving outcomes from acute kidney injury.

Mozafari M., Nekooeian A.A., Panjeshahin M.R., & Zare H.R. (2015). The effects of resveratrol in rats with simultaneous type 2 diabetes and renal hypertension: a study of antihypertensive mechansims. *Iran Journal of Medical Science.* 40:152-160

N'guessan, B. B., Amponsah, S. K., Dugbartey, G. J., Awuah, K. D., Dotse, E., Aning, A., … Appiah-Opong, R. (2018). In Vitro Antioxidant Potential and Effect of a Glutathione-Enhancer Dietary Supplement on Selected Rat Liver Cytochrome P450 Enzyme Activity. *Evidence-Based Complementary and Alternative Medicine*, 1–8. https://doi.org/10.1155/2018/7462839

Nagasawa HT, inventor; Max International, Llc, assignee. (2015). Method to enhance delivery of glutathione and apt levels in cells. *United States patent application US 14/838,27*

Nasri H and Rafieian-Kopaei M. (2014). Metformin: Current knowledge. *Journal Research Medicine Science.* 19: 658-664

National Diabetes Association (*NDA*) (2016). 4 million suffer from Diabetes - National Diabetes Association | Health. *https://www.ghanaweb.com*

Navale, A. M., & Paranjape, A. N. (2016). Glucose transporters: physiological and pathological roles. *Biophysical reviews*, *8*(1), 5-9.

Nazar, C. M. J. (2014). Diabetic nephropathy; principles of diagnosis and treatment of diabetic kidney disease. *Journal of nephropharmacology*, *3*(1), 15.

Negi G, Kumar A, Joshi RP, Ruby PK, Sharma SS (2011). Oxidative stress and diabetic neuropathy: current status of antioxidants. *Institute of Integrative Omics and Applied Biotechnology Journal*. 2(6):71-78.

Negre-Salvayre, A., Coatrieux, C., Ingueneau, C., & Salvayre, R. (2008). Advanced lipid peroxidation end products in oxidative damage to proteins. Potential role in diseases and therapeutic prospects for the inhibitors. *British journal of pharmacology*, *153*(1), 6-20.

Nelson, R. G., & Tuttle, K. R. (2007). The new KDOQITM clinical practice guidelines and clinical practice recommendations for diabetes and CKD. *Blood purification*, *25*(1), 112-114.

NIDDM. *Diabetes*, Vol. 37, No.6, pp. 667-684, ISSN 0012-1797

Ofori, S. N., & Unachukwu, C. N. (2014). Holistic approach to prevention and management of type 2 diabetes mellitus in a family setting. *Diabetes, metabolic syndrome and obesity: targets and therapy*, *7*, 159–168. doi:10.2147/DMSO.S62320

Owen JB, Butterfield DA. (2010). Measurement of oxidized/reduced glutathione ratio. *Methods Molecular Biology*.

Oyenihi, A. B., Ayeleso, A. O., Mukwevho, E., & Masola, B. (2015). Antioxidant strategies in the management of diabetic neuropathy. *BioMed Research International*, *2015*. https://doi.org/10.1155/2015/515042

Ozbek, E. (2012). Induction of oxidative stress in kidney. *International Journal of Nephrology*, 1-9.

Özcan, S. (2004). Improved and simplified liquid chromatography/atmospheric pressure chemical ionization mass spectrometry method for the analysis of underivatized free amino acids in various foods. *Journal of chromatography A*, *1135*(2), 179-185.

Palipoch, S., Punsawad, C., & Suwannalert, P. (2013). Thunbergia laurifolia, a new choice of natural antioxidant to prevent oxidative stress-related pathology: A review. *Journal of Medicinal Plants Research*, *7*(12), 698-701.

Pálsson, R., & Patel, U. D. (2014). Cardiovascular complications of diabetic kidney disease. *Advances in chronic kidney disease*, *21*(3), 273–280. doi:10.1053/j.ackd.2014.03.003

Pari, L. & Srinivasan, S. (2010). Antihyperglycaemic effect of diosmin on hepatic key enzymes of carbohydrate metabolism in streptozotocin-nicotinamide induced diabetic rats. *Biomedical Pharmacotherapy*, 64: 477 – 478

Pavkov, M. E., Bennett, P. H., Knowler, W. C., Krakoff, J., Sievers, M. L., & Nelson, R. G. (2006). Effect of youth-onset type 2 diabetes mellitus on incidence of end-stage renal disease and mortality in young and middle-aged Pima Indians. *Jama*, *296*(4), 421-426.

Pitetti, R., Singh, S., Hornyak, D., Garcia, S. E. and Herr. S. (2001). "Complementary and alternative medicine use in children," *Pediatric Emergency Care*, 17: 165–169

Poljsak, B., & Fink, R. (2014). The protective role of antioxidants in the defence against ROS/RNS-mediated environmental pollution. *Oxidative Medicine and Cellular Longevity*. 10.1155/2014/671539

Pourghasem, M., Shafi, H., & Babazadeh, Z. (2015). Histological changes of kidney in diabetic nephropathy. *Caspian journal of internal medicine*, *6*(3), 120.

Rahman, S., Rahman, T., Ismail, A. A. S., & Rashid, A. R. A. (2007). Diabetes-associated macrovasculopathy: pathophysiology and pathogenesis. *Diabetes, Obesity and Metabolism*, *9*(6), 767-780.

Rask-Madsen, C., & King, G. L. (2010). Kidney complications: factors that protect the diabetic vasculature. *Nature medicine*, *16*(1), 40.

Reliene R.1., Schiestl R.H. (2006). Glutathione depletion by buthionine sulfoximine induces DNA deletions in mice. *Carcinogenesis.*

Remuzzi G, Bertani T (1998) Pathophysiology of progressive nephropathies. *The New England Journal of Medicine.* 339:1448–1456

Röder, P. V., Wu, B., Liu, Y., & Han, W. (2016). Pancreatic regulation of glucose homeostasis. *Experimental & molecular medicine*, *48*(3), e219.

Rodrigues, C., & Percival, S. S. (2019). Immunomodulatory effects of glutathione, garlic derivatives, and hydrogen sulfide. *Nutrients*, *11*(2), 295.

Rosenberg, A. Z., & Kopp, J. B. (2017). Focal segmental glomerulosclerosis. *Clinical Journal of the American Society of Nephrology*, *12*(3), 502-517.

Rui L. (2014). Energy metabolism in the liver. *Comprehensive Physiology*, 4:177–197. doi:10.1002/cphy.c130024

Ruiz, S., Pergola, P. E., Zager, R. A., & Vaziri, N. D. (2013). Targeting the transcription factor Nrf2 to ameliorate oxidative stress and inflammation in chronic kidney disease. *Kidney international*, *83*(6), 1029-1041.

Ruiz-Ortega M, Lorenzo O, Suzuki Y, Ruperez M, Egido J (2001) Pro-inflammatory actions of angiotensins. *Current Opinion in Nephrology and Hypertension - LWW Journals.* 10:321–329

Sabbatini, M., Sansone, G., Uccello, F., Giliberti, A., Conte, G., & Andreucci, V. E. (1992). Early glycosilation products induce glomerular hyperfiltration in normal rats. *Kidney international*, *42*(4), 875-881.

Sadowska-Bartosz, I., & Bartosz, G. (2014). Effect of antioxidants supplementation on aging and longevity. *BioMed research international*. 404680. doi:10.1155/2014/404680

Salim S. (2017). Oxidative Stress and the Central Nervous System. *The Journal of pharmacology and experimental therapeutics*, *360*(1), 201–205. doi:10.1124/jpet.116.237503

Santos, R. A. S., Oudit, G. Y., Verano-Braga, T., Canta, G., Steckelings, U. M., & Bader, M. (2019). The renin-angiotensin system: going beyond the classical paradigms. *American Journal of Physiology-Heart and Circulatory Physiology*, *316*(5), H958-H970.

Satirapoj, B., & Adler, S. G. (2014). Comprehensive approach to diabetic nephropathy. *Kidney research and clinical practice*, *33*(3), 121-131.

Schalm, O. W., Jain, N. C., & Carroll, E. J. (1975). *Veterinity haematology* (3rd ed., p.15- 218). USA: Lea & Fabiger, Philadelphia.

Sekhar, R. V., McKay, S. V., Patel, S. G., Guthikonda, A. P., Reddy, V. T., Balasubramanyam, A., & Jahoor, F. (2011). Glutathione synthesis is diminished in patients with uncontrolled diabetes and restored by dietary supplementation with cysteine and glycine. *Diabetes care*, *34*(1), 162–167. doi:10.2337/dc10-1006

Sheshala, R., Peh, K. K., & Darwis, Y. (2009). Preparation, characterization, and in vivo evaluation of insulin-loaded PLA–PEG microspheres for controlled parenteral drug delivery. *Drug development and industrial pharmacy*, *35*(11), 1364-1374.

Shrivastava, S. R., Shrivastava, P. S., & Ramasamy, J. (2013). Role of self-care in management of diabetes mellitus. *Journal of diabetes and metabolic disorders*, *12*(1), 14. doi:10.1186/2251-6581-12-14

Siddharthan, T., Ramaiya, K., Yonga, G., Mutungi, G. N., Rabin, T. L., List, J. M., ... & Schwartz, J. I. (2015). Noncommunicable diseases in East Africa: assessing the gaps in care and identifying opportunities for improvement. *Health Affairs*, *34*(9), 1506-1513.

Sies, H. (1999). "Glutathione and its role in cellular functions," *Free Radical Biology and Medicine.* 27:916–921

Simonson, D. C., Ferrannini, E., Bevilacqua, S., Smith, D., Barrett, E., Carlson, R., & DeFronzo, R. A. (1984). Mechanism of improvement in glucose metabolism after chronic glyburide therapy. *Diabetes*, *33*(9), 838-845.

Steyn NP, Mann J, Bennett PH, Temple N, Zimmet P, Tuomilehto J, Lindström J and Louheranta A. (2004) Diet, nutrition and the prevention of type 2 diabetes. *Public Health Nutrition*. Feb; 7(1A): 147-65

Straub, L. G., Efthymiou, V., Grandl, G., Balaz, M., Challa, T. D., Truscello, L., ... & Sun, W. (2019). Antioxidants protect against diabetes by improving glucose homeostasis in mouse models of inducible insulin resistance and obesity. *Diabetologia*, 1-12.

Sundaram RK, Bhaskar A, Vijayalingam S, Viswanathan M, Mohan R, Shanmugasundaram KR. 1996: Antioxidant status and lipid peroxidation in type II diabetes mellitus with and without complications. *Clinical Science* (London); 90:255–260

Szablewski L. (2011). Glucose homeostasis and insulin resistance, *Bentham E-Books*, ISBN 978-1-60805-189-2. Available from http:www.intechopen.com/books/diabetes-damages-and treatments/glucose-homeostasis-mechanism-and-defects

Szkudelski T. (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiological Research*. 50: 536-546

Tahara, A., Matsuyama-yokono, A., Nakano, R., Someya, Y. & Shibasaki, M. (2008). Hypoglycaemic effects of antidiabetic drugs in streptozotocin-nicotinamide-induced mildly diabetic and streptozotocin-induced severely diabetic rats. *Basic and Clinical Pharmacology and Toxicology*, 103: 560-568

Tervaert, T. W. C., Mooyaart, A. L., Amann, K., Cohen, A. H., Cook, H. T., Drachenberg, C. B., ... & Joh, K. (2010). Pathologic classification of diabetic nephropathy. *Journal of the American Society of Nephrology*, *21*(4), 556-563.

Thomas, M. C., Cooper, M. E., Rossing, K., & Parving, H. H. (2006). Anaemia in diabetes: is there a rationale to TREAT?. *Diabetologia*, *49*(6), 1151.

Thomas, M. C., Forbes, J. M., & Cooper, M. E. (2005). Advanced glycation end products and diabetic nephropathy. *American journal of therapeutics*, *12*(6), 562-572.

Togun, V. A., Oseni, B. S. A., Ogundipe, J. A., Arewa, T. R., Hammed, A. A., Ajonijebu, D. C., . . . Mustapha, F. (2007). Effects of chronic lead administration on the haematological parameters of rabbits – a preliminary study (p. 341). *Proceedings of the 41st Conferences of the Agricultural Society of Nigeria.*

Tomaiuolo, G. (2014). Biomechanical properties of red blood cells in health and disease towards microfluidics. *Biomicrofluidics*, *8*(5), 051501.

Traverso, N., Ricciarelli, R., Nitti, M., Marengo, B., Furfaro, A. L., Pronzato, M. A., …Domenicotti, C. (2013). Role of glutathione in cancer progression and chemoresistance. *Oxidative Medicine and Cellular Longevity*. https://doi.org/10.1155/2013/972913

Tripathi B.K. & Srivastava *A.K. (*2006). Diabetes mellitus: complications and therapeutics. *Medical Science Monito*r, 12(7):RA130-47.

Triplitt, C.L. (2012). Examining the mechanisms of glucose regulation. *The American Journal of Managed Care*, 18: S4-10

Turanov A.A., Kehr S. Marino S.M., Yoo M.H., Carlson B.A., Hatfield D., Gladyshev V.N. (2010). Mammalian thioredoxin reductase 1: roles in redox homeostasis and characterization of cellular targets. *Journal of Biochemistry*. 430:285–293

UK Prospective Diabetes Study (UKPDS) Group. (1998). Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *The lancet*, *352*(9131), 837-853.

Valko M., Leibfritz D., Moncol J., Cronin M.T., Mazur M., & Telser J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry & Cell Biology* – Elsevier. 39(1): 44–84. doi: 10.1016/j.biocel.2006.07.001

Vallon, V. (2011). The proximal tubule in the pathophysiology of the diabetic kidney. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology,* 300(5), R1009-R1022

Vijayalingam S., Parthiban A., Shanmugasundaram K.R., Mohan V. (1996). Abnormal antioxidant status in impaired glucose tolerance and non-insulin-dependent diabetes mellitus. *Diabetes Medicine.* 13:715–719

Viollet, B., Guigas, B., Sanz Garcia, N., Leclerc, J., Foretz, M. & Andreelli, F. (2012). Cellular and molecular mechanisms of metformin: an overview. *Clinical Science* (London, England: 1979), 122(6), 253-70

Wang, Z., Yang, Y., Xiang, X., Zhu, Y., Men, J., & He, M. (2010). Estimation of the normal range of blood glucose in rats. Journal of Hygiene Research, 39(2), 133-137

Wang, P.R., Kitamura, H., Shimizu A., & Yamanaka N. (2015). Glomerular damage in experimental proliferative glomerulonephritis under glomerular capillary hypertension, *Kidney, Blood Pressure Research*. 40:188-199. doi. org.10.1159

Watkins, P.J. (2003). ABC of Diabetes (5th edition). London: B.M.J. Publishing group

Wei, M., Ong, L., Smith, M. T., Ross, F. B., Schmid, K., Hoey, A. J., (2003). The streptozotocin-diabetic rat as a model of the chronic complications of human diabetes. *Heart, Lung and Circulation*, 12 (1), 44-50.

West, I.C. (2000). Radicals and oxidative stress in diabetes. *Diabetic Medicine*. 17:171-180

Whiting P.H., Kalansooriya A, Holbrook I, Haddad F, Jennings PE. 2008: The relationship between chronic glycaemic control and oxidative stress in type 2 diabetes mellitus. *British Journal of Biomed Science*. 65:71–74

WHO, (2008). website:www.who.int/entity/diabetes/actionnow/en/mapdiabprev.pdf; retrieved 20th August, 2018.

Wiecek, A., Chudek, J., & Kokot, F. (2003). Role of angiotensin II in the progression of diabetic nephropathy – therapeutic implications. *Nephrology, Dialysis, Transplantation*, 18 (Suppl 5), 16-20

Wiernsperger N.F. (2003). Oxidative stress as a therapeutic target in diabetes: revisiting the controversy. *Diabetes Metabolism*. 29: 579 – 85

Wild, S., Sicree, R., Roglic, G., King, A., (2004). Green, Global prevalence of diabetes. Estimates for the year 2000 and projections for 2030, *Diabetes Care*. 27: 1047-1053.

Williams, M. E. (2005). Diabetic nephropathy: the proteinuria hypothesis. *American journal of nephrology*, *25*(2), 77-94.

Wolf,G. (2004). New insights into the pathophysiology of diabetic nephropathy: From haemodynamics to molecular pathology. *European Journal of Clinical Investigation*, 34(12), 785-796

World Health Organization (WHO) (2014). WHO About Diabetes. Retrieved June 12, 2018, from http://www.who.int/diabetes/action_online/basics/en/index1.html

Wright, E., Scism-Bacon, J. L., & Glass, L. C. (2006). Oxidative stress in type 2 diabetes: The role of fasting and postprandial glycaemia. International Journal of Clinical Practice, 60(3), 308–314. https://doi.org/10.1111/j.1368-5031.2006.00825.x

Wu Y, Ding Y, Tanaka Y, Zhang W. (2014). Risk factors contributing to Type 2 diabetes and recent advances in the treatment and prevention. *International Journal of Medical Sciences*, 11(11), 1185–1200

Wu, Y., Tang, L., & Chen, B. (2014). Oxidative Stress: Implications for the Development of Diabetic Retinopathy and Antioxidant Therapeutic Perspectives. *Oxidative Medicine and Cellular Longevity*, 2014, 1–12. https://doi.org/10.1155/2014/752387

Xu, J., & Rajaratnam, R. (2017). Cardiovascular safety of non-insulin pharmacotherapy for type 2 diabetes. *Cardiovascular diabetology. 16*(1), 18. doi:10.1186/s12933-017-0499-5

Yakhchalian, N., Hatami, K., Nosrati, H., & Yousofvand, N. (2018). Diabetes Mellitus in Male Adult Wistar Rats.

Yarandi, S. S., Zhao, V. M., Hebbar, G., & Ziegler, T. R. (2011). Amino acid composition in parenteral nutrition: what is the evidence?. *Current opinion in clinical nutrition and metabolic care*, *14*(1), 75–82. doi:10.1097/MCO.0b013e328341235a

Yates, M. S., Tauchi, M., Katsuoka, F., Flanders, K. C., Liby, K. T., Honda, T., ... & Guilarte, T. R. (2007). Pharmacodynamic characterization of chemopreventive triterpenoids as exceptionally potent inducers of Nrf2-regulated genes. *Molecular cancer therapeutics*, *6*(1), 154-162.

Yeom, E., Byeon, H., & Lee, S. J. (2016). Effect of diabetic duration on hemorheological properties and platelet aggregation in streptozotocin-induced diabetic rats. Scientific Reports, 6(February), 1–11. https://doi.org/10.1038/srep21913

Yoshioka T, Rennke HG, Salant DJ, Deen WM, Ichikawa I (1987) Role of abnormally high transmural pressure in the permselectivity defect of glomerular capillary wall: a study in early passive Heymann nephritis. *Circulation Research*. 61:531–538

Yuksek, V., Dede, S., & Ceylan, E. (2013). The electrophoretical determination of serum protein fractions in lycopene treated experimental diabetic rats. *Cell biochemistry and biophysics*, *67*(3), 1283-1289.

Zhang S., Xu H., Yu X., Wu Y., & Sui D. (2017). Metformin ameliorates diabetic nephropathy in a rat model of low-dose streptozotocin-induced diabetes. *Experimental and Therapeutic Medicine*. 14: 383-390

Zheng Y., Ley S.H., Hu F.B., (2018). Global aetiology and epidemiology of type 2 diabetes mellitus. *Nature Reviews Endocrinology* 14(2):88-98. doi: 10.1038

Zheng, X., Zhu, S., Chang, S., Cao, Y., Dong, J., Li, J., ... & Zhou, Y. (2013). Protective effects of chronic resveratrol treatment on vascular inflammatory injury in streptozotocin-induced type 2 diabetic rats: Role of NF-kappa B signaling. *European journal of pharmacology*, *720*(1-3), 147-157.

Zheng, Y., Sylvia H. L., Frank B. H., (2018). Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *https:// www. researchgate.net /publication/321674052*

Ziegler, D., Nowak, H., Kempler, P., Vargha, P., & Low, P. A. (2004). Treatment of symptomatic diabetic polyneuropathy with the antioxidant α-lipoic acid: a meta-analysis. *Diabetic Medicine*, *21*(2), 114-121.

Ziyadeh F.N. and Wolf G. (2008). Pathogenesis of the podocytopathy and proteinuria in diabetic glomerulopathy. *Current Diabetes Review*. 4, 39-45.

Ziyadeh, F. N. (2004). Mediators of diabetic renal disease: the case for TGF-β as the major mediator. *Journal of the American Society of Nephrology*, *15*(1 suppl), S55-S57.

Zojac, A. J., Blattman, J. N., Murali-Krishna, K., Sourdive, D. J., Suresh, M., Altman, J. D., & Ahmed, R. (1998). Viral immune evasion due to persistence of activated T cells without effector function. *Journal of Experimental Medicine*, *188*(12), 2205-2213.

APPENDIX I. – COLLECTION OF BLOOD FOR FASTING BLOOD GLUCOSE (FBG)

i. The tail of rat was thoroughly cleaned with methylated spirit

ii. A vein close to the tip was identified and pricked with a fresh needle

iii. The appropriate side with the test strip analyser was used to fetch the blood after a significant drop of blood was seen at the sight of prick

iv. Blood glucose reading was taken immediately and recorded

APPENDIX II. – INTRAPERITONEAL INJECTION (*i.p.*)

i. The rats were tightly held at the back so that the head was slightly lower than the back feet.

ii. The rat's left lower quadrant of abdomen was cleaned with alcohol swab

iii. A 0.5ml syringe was inserted at angle of less than 15 into the left lower quadrant

iv. The syringe was slightly drawn back to make sure a blood vessel had not been entered

v. Injection was then administered

APPENDIX III. – PREPARATION OF 10 % SUGAR SOLUTION

Dissolve 10g of sugar in 100ml of normal tap water. Put the prepared solution in the rat water trough for the next 24hours to prevent hypoglycaemia induced deaths

APPENDIX IV. – PREPARATION OF NICOTINAMIDE

Weigh the required dose of nicotinamide using the sensitive scale and put it in an Eppendorf tube. Add 1-2ml NaCl 0.9% and mix it together using a vortex mac33.3ne. Nicotinamide solution must be prepared just before use

Calculations:

Dose to be administered is 110mg/kg

For every 110mg of NA= 1000g of rat weight

Therefore for a rat weig33.3ng 273, the amount of NA that will be equivalent to 110mg/kg is

mass (NA) = (273×110) /1000

To calculate for any rat weight, m (NA) = (W (mg) ×110) /1000

Dissolve the amount calculated in 1ml of normal saline solution and mix using vortex mac33.3ne.

APPENDIX V. – PREPARATION CITRATE BUFFER AND STREPTOZOTOCIN

Preparation of Citrate buffer

Dissolve 2.94 g of sodium citrate (MW 294.10) in 100ml distilled water to make a 0.1M sodium citrate solution. Dissolve 2.10g of citric acid (MW 210.1) in 100 ml distilled water to make 0.1M citric acid solution. Add citric acid solution to sodium citrate solution until the pH is 4.5

Preparation of Streptozotocin

Weigh streptozotocin desired dose using the sensitive scale and put it in Eppendorf tubes. Add 1-2ml of sodium citrate buffer (pH4.5) and mix it together using a vortex mac33.3ne. Streptozotocin must be prepared just before use and administered wit33.3n 10-15ml just before.

Calculations:

For weight of STZ administered in every volume given:

For weigh equivalent to 60mg/kg of STZ

If 60mg of STZ = 1000g of rat weight

Then 290.68g of rat will require an equivalent of = (60×290.68)/1000

Therefore to calculate for every weigh equivalent of STZ required is =60×W (mg) /1000

For volume of STZ to be administered

33.3ghest weigh of rat =290.68mg

If 290.68mg = 0.5ml

Therefore Vml= (Wmg×0.5) / 290.68

APPENDIX VI. – PREPARATION OF GLIBENCLAMIDE, CELLGEVITY AND CAPTOPRIL

Weigh the appropriate mass of the powdered drug needed to be administered to the various groups and add 10ml of distilled water. Determine the volume each animal has to take per body weight and administer using the oral gavage method. ***Note that, groups 1 and 3 will be treated with 1 ml distilled water (p.o) for each***.

Calculations:

Group 2

Cellgevity 125mg/capsule

Dose to administered is 80mg/kg

$$80mg \rightarrow 1000g$$

$$X \quad \rightarrow w\ (g)$$

$$X = 80 \times w(g)/\ 1000$$

Each capsule of Cellgevity contains approximately 500mg of ingredient and 125mg of active

125→ 500mg

Therefore one capsule of Cellgevity will be required

$$Dissolve\ 500mg \rightarrow 10ml$$

$$Then\ w(g) \rightarrow v(ml)$$

$$V(ml) = 10 \times w(mg)/500$$

Group 4

Cellgevity 125mg/capsule

Dose to administered is 40mg/kg

$$40mg \rightarrow 1000g$$

$$X \rightarrow w\,(g)$$

$$X = 40 \times w(g)/\,1000$$

Each capsule of Cellgevity contains approximately 500mg of ingredient and 125mg of active

$$If\ 125mg \rightarrow 500mg$$

$$Then\ 60mg \rightarrow xmg$$

$$X = 60 \times 500/125$$

$$= 240mg\ of\ powder$$

Therefore one capsule of Cellgevity will be required

$$Dissolve\ 240mg \rightarrow 10ml$$

$$Then\ w(g) \rightarrow v(ml)$$

$$V(ml) = 10 \times w(mg)/240$$

Group 5

Cellgevity 125mg/capsule

Dose to administered is 80mg/kg

$$80mg \rightarrow 1000g$$

$$X \rightarrow w\ (g)$$

$$X = 80 \times w(g)/\ 1000$$

Each capsule of Cellgevity contains approximately 500mg of ingredient and 125mg of active

$$If\ 125 \rightarrow 500mg$$

$$Then\ 125 \rightarrow 500mg\ of\ drug\ powder$$

Therefore one capsule of Cellgevity will be required

$$Dissolve\ 500mg \rightarrow 10ml$$

$$Then\ w(g) \rightarrow v(ml)$$

$$V(ml) = 10 \times w(mg)/500$$

Group 6

Cellgevity 125mg/capsule

Dose to administered is 160mg/kg

$$160mg \rightarrow 1000g$$

$$X \rightarrow w\,(g)$$

$$X = 160 \times w(g)/\,1000$$

Each capsule of Cellgevity contains approximately 500mg of ingredient and 125mg of active

$$If\ 125 \rightarrow 500mg$$

$$Then\ 250 \rightarrow x$$

$$X = 500 \times 250/125$$

$$= 1000g$$

Therefore two capsules of Cellgevity will be required

$$Dissolve\ 1000mg \rightarrow 10ml$$

$$Then\ w(g) \rightarrow v(ml)$$

$$V(ml) = 10 \times w(mg)/1000$$

Group 7

Glibenclamide 5mg/tablet

Dose to administered is 15mg/kg

$$15mg \rightarrow 1000g$$

$$X \rightarrow w\,(g)$$

$$X = 15 \times w(g)/\,1000$$

Each tablet of Glibenclamide contains approximately 5.73mg of ingredient and 5mg of active

$$If\ 5mg \rightarrow 5.73mg$$

$$Then\ 15mg \rightarrow x$$

$$X = 15 \times 5.73/5$$

$$= 17.19\ mg\ of\ powdered\ drug$$

Therefore one tablet of Glibenclamide will be required

$$Dissolve\ 17.19mg \rightarrow 10ml$$

$$Then\ w(g) \rightarrow v(ml)$$

$$V(ml) = 10 \times w(mg)/17.19$$

Group 8

Captopril 25mg/tablet

Dose to administered is 20mg/kg

$$20mg \rightarrow 1000g$$

$$X \rightarrow w\,(g)$$

$$X = 20 \times w(g)/\,1000$$

Each capsule of Cellgevity contains approximately 180mg of ingredient and 25mg of ative

$$If\ 25 \rightarrow 180$$

$$Then\ 26mg \rightarrow x$$

$$X = 26 \times 180/25$$

$$= 195.00mg\ app$$

Therefore two tablets of Captopril will be required

$$Dissolve\ 195mg \rightarrow 10ml$$

$$Then\ w(g) \rightarrow v(ml)$$

$$V(ml) = 10 \times w(mg)/195$$

Group 9

Glibenclamide 5mg/tablet

Dose to administered is 10mg/kg

$$10mg \rightarrow 1000g$$

$$X \rightarrow w\,(g)$$

$$X = 10 \times w(g)/\,1000$$

Each tablet of Glibenclamide contains approximately 5.73mg of ingredient and 5mg of active

$$If\ 5mg \rightarrow 5.73mg$$

$$Then\ 10mg \rightarrow x$$

$$X = 10 \times 5.73/5$$

$$= 11.46\ mg\ of\ powdered\ drug$$

Therefore one tablet of Glibenclamide will be required

$$Dissolve\ 14.46\ mg \rightarrow 10ml$$

$$Then\ w(g) \rightarrow v(ml)$$

$$V(ml) = 10 \times w(mg)/11.46$$

Captopril 25mg/tablet

Dose to administered is 20mg/kg

$$20mg \rightarrow 1000g$$

$$X \rightarrow w\,(g)$$

$$X = 20 \times w(g)/\,1000$$

Each capsule of Cellgevity contains approximately 180mg of ingredient and 25mg of ative

$$If\ 25 \rightarrow\ 180$$

$$Then\ 30mg \rightarrow x$$

$$X = 30 \times 180/25$$

$$= 230.00mg\ app$$

Therefore two tablets of Captopril will be required

$$Dissolve\ 230mg \rightarrow\ 10ml$$

$$Then\ w(g) \rightarrow\ v(ml)$$

$$V(ml) = 10 \times w(mg)/230$$

Group 10

Glibenclamide 5mg/tablet

Dose to administered is 10mg/kg

$$10mg\ \rightarrow\ 1000g$$

$$X\ \ \ \rightarrow w\ (g)$$

$$X\ = 10 \times w(g)/\ 1000$$

Each tablet of Glibenclamide contains approximately 5.73mg of ingredient and 5mg of active

$$If\ 5mg\ \rightarrow\ 5.73mg$$

$$Then\ 10mg\ \rightarrow x$$

$$X = 10 \times 5.73/5$$

$$= 11.46\ mg\ of\ powdered\ drug$$

Therefore one tablet of Glibenclamide will be required

$$Dissolve\ 14.46\ mg \rightarrow\ 10ml$$

$$Then\ w(g) \rightarrow\ v(ml)$$

$$V(ml) = 10 \times w(mg)/11.46$$

Cellgevity 125mg/capsule

Dose to administered is 160mg/kg

$$160mg\ \rightarrow\ 1000g$$

$$X\ \ \ \ \rightarrow w\ (g)$$

$$X\ = 160 \times w\ (g)/\ 1000$$

Each capsule of Cellgevity contains approximately 500mg of ingredient and 125mg of active

$$If\ 125 \rightarrow\ 500mg$$

$$Then\ 220 \rightarrow x$$

$$X =\ 500 \times 220/125$$

$$= 880.00mg$$

Therefore two capsules of Cellgevity will be required

$$Dissolve\ 880mg \rightarrow\ 10ml$$

$$Then\ w\ (g)\ \rightarrow\ v(ml)$$

$$V\ (ml) = 10 \times w(mg)/880$$