EFFECTS OF LYCOPENE ON BLOOD GLUCOSE AND PHYSIOLOGICAL PARAMETERS OF STREPTOZOTOCIN-INDUCED DIABETIC WISTAR RATS

BY

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DECLARATION

I, Eze Ejike Daniel declare that the work in this dissertation entitled: **Effects of Lycopene on Blood Glucose and Physiological Parameters of Streptozotocin-induced Diabetic Wistar Rats** was carried out by me in the Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria, under the supervision of Dr. Aliyu Mohammed, Dr. Yusuf Tanko and Dr. Ahmad Abubakar. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

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CERTIFICATION

This dissertation titled "**Effects of Lycopene on Blood Glucose and Physiological Parameters of Streptozotocin-induced Diabetic Wistar Rats**" by Eze Ejike Daniel, meets the regulation governing the award of Doctorate of Philosophy degree in Human Physiology of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This research work is dedicated to God Almighty for His grace upon my life

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ABSTRACT

Diabetes mellitus is a major health problem in the world and is diagnosed by the presence of sustained high glucose levels in the blood. Oxidative stress is known to be actively involved in the onset and progression of diabetes and its complications. Antioxidants have important roles in biological systems by scavenging free radicals which may result in oxidative damage of biological molecules such as lipids, proteins and DNA. The aim of the study was to investigate the effects of lycopene on blood glucose level and some physiological parameters of streptozotocin-induced hyperglycaemic Wistar rats as model. The animals were made diabetic by single intraperitoneal injection of STZ (60 mg/kg). Diabetes was confirmed by the presence of high blood glucose ≥ 200 after 72 hours. Thirty Wistar rats of both sexes were used in the study of which twenty five of them were diabetic. They were divided into six groups (1, 2, 3, 4, 5 and 6) comprising five animals each. Animals in Group 1 (Diabetic control) and Group 2 (Normal control) received 0.5 ml of live oil while those in groups 2, 3, 4, 5 and 6 were administered 10, 20, 40 and 2 mg/kg b w of lycopene and glibenclamide respectively orally once daily for a period of four weeks. At the end of the treatment, all animals were sacrificed; blood samples collected and the serum separated for determination of biochemical and physiological parameters. The liver and kidney tissues were excised and subjected to routine histological investigation for hepatic glycogen alteration and histo-pathological changes. The results showed that lycopene at all doses significantly ($P < 0.05$) decreased the blood glucose concentration from (431.4 ± 48.84 mg/dL) to (171.1 ± 7.65, 118.4 ± 1.97 and 100.8 ± 6.89 mg/dL) after four weeks of treatment. The Serum insulin level was increased from (3.02 ± 0.24 µIU/mL) to (4.02 ± 0.70, 3.96 ± 1.41 and 5.06 ± 0.96 µIU/mL) but however, was not significant ($P < 0.05$), while the activity of hepatic glucokinase was significantly ($P < 0.05$) increased in diabetic animals from (8.78 ± 1.11 ng/mL) to (11.96 ± 0.54, 14.23 ± 0.88 and 15.78 ± 0.27 ng/mL) when compared with diabetic control group. The hepatic glycogen content was significantly ($P < 0.05$) elevated from (4.10 ± 0.09 mg/g tissue) to (6.00 ± 0.18, 6.28 ± 0.19, 7.18 ± 0.17 mg/g tissue) as reflected by significantly ($P < 0.05$) dose dependent increase in percentage hepatic glycogen levels (44.40 ± 4.81, 53.61 ± 6.63, 78.24 ± 4.88 %). Histological observation also revealed improvement on hepatic glycogen stores. There was significantly ($P < 0.05$) decreased activities of serum liver enzymes from (91.20 ± 2.06 IU/L) to (47.40 ± 1.81, 38.20 ± 0.86 and 36.20 ± 0.86 IU/L) for

AST, (93.60 ± 3.25 IU/L) to (51.00 ± 1.73, 40.80 ± 1.59 and 39.00 ± 3.15 IU/L) for ALT and (134.80 ± 3.14 IU/L) to (78.40 ± 2.38, 59.20 ± 7.33 and 72.80 ± 2.65 IU/L) for ALP. The serum levels of TC, TRIG and LDL-c in diabetic rats were significantly ($P < 0.05$) reduced to (2.30 ± 0.09 and 2.30 ± 0.08 mmol/L), (1.00 ± 0.07, 0.72 ± 0.06 and 0.52 ± 0.04 mmol/L) and (1.16 ± 0.06, 0.89 ± 0.20 0.53 ± 0.22 and 1.11 ± 0.05 mmol/L) while the HDL-c level was improved to (1.18 ± 0.05, 1.26 ± 0.16 and 1.44 ± 0.17 mmol/L) when compared with diabetic control group. The atherogenic risk predictor indices (LDL-cholesterol/HDL-cholesterol), log (TRIG/HDL-cholesterol) and (CRR) (TC/HDL-cholesterol) were significantly ($P < 0.05$) decreased to (1.00 ± 0.10, 0.81 ± 0.22, 0.45 ± 0.21 mmol/L), (0.89 ± 0.09, 0.62 ± 0.11, 0.39 ± 0.07 mmol/L) and (2.17 ± 0.11, 1.94 ± 0.24 and 1.70 ± 0.23 mmol/L), while (HDL-cholesterol/TC) was significantly ($P < 0.05$) elevated to (0.46 ± 0.02, 0.56 ± 0.08, 0.63 ± 0.09 mmol/L) when compared with diabetic control group. There was a corresponding increased percentage protections of (31.75 ± 7.05, 37.97 ± 9.11, 44.04 ± 9.92 %) in diabetic animals. Serum cortisol and MDA levels were significantly ($P < 0.05$) reduced to (5.36 ± 0.45, 6.42 ± 0.48 and 8.86 ± 0.44 µg/dL) and (2.22 ± 0.07, 1.76 ± 0.20, 1.52 ± 0.19 µg/dL) while there was up-regulated activities of serum endogenous enzymes: SOD to (1.90 ± 0.05, 2.14 ± 0.09, 2.40 ± 0.14 nmol/ml), CAT to (46.60 ± 1.503, 50.40 ± 1.75, 55.20 ± 1.43 nmol/ml) and GSH-Px to (40.00 ± 2.09, 43.80 ± 2.04 and 47.60 ± 0.68 nmol/ml) in diabetic treated animals when compared with diabetic control group. There was significantly ($P < 0.05$) elevation of serum total protein, albumin and globulin levels to (65.80 ± 1.28, 65.60 ± 1.33, 66.80 ± 1.39 g/L), (37.80 ± 1.39, 36.00 ± 1.58, 38.20 ± 1.39 g/L) and (28.00 ± 0.32, 29.60 ± 1.03, 27.60 ± 0.51 g/L). However, albumin/globulin ratio was significantly ($P < 0.05$) reduced to (1.35 ± 0.06, 1.23 ± 0.08, 1.39 ± 0.06 g/L) and (1.37 ± 0.35 g/L) in diabetic treated rats when compared with diabetic control group. There was a significant ($P < 0.05$) increase of (138.80 ± 0.97, 135.20 ± 3.93 and 130.40 ± 4.69 mmol/L) in the serum sodium ion level and reduction of (3.14 ± 0.12, 2.20 ± 0.31 and 2.40 ± 0.18 mmol/L) in the serum urea level. There was significantly ($P < 0.05$) lowered erythrocytes MDA concentration to (1.48 ± 0.07, 1.26 ± 0.08, 1.02 ± 0.09 µmol/g Hb) in diabetic treated groups. At 0.5, 0.7 and 0.9 % Nacl concentrations, a significantly ($P < 0.05$) lowered erythrocyte osmotic fragility of (12.20 ± 0.66, 8.80 ± 0.37, 8.40 ± 0.75 and 10.6 ± 1.25 %), (3.00 ± 0.45, 2.00 ± 0.32, 1.60 ± 0.25 and 3.40 ± 0.75 %) and (1.60 ± 0.25, 1.80 ± 0.49, 1.40 ± 0.25 and 1.40 ± 0.25 %) were produced in diabetic treated animals when compared with diabetic control group. There

were significantly ($P < 0.05$) elevated Hb concentration to (12.56 ± 0.32, 12.68 ± 0.36, 13.30 ± 0.35 g/dL) and (11.52 ± 0.51 g/dL), RBC (6.65 ± 0.28, 6.88 ± 0.31, 6.77 ± 0 .26) and (5.77 ± 0.29), PCV (35.62 ± 0.83, 35.84 ± 1.10, 37.46 ± 1.03 %) and (37.14 ± 2.80 %). In addition, MCV (53.92 ± 2.15, 55.82 ± 4.09, 64.58 ± 3.51 fl) and (61.64 ± 2.48 fl), MCH (18.90 ± 0.48, 18.96 ± 0.52, 19.78 ± 0.35 Pg) and (18.84 ± 0.21 Pg) and MCHC (33.09 ± 0.13, 34.64 ± 0.25, 36.42 ± 1.59 g/dL) and (34.06 ± 0.63 g/dL) were significantly ($P < 0.05$) higher in diabetic lycopene when compared DC group. There was a significantly ($P < 0.05$) increased Total WBC count from (6.70 ± 0.26) to (13.12 ± 1.65, 11.08 ± 1.58, 10.88 ± 0.59) and (10.72 ± 1.14), lymphocytes count from (4.26 ± 0.27) to (8.08 ± 0.69, 7.64 ± 1.33, 7.54 ± 0.53) and (7.92 ± 1.21) and neutrophils count from (1.50 ± 0.23) to (2.09 ± 0.10, 2.42 ± 0.34, 2.40 ± 0.16) and (2.30 ± 0.29) when compared with diabetic control group. Conversely, treatment of diabetic animals with lycopene and glibenclamide produced a significantly ($P < 0.05$) decreased neutrophli/lymphocyte ratio from (0.50 ± 0.04) to (0.32 ± 0.04, 0.28 ± 0.03, 0.32 ± 0.02) and (0.22 ± 0.03) and platelets counts from (892.60 ± 20.10) to (483.00 ± 52.92, 568.80 ± 80.20, 586.20 ± 40.65) and (509.20 ± 58.14) when compared with diabetic control group. In conclusion lycopene ameliorated the physiological and biochemical alterations in STZ-induced diabetic Wistar rats.

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LIST OF ABBREVIATIONS

ADP	-	Adenosine 5’-Diphosphate
AGEs	-	Advanced glycation end-products
AI	-	Atherogenic index
ALP	-	Alkaline phosphate
ALT	-	Alanine aminotransferase
ANOVA	-	One way analysis of variance
AST	-	Aspartate aminotransferase
ATP	-	Adenosine triphospahte
ATP	-	Adenosine 5’-Triphosphate
BCG	-	Bromocresol green
CAT	-	Catalase
CHD	-	Coronary Heart Disease
CVD	-	Cardiovascular disease
DM	-	Diabetes mellitus
DME	-	Diabetic macular edema
DNA	-	Deoxyribonucleic acid
DPPH	-	1-1- diphenyl 2-picryl hydrazyl
EDTA	-	Ethylenediaminetetraacetic acid
G-6PDH	-	Glucose-6-Phosphate Dehydrogenase
GH	-	Glycogen hepatopathy
GLB	-	Glibenclamide
GLCK	-	Glucokinase

GLUT 2	-	Glucose transporter 2
GSH-Px	-	Glutathione peroxidase,
GR	-	Glutathione reductase
GSH	-	Glutathione
GSSG	-	Oxidized glutathione
H & E stain	-	Hematoxylin and Eosin Stain
H_2O_2	-	Hydrogen peroxide
H_2O_2	-	Hydrogen peroxide
HCO_3^-	-	Bicarbonate
HDL-C	-	High-density lipoprotein cholesterol
HIT-T15	-	Hamster pancreatic beta cells
HNO_2	-	Nitrous oxide
HOCl	-	Hydrochlorous acid
HRO_2^-	-	hydroperoxyl
ICH	-	Insulin control high
ICL	-	Insulin control low
IDDM	-	Insulin dependent diabetes mellitus
LDL-C	-	Low-density lipoprotein cholesterol
LYC	-	Lycopene
MCHC	-	Mean corpuscular haemoglobin concentration
MCV	-	Mean corpuscular volume
MDA	-	Malodialdehyde concentration
mg/dl	-	Milligram per deciliter

$MgCl_2$	-	Magnesium chloride
MHC	-	Mean haemoglobin concentration
MI	-	Myocardial infarction
Mmol/L	-	millimol per litre
MNU	-	Methyl-nitrosourea
mRNA	-	messenger ribonucleic acid
NaCl	-	Sodium chloride
NADPH	-	Nicotinamide adenine dinuleotide phosphate
NAFLD	-	Non-alcoholic fatty liver disease
NaOH	-	Sodium hydroxide
NIDDM	-	Non-insulin dependent diabetes mellitus
NO	-	Nitric oxide
NO_2^-	-	Nitrogen dioxide
O-GlcNAcase-		N- acetylglucosaminase
O_2^-	-	Superoxide
O-GlcNAc	-	N- acetylglucosamine
OGT:	-	*O*- GlcNAc transferase
OH^-	-	Hydroxyl
ONOO	-	Peroxynitrite
PAS	-	Periodic Acid Schiff
PCV	-	Packed cell volume
PDR	-	Proliferative diabetic retinopathy
PO_4^{2+}	-	Phosphate

PSA	-	Prostate specific antigen
RBC	-	Red cell count
RO_2	-	Peroxyl
RONOO	-	Alkyl peroxynitrates
ROS	-	Reactive oxygen species
SEM	-	Standard error of mean
SO_4-	-	Sulphate ion
SOD	-	Superoxide dismutase
STZ	-	Streptozotocin
TC	-	Total Cholesterol
TRIG	-	Triacylglyceride
VLDL	-	Very- low density lipoprotein
WBC	-	White Blood Cell
β-NADP	-	Beta -nicotinamide adenine dinucleotide phosphate
(Cu/Zn) SOD-		Copper/zinc superoxide dismutase
(Mn) SOD	-	Manganese superoxide dismutase

CHAPTER ONE

1.0 INTRODUCTION

Hyperglycaemia is a condition in which a high amount of glucose circulates in the blood. Chronic hyperglycaemia is the defining characteristic of the disease known as diabetes mellitus (DM) (Conget, 2002). Uncontrolled chronic hyperglycaemia as a result of absolute insulin deficiency (type 1 diabetes) or insulin resistance with or without insulin deficiency (type 2 diabetes) is one of the primary causes of diabetic complications in a number of organs (Wang *et al.,* 2012). DM is a complex, progressive disease, which is accompanied by multiple complications. It is a metabolic disorder of the endocrine system (Li *et al*., 2004) and among the most common disorders in both developed and developing countries (Mukund *et al*., 2008; Zhou *et al.,* 2009). It has become a global metabolic epidemic, affecting important biochemical activities in nearly every age group (Gupta, 2008; Singh *et al.,* 2012). The number of people affected by diabetes was estimated to have risen by 50% by 2010, and will almost be doubled by 2025 (Zimmet *et al*., 2001; Bethel *et al*., 2007). It has been estimated that the number of people with diabetes will rise from the present 150 to 230 million in 2025 (Iraj *et al*., 2009; Abu-Zaiton, 2010). Some of the causes of an increased risk of diabetes are due to an increase in sedentary lifestyle, consumption of an energy rich diet and obesity (Deore *et al.,* 2012). There is also an increasing evidence for the role of genetic factors in several diabetic complications, particularly diabetic nephropathy and cardiovascular complications of diabetes (Bowden, 2002).

Prolonged hyperglycaemia results in the formation of advanced glycation end-products (AGE) in body tissues of patients (Deepralard *et al*., 2009). During the hyperglycaemic states, the antioxidant defence system that exists naturally in humans is altered (Jabeen *et al.,* 2012). Chronic hyperglycaemia of diabetes is linked to long term damage, dysfunction and damage to

various organs (Lyra *et al.,* 2006; Murti *et al.,* 2012). Chronic hyperglycaemia leads to many long-term complications in the eyes, kidneys, nerves, heart, and blood vessels (Laakso, 2010). It has been reported that diabetes is a risk factor for cardiovascular disease (Oguntibeju *et al.*, 2009b; Laakso, 2010) and more than 70% of type 2 diabetic patients die of cardiovascular diseases (Laakso, 2001). Oxidative stress has been suggested to be a common pathway linking diverse mechanisms for the pathogenesis of complications in diabetes (Ha and Lee, 2000; Mehrotra *et al*, 2001; Shih *et al*., 2002). It has been reported that oxidative stress participates in the progression of insulin resistance (Evans *et al*., 2002).

Diabetes is known to have a multifactorial pathogenicity and therefore, demands a multi-modal therapeutic approach. Great efforts have been made in the understanding and management of diabetes but serious problems like diabetic neuropathy (Shaikh and Somani, 2010), diabetic retinopathy (Schwartz and Flynn Jr., 2007), diabetic nephropathy (Djordjevi´c, 2001), hepatopathy (Levinthal and Tavill, 1999), cardiovascular diseases (Stratmann and Tschoepe, 2009) and reproductive problems (Baccetti *et al.,* 2002) continue to confront diabetic patients. The recognition of the potential role for nutraceuticals and dietary supplements in helping to reduce health risks and improve health quality is on the increase (Singh *et al.,* 2012). The control of diabetes can be attained by diet, exercise, insulin replacement therapy and by using herbal hypoglycaemic agents (Mallick *et al.,* 2007). Many drugs are available for use in the treatment of diabetes, but their long-term use may cause adverse side effects and hence, the increased search for natural remedies for the effective treatment of diabetes continues (Nabeel *et al*., 2010).

In both experimental and clinical models of diabetes, antioxidants have been reported to reduce markers of oxidative stress (Johansen *et al.,* 2005; Fenercioglu *et al.,* 2010; Neri *et al.,* 2010). Besides, some studies have showed that antioxidants are effective and cheaper than conventional

therapy in management of some diseases suh as diabetes mellitus (Trevithick *et al.,* 2004). Therefore, antioxidants or nutrients with high antioxidant capacity may offer additional health benefits with potential for limiting the progression of diabetes and its related complications (Maritim *et al.,* 2003; Johansen *et al.,* 2005; Fenercioglu *et al.,* 2010). Until now, the possible beneficial effect of lycopene for the control of progression of diabetes has not been given much serious attention. Lycopene is a red pigment that occurs naturally in certain fruits, vegetables, algae, and fungi. It belongs to a large group of pigments known as carotenoids (Ernst, 2002). Tomatoes and tomato-based products are the major sources of natural lycopene in the human diet. Other significant sources of lycopene include watermelon, pink grapefruit, pink guava, and apricots (Nguyen and Schwartz, 1999). Lycopene occurs in the all-*trans* and various *cis* configurations. Naturally-occurring lycopene consists predominantly of all-*trans*-lycopene. For example, lycopene present in red tomato fruits typically contains 94-96% of all-*trans*-lycopene (Schierle *et al.,* 1997). Lycopene is one of the most potent antioxidants among dietary carotenoids. Although chemically a carotenoid, lycopene has no vitamin A activity. It is the red pigment of ripe tomatoes with strong antioxidant property, which can neutralize oxygen derived free radicals (Rafi *et al.,* 2007).

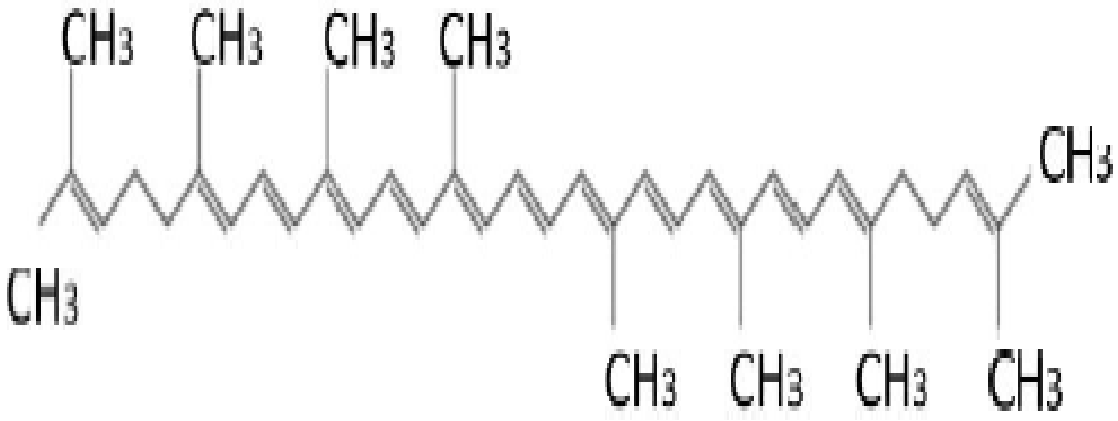


Figure 1.1: Chemical formula of all-*trans*-lycopene
(Rao and Agarwal, 1998)

1.1 Statement of Research Problem

Diabetes mellitus is a chronic metabolic disorder and its incidence has been increasing around the world. In 2010 it was estimated that more than 200 million people worldwide have diabetes mellitus and 300 million will subsequently have the disease by 2025. Several mechanisms are involved in the pathogenesis of diabetes and its complications. The most commonly accepted cause of diabetes is the oxidative damage that is caused by free radicals (Maritim *et al.*, 2003). It has been shown that people who have diabetes have higher levels of free radicals, which can cause diabetic complications (Memişoğulları and Bakan, 2004). During diabetes, persistent hyperglycemia causes an increased production of free radicals, especially reactive oxygen species (ROS) (Bonnefont-Rousselot *et al.*, 2000; Robertson *et al*., 2003). Oxidative stress, mediated mainly by hyperglycemia-induced generation of free radicals, contributes to the development and progression of diabetes mellitus and its related complications. Management of diabetes without any side effects is still a challenge to the medical system. This leads to increasing demand for natural products with potent anti-diabetic activity and fewer side effects (Grover *et al*., 2002). It has become clear that ameliorating oxidative stress using antioxidants might be an effective strategy for the treatment of diabetes mellitus and also reducing diabetic complications (Giugliano *et al*., 1996; Mohamed *et al.*, 2009). Lycopene being an antioxidant has been suggested to protect critical bimolecules including lipids, protein and DNA from free radicals (Ashwani and Prachi, 2013). However, there is paucity of information on the benefit of lycopene in the management of diabetes mellitus. This research, therefore, is aimed at investigating the anti-diabetic activity of lycopene and its effects on biochemical parameters in streptozotocin-induced diabetes in wistar rats.

1.2 Significance of the Study

Current research encourages the use of prescription medications and lifestyle changes in the management of diabetes mellitus (DM); however, emerging scientific evidence strongly suggests that nutraceuticals and functional foods have an important role to play in both the primary management and adjunctive treatment of this disease. There has been increased interest in finding naturally occurring antioxidants for use in pharmaceutical applications, which can protect the human body from free radicals and retard the progress of many diseases such as diabetes mellitus (Prior, 2003); and lycopene is one of such. This compound has great importance for their ability to prevent oxidation and is usually used as major ingredients in foods (Slusarczyk *et al*., 2009). Oxidative stress is itself known to be a root cause for the progression and development of many diseases such as diabetes mellitus and its associated complications (Tiwari, 2004; Kar and Panda, 2004; Kar and Panda, 2005). Diabetes mellitus has remained one of the serious health problems in the world. However, there have been no satisfactory anti-diabetic drugs in use for the management of this disease. And there is an emerging trend in research to support the biological activities of dietary products in the management of many diseases such as diabetes mellitus. The philosophy that food can be health-promoting beyond its nutritional value is gaining acceptance within the public arena and among the scientific community as mounting research links diet or food supplements to disease prevention and treatment such as in diabetes mellitus. Chemoprevention by dietary means continues to attract major attention for the management of chronic diseases such as of diabetes mellitus (Aruoma *et al.*, 2007). Therefore, the present investigation explores the antidiabetic potentials of lycopene in experimentally induced diabetes in Wistar rats as models.

1.3 General Aim of the Study

This study is designed to study the effects of lycopene on blood glucose and physiological parameters in streptozotocin-induced diabetic Wistar rats.

Objectives of the study

1. To evaluate the effects of lycopene on blood glucose level and lipid profile (total cholesterol, triglyceride, high density-lipoprotein and low-density lipoprotein) and atherogenic indices in STZ-induced diabetic Wistar rats.
2. To evaluate the effect of lycopene on biomarkers of oxidative stress in STZ-induced diabetic Wistar rats such as:
   (a) Lipid peroxidation
   (i) Serum malondialdehyde concentration
   (ii) Erythrocytes osmotic fragility
   (iii) Erythrocyte MDA concentration
   (b) Serum cortisol levels
   (c) Serum and kidney antioxidant enzyme activities
   (i) Superoxide dismutase
   (ii) Catalase
   (iii) Glutathione peroxidase
3. To evaluate the effects of lycopene on serum liver enzyme activities such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) in STZ-induced diabetic Wistar rats.
4. To determine the effects of lycopene on renal function status (urea and electrolytes), serum total protein, albumin and globulin level in STZstrep-induced diabetic Wistar rats.

5. To determine the effect of lycopene on haematological parameters such as red cell count, PCV, Hb concentration, MCV, MCH, MCHC, white blood cell count and its differentials in STZ-induced diabetic Wistar rats.
6. To provide possible mechanism(s) of anti-diabetic action of lycopene in STZ-induced diabetic Wistar rats by:
   (a) Determining effects lycopene on hepatic glucokinase enzyme activity in the liver of STZ-induced diabetic wistar rats.
   (b) Evaluating the effects of lycopene on hepatic glycogen content in STZ-induced diabetic Wistar rats.
   (c) Evaluating the effects of lycopene on circulating serum insulin levels in STZ-induced diabetic Wistar rats.

1.4 Research Hypothesis

Lycopene does have effect on blood glucose levels and physiological parameters in STZ-induced diabetic Wistar rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

The fundamental mechanism underlying diabetes mellitus is the lack of biologically active insulin which leads to alterations in the uptake and storage of glucose and reduced usage of glucose for energy purposes (Saravanan and Ponmurugan, 2012). Increased oxidative stress contributes to the deterioration of pancreatic β-cells due to glucose toxicity which consequently leads to severe impairment of glucose-stimulated insulin secretion and β-cell damage (Likidlilid *et al.,* 2010). The liver is a vital insulin-dependent tissue that plays a critical role in glucose and lipid homeostasis and is severely affected during diabetes (Rajasekaran *et al.,* 2006). Red blood cells are distinctive, highly specialized and the most abundant cells in humans and contain high levels of both enzymatic and non-enzymatic cytoplasmic antioxidants (Pandey and Rizvi, 2010). They are the first cells in the body to be exposed to stressful stimuli and hence, prone to oxidative stress (Pandey and Rizvi, 2010). Abnormally elevated levels of free radicals and a concurrent decrease in antioxidant defence system can lead to destruction of cellular organelles and enzymes, increased lipid peroxidation and development of insulin resistance (Maritim *et al.,* 2003). The most important defence mechanism against free radicals in the body is mediated by the actions of antioxidants (Astaneie *et al.,* 2005). Much interest in the role and usage of natural antioxidants as a means to prevent oxidative damage in diabetes is presently on the increase (Babujanarthanam *et al.,* 2011).

2.1 Mechanism of Induction of Diabetes by Streptozotocin

Streptozotocin (STZ), originally identified in the late 1950`s as an antibiotic is a naturally occurring compound that is produced by the bacterium *Streptomyces achromogenes* and shows broad spectrum antibacterial properties (Vavra *et al.,* 1959; Sharma, 2010). STZ was later discovered to be particularly toxic to pancreatic β-cells that secrete insulin and has since been used extensively to create animal models of type I diabetes (Mansford and Opie, 1968; Pathak *et al.,* 2008). It induces diabetes which resembles human hyperglycaemic non-ketotic diabetes mellitus in animal models (Weir *et al.*, 1981). STZ selectively destroys the insulin producing β-cells by inducing necrosis and hence, it is diabetogenic (Sharma, 2010). Its action on β-cells is accompanied by characteristic alterations in blood insulin and glucose concentrations (Szkudelski, 2001). The glucose moiety in the structure of STZ enables it to be transported through GLUT 2 (Elsner *et al.,* 2000) and thus, insulin-producing cells that do not express this glucose transporter are resistant to STZ (Lenzen, 2007). STZ is able to produce nitric oxide (NO), a bioregulatory and cytotoxic molecule and it has been indicated that direct NO-generation may be a mechanism of STZ toxicity in diabetogenesis (Kwon *et al.,* 1994). Wada and Yagihashi (2004) also reported that nucleic acid alkylation or excessive nitric oxide (NO) generation has been proposed to contribute to STZ-induced beta-cell damage. The production of NO by STZ could damage genomic DNA and may cause beta-cell dysfunction by inhibiting mitochondrial enzymes (Wada and Yagihashi, 2004). The DNA damage caused by alkylation that is mediated by STZ is repaired by an excision repair process and requires the activation of the NAD dependent enzyme poly (ADP-ribose) synthetase (Wilson and Leiter, 1990; Sharma, 2010). This process leads to depletion of cellular NAD and ATP and the increased ATP dephosphorylation provides substrate for xanthine oxidase which leads to generation of superoxide radicals and

consequently leads to the formation of hydrogen peroxide and hydroxyl radicals (Szkudelski, 2001).

The link between STZ and a cytosolic protein post-translational modification through O-glycosylation with N-acetylglucosamine (O-GlcNAc) has recently been proposed to be a mechanism of STZ toxicity effects and it is referred to as O-GlcNAc-dependent model of STZ toxicity (Pathak *et al.* 2008). Streptozotocin is proposed to induce apoptosis by inhibiting O-GlcNAcase, the enzyme that, together with O-GlcNAc transferase, is responsible for the reversible intracellular O-GlcNAc post-translational modification (Pathak *et al.* 2008; He *et al.,* 2009). *O*-GlcNAc-selective *N*-acetyl-b-d-glucosaminidase (OGlcNAcase) removes *O*-GlcNAc from protein and is the final enzyme in the pathway of O-glycosylation in the β-cells (Konrad *et al.,* 2001). Streptozotocin elevated *O*-GlcNAc levels in pancreatic islets contributes to the destruction of β-cells (Liu *et al.,* 2000).

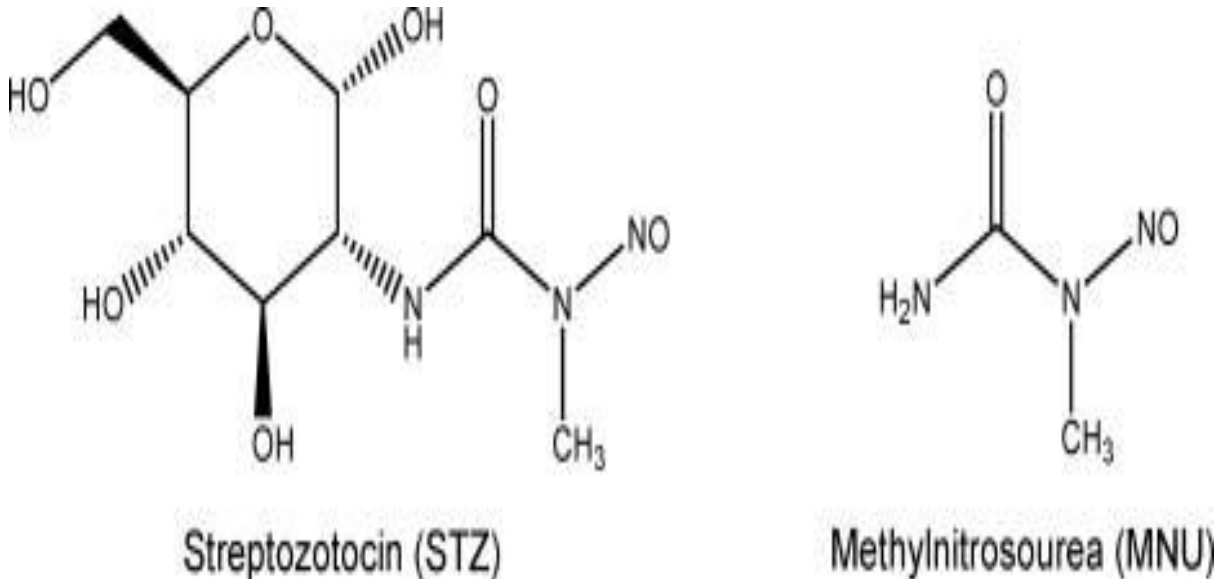


Figure 2.1: Chemical formula of streptozotocin and methyl-nitrosourea (Vavra *et al.*, 1959)

2.2 Hyperglycaemia and Diabetes Mellitus

Glucose is the major fuel for most body tissues and it is largely derived from the ingestion of carbohydrates into the body. Glucose in solution is a ring structure, in equilibrium with an open-chain aldehyde form in small amount (Mohora *et al.,* 2007). Hyperglycaemia (high glucose level) is the net result of higher glucose influx than glucose outflow from the plasma compartment and it is directly linked to increased hepatic glucose production in the fasting state (Inzucchi *et al.,* 2012). Diabetes mellitus is a complex metabolic disorder in the endocrine system characterized by abnormalities in insulin secretion and/or insulin action that leads to the progressive deterioration of glucose tolerance which causes hyperglycaemia. Symptoms of the endocrine disorder include glucosuria, ketoacidosis, hypercholesterolaemia and hypertriglyceridaemia with loss of weight and caloric deficits (Granner, 2000; Eteng *et al.,* 2008). There are two main categories of the disease, type 1 diabetes mellitus also called insulin-dependent diabetes mellitus (IDDM) and type 2, the non-insulin dependent diabetes mellitus (NIDDM) (Raubenheimer, 2010). The most prevalent form of diabetes mellitus is type 2 diabetes and it typically makes its appearance at the later stage of life (Grundy *et al*., 1999). The cause of type 2 diabetes may be due to the combined effects of impairment in the insulin-mediated glucose disposal and defective secretion of insulin by the β-cells of the pancreas (Grundy *et al*., 1999). Diabetes affects numerous organs and persistent hyperglycaemia can lead to destruction of non-insulin sensitive organs where there are no "gate keepers" in the form of insulin receptors that restrict the entry of glucose into the cell (Albright and Bell, 2003). Hyperglycaemia has tissue-damaging effects on a subset of cell types such as capillary endothelial cells of the retina, mesangial cells in the renal glomerulus, and neurons in the peripheral nerves (Brownlee, 2005).

2.3 Oxidative Stress in Diabetes Mellitus

Oxidative stress, an imbalance between the generation of reactive oxygen species/ reactive nitrogen species and antioxidant defence capacity of the body, is actively involved in the pathogenesis of diabetes and its complications (Ha and Lee, 2000; Bonnefont-Rousselot, 2002; Johansen *et al.*, 2005; Oguntibeju *et al.*, 2010). Reactive oxygen species/reactive nitrogen species include free radicals such as superoxide (O_2^-), hydroxyl (OH^-), peroxyl (RO_2), hydroperoxyl (HRO_2^-), nitric oxide (NO) and nitrogen dioxide (NO_2^-) and non-free radical such as hydrogen peroxide (H_2O_2), hydrochlorous acid (HOCl), peroxynitrite (ONOO), nitrous oxide (HNO_2) and alkyl peroxynitrates (RONOO) (Johansen *et al.*, 2005; Oguntibeju *et al*., 2010). Diabetes has been associated with increased oxidative stress, which may contribute to microvascular and macrovascular complications (Giugliano *et al*., 1996). Hyperglycaemia is mediated in large part, by a state of enhanced oxidative stress which results in the excessive production of reactive oxygen species which can cause adverse structural and functional changes in tissues (Mehta *et al*., 2006).

Several mechanisms appear to be involved in hyperglycaemia such as glucose autoxidation, stimulation of the polyol pathway, activation of the reduced form of nicotinamide adenine dinucleotide phosphate oxidase, and production of advanced glycation end-products (AGEs) which leads to increased generation of reactive oxygen species (Bonnefont-Rousselot *et al.*, 2000; Bonnefont-Rousselot, 2002). Glycation is a major source of reactive oxygen species and reactive carbonyl species that are caused by both oxidative (glycoxidative) and non-oxidative pathways (Rahbar and Figarola, 2003). Elevated non-enzymatic glycation of proteins, lipids and nucleic acids due to the formation of advanced AGEs is accompanied by oxidative, radical-generating reactions and therefore represents a major source for oxygen free radicals under hyperglycaemic conditions (Mohamed *et al*., 1999). It has been reported that glycation may

result in the production of superoxide (Jones *et al*., 1987; Sakurai and Tsuchiya, 1988). The formation of AGEs is also accompanied by an increased oxidation of low density lipoprotein (LDL) and an increase in atherogenic oxidized LDL occurs in diabetes (Bucala, 1997). The non-enzymatic glycation of haemoglobin has been established and shown to be significantly increased in diabetes (Goldstein, 1995). Protein glycation alters protein and cellular function, and binding of AGEs to their receptors can lead to modification in cell signalling and further production of free radicals (Penckofer *et al*., 2002). Glycoxidation of collagens contributes to development of vascular complications in diabetes (Urios *et al.,* 2007). In an *in vitro* study on HIT-T15 cells, induced glycation suppressed insulin gene promoter activity and its mRNA levels by provoking oxidative stress through glycation reaction (Matsuoka *et al*., 1997).

Reactive oxygen species, particularly superoxide anions could inactivate endothelium-derived NO to form potent oxidant $ONOO^-$ which contributes to the development of endothelial dysfunction in diabetes (Kodja and Harrison, 1999; Laight *et al*., 2000; Johansen *et al*., 2005). O_2- can also activate several damaging pathways in diabetes including accelerated formation of AGEs, polyol pathway, hexosamine pathway and protein kinase C, all of which have been proven to be involved in micro and macro vascular complications (Johansen *et al*., 2005). Oxidative stress can alter insulin action through a change in the physical state of the plasma membrane of target cells, increased intracellular calcium content and reduction in NO availability (Paolisso and Giugliano, 1996). Mitochondrial overproduction of free radicals is possibly a potential mechanism causing impaired first phase of glucose-induced insulin secretion (Knight, 1998; Sakai *et al.,* 2003) and this process has been associated with the onset of type 1 diabetes via apoptosis of pancreatic β-cells, and the onset of type 2 diabetes via insulin resistance (Bonnefont-Rousselot *et al*., 2000). Oxidative stress caused by short exposure of β- cell

preparations to H_2O_2 has been shown to increase the production of p21 and decreases insulin mRNA, cytosolic ATP, and calcium flux in the cytosol and mitochondria (Maechler *et al.*, 1999). β-cells are particularly sensitive to reactive oxygen species due to their low free-radical quenching (antioxidant) enzymes such as catalase, glutathione peroxidase, and superoxide dismutase (Tiedge *et al.*, 1997).

2.4 Diabetes Mellitus and Body Antioxidant Defence System

Aerobic metabolism is always accompanied by the production of reactive oxygen species and all living organisms have developed antioxidant defence systems against injury as a result of oxidative stress. Free radicals that are formed are rapidly scavenged by natural cellular defence mechanisms which include enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), catalase (CAT) (Kumawat *et al.*, 2009).

2.4.1 Catalase

Catalase (CAT) is an antioxidant enzyme that is produced naturally in the body and found in peroxisomes in eukaryotic cells. It is particularly important in conditions where glutathione (GSH) is limited or the activity of GPx is diminished (Caldwell *et al.*, 2008). CAT degrades Hydrogen peroxide (H_2O_2) to water and oxygen and hence, finishes the detoxification reaction started by SOD. Each catalase molecule can convert millions of H_2O_2 molecules every second. H_2O_2 is a powerful oxidizing agent and is potentially damaging to cells. CAT allows for important cellular processes which produce H_2O_2 as a by-product to occur by preventing excessive build up of hydrogen peroxide and also protect against hydrogen peroxide mediated oxidative damage. In the small intestine, CAT activity was significantly increased in the diabetic rats (Bohr *et al.*, 2004). CAT activity has been shown to be significantly high in diabetic patients (Kumawat *et al.*, 2005) and found to be significantly decreased in the liver of diabetic rats (Genet

et al., 2002: Sathishsekar and Subramanian, 2005; Jeyashanthi and Ashok, 2010; Meenakshi *et al.,* 2010; Pari *et al.,* 2010; Sancheti *et al.,* 2010; Babujanarthanam *et al.,* 2011; Makni *et al.,* 2011a, 2011b; Atangwho *et al.,* 2012). Decrease in the activity of catalase in the plasma (Jeyashanthi and Ashok, 2010; Makni *et al.,* 2011a), pancreas (Abdelmoaty *et al.,* 2010; Babujanarthanam *et al.,* 2011), kidney (Jeyashanthi and Ashok, 2010; Pari *et al.,* 2010; Sancheti *et al.,* 2010), brain and sciatic nerve tissues (Uzar *et al.,* 2012) of diabetic rats have been shown. Increased activities of catalase in the heart (Genet *et al.,* 2002) and hippocampus (Ceretta *et al.,* 2012) have been reported in diabetic animals. No significant effects in the activities of catalase in the kidney (Sadi *et al.,* 2012) and liver (Ugochukwu *et al.,* 2003) have been shown in diabetic rats. The uncontrolled generation of H_2O_2 as a result of the auto-oxidation of glucose, protein glycation and lipid oxidation in diabetes is markedly responsible for the decline in catalase activity (Saravanan and Ponmurugan, 2012).

2.4.2 Superoxide Dismutase

Superoxide (SOD) is an antioxidant enzyme that catalyzes the conversion of two superoxides into H_2O_2 and oxygen. It acts as a major defence system against the cytotoxic effects of superoxide radicals (Caidwell *et al.,* 2008). SOD is metal-containing enzyme that depends on bound trace metals for antioxidant activity. They are of two types: copper/zinc (Cu/Zn) SOD and manganese (Mn) SOD and each type of SOD plays a different role in keeping cells healthy. Different isoforms of SOD are located at different sites within the cells (Caldwell *et al.,* 2008). Cu/Zn SOD protects the cell`s cytoplasm, and Mn SOD protects the mitochondria from free radical damage. A non-significant effect was observed in the kidney SOD of diabetic rats (Sadi *et al.,* 2012). In another study, Bohr *et al.* (2004) showed a significant increase in small intestine SOD activity of diabetic rats. A decrease in the activity of SOD in the liver of diabetic rats has

been shown by several studies (Genet *et al.,* 2002; Ugochukwu *et al.,* 2003; Sathishsekar and Subramanian, 2005; Jeyashanthi and Ashok, 2010; Meenakshi *et al.,* 2010; Pari *et al.,* 2010; Sancheti *et al.,* 2010; Babujanarthanam *et al.,* 2011; Makni *et al.,* 2011a, 2011b). A similar decrease in the activity of SOD in the plasma (Jeyashanthi and Ashok, 2010; Makni *et al.,* 2011a), pancreas (Abdelmoaty *et al.,* 2010; Babujanarthanam *et al.,* 2011), kidneys (Pari *et al.,* 2010; Sancheti *et al.,* 2010) and striatum and amygdala (Ceretta *et al.* 2012) of diabetic rats have been shown. Increased activity of SOD in the brain of diabetic rats has also been reported (Genet *et al.,* 2002). Decrease in the activity of SOD in diabetes could possibly be a response to increased generation of H_2O_2 and O_2 by the autoxidation of glucose and non-enzymatic glycation (Pari and Latha, 2004). Kumawat *et al.* (2005) has also reported that the reduced activity of SOD in the erythrocytes of diabetic rats could be due to ageing or an increase in the glycation of SOD.

2.4.3 Glutathione Peroxidase

Glutathione peroxidase (GPx) is a group of enzymes of which most contain selenium. It helps to protect the cell from damage due to free radicals like hydrogen and lipid peroxides and its actions take place in the presence of glutathione, the master antioxidant. They act like catalase by degrading hydrogen peroxide. GPx metabolizes hydrogen peroxide to water with the usage of reduced glutathione as a hydrogen donor (Maritim *et al.,* 2003; Caldwell *et al.,* 2008). They also reduce organic peroxides to alcohols, providing another way for the removal of toxic oxidants. Decreased activity of GPx in the liver of diabetic rats has been reported (Genet *et al.,* 2002; Sathishsekar and Subramanian, 2005; Lapshina *et al.,* 2006; Jeyashanthi and Ashok, 2010; Meenakshi *et al.,* 2010; Pari *et al.,* 2010; Atangwho *et al.,* 2012) while its activity was significantly high in diabetic patients (Kumawat *et al.,* 2005). Increase in the activities of GPx in the kidney (Genet *et al.,* 2002; Sadi *et al.,* 2012) and muscle tissues (Kurt *et al.,* 2011) of diabetic

rats has been documented. A decline in GPx activity of the small intestine (Bohr *et al.*, 2004), kidney (Jeyashanthi and Ashok, 2010; Pari *et al.,* 2010), lens (Preet *et al.,* 2006), brain (Nakhaee *et al.*, 2010) and plasma (Jeyashanthi and Ashok, 2010) have been shown in diabetic rats. A decrease in the activity of GPx in the pancreas of diabetic rats has also been reported (Ugochukwu *et al.,* 2003; Abdelmoaty *et al.,* 2010; Babujanarthanam *et al.,* 2011). Reduced activity of GPx could be due to low content of glutathione in diabetic state, since glutathione serves as a substrate and cofactor of GPx (Saravanan and Ponmurugan, 2012). Decrease in GPx activity could be a result of a number of deleterious effects due to the accumulation of toxic products (Saravanan and Ponmurugan 2011; 2012).

2.4.4 Glutathione Reductase

Glutathione reductase (GR) is a cellular antioxidant enzyme that regenerates glutathione from glutathione disulfide by recycling using NADPH (Maritim *et al.,* 2003). For every mole of oxidized glutathione (GSSG), one mole of NADPH is needed to reduce GSSG to reduced glutathione (GSH). GR maintains the levels of glutathione in the cells (Blakytny and Harding, 1992). GR plays an essential function in the protection of haemoglobin, red cell enzymes and biological cell membranes against oxidative damage (Waggiallah and Alzohairy, 2011). Bohr *et al.* (2004) showed no significant difference in the activity of GR in the small intestine of diabetic rats. Similarly, no significant difference in the activity of GR in the cardiomyocyte has also been shown in diabetic rats (Ghosh *et al.,* 2004). An increase in the activity of GR in muscle tissues of diabetic rats has been shown (Kurt *et al.,* 2011). In diabetic patients, the level of GR in the erythrocytes was significantly decreased (Kumawat *et al.,* 2005). A decrease in the activity of GR in the pancreas (Babujanarthanam *et al.,* 2011), brain (Nakhaee *et al.,* 2010) and lens (Preet *et al.,* 2006) of diabetic rats has been reported. Decrease in GR is due to reduced GSH

concentration which results in increased free radicals and leads to oxidative stress in diabetes (Waggiallah and Alzohairy, 2011).

2.4.5 Glutathione

Glutathione (GSH) is classified as a tripeptide because it is made up of three amino acids: cysteine, glutamic acid, and glycine. It can be found in every part of the body, particularly the lungs, intestinal tract, and liver. It can be found in large concentrations in the liver where it is used to detoxify harmful compounds so that they can be removed from the body through the bile. Reduced GSH is a major component of the intracellular defence system (Caldwell *et al.,* 2008). GSH functions as a direct free-radical scavenger, co-substrate for glutathione peroxidase activity, co-factor for many enzymes and also forms conjugate in endo- and xenobiotic reactions (Maritim *et al.,* 2003). Not only does it protect the body against free radical attacks, it is also helpful in a well functioning immune system. Sakamaki *et al.* (1999) reported a decrease in GSH of embryonic tissues of diabetic pregnant rats. The level of GSH is significantly decreased in kidneys of diabetic rats (de Cavanagh *et al.,* 2001; Sathishsekar and Subramanian, 2005; Sancheti *et al.,* 2010; Sadi *et al.,* 2012). A decrease in the level of GSH in the liver of diabetic rats has been reported (de Cavanagh *et al.,* 2001; Ozsoy-Sacan *et al.,* 2004; Patriarca *et al.,* 2005; Sathishsekar and Subramanian, 2005; Sancheti *et al.,* 2010; Babujanarthanam *et al.,* 2011; Makni *et al.,* 2011a, 2011b). Similar reduction of GSH in the blood of diabetic rats has also been shown (Ozsoy-Sacan *et al.,* 2004). In another study, the GSH level in the livers of diabetic rats did not change (Lapshina *et al.,* 2006). A decrease in the levels of GSH in the plasma (Makni *et al.,* 2011a), cardiomyocyte (Ghosh *et al.,* 2004) and pancreas (Babujanarthanam *et al.,* 2011) has been reported in diabetic rats. In diabetes, decrease in GSH levels could probably be due to its

increased use by the hepatic cells as a result of decreased synthesis or increased degradation of GSH by oxidative stress (Saravanan and Ponmurugan, 2012).

2.5 Diabetes Mellitus and Its Complications

Hyperglycaemia is the initiating cause of diabetic tissue damage and the process is modified by both genetic determinants of individual susceptibility and by independent accelerating factors such as hypertension and hyperlipidaemia (Brownlee, 2005).

2.5.1 Neuropathy

Neuropathy (disease or abnormality of the nervous system) is a microvascular complication of diabetes mellitus which results in considerable morbidity and a decreased quality of life (Van Acker *et al.,* 2009; Mijnhout *et al.,* 2010; Bertolotto and Massone, 2012). It is characterized by a slowly progressive, length-dependent loss of sensation that correlates with duration of diabetes and glycaemic control (Kern *et al.,* 2009). Neuropathy is the most common complication of diabetes mellitus and occurs in 60% of the patients and affects their quality of life (Shaikh and Somani, 2010). Diabetic neuropathy causes foot ulceration, may lead to amputation and chronic pain with reduced quality of life and the most common among the diabetic neuropathies are diabetic peripheral neuropathy (DPN) and diabetic autonomic neuropathy (DAN) (Sasase and Ohta, 2011). Shaikh and Somani (2010) reported that many abnormalities that are found in diabetic patients with neuropathy, including hyperalgesia (extreme sensitivity to pain), allodynia (pain that results from a non-injurious stimulus to the skin), slow nerve conduction velocity and progressive sensory and sensory motor deficit are seen in diabetic rodents.

2.5.2 Retinopathy

Diabetic retinopathy (disorder of retinal blood vessels) is often the cause of new cases of blindness among adults aged 20–74 years and the duration of diabetes is probably the strongest predictor for development and progression of retinopathy (Fong *et al.*, 2004). Diabetic retinopathy is duration-dependent which develops in stages and it is often not detected in the first few years of diabetes, but increases to 50% by 10 years and to 90% by 25 years of diabetes (Kowluru and Chan, 2007). It is regarded as a disease of the retinal microvasculature and has been divided into an early, non-proliferative (or background) stage, and a later, proliferative stage (Kern, 2007). The two most vital visual complications of diabetic retinopathy are diabetic macular edema (DME) and proliferative diabetic retinopathy (PDR) (Schwartz and Flynn Jr., 2007). Retinopathy is characterized by a spectrum of retinal lesions and abnormalities that show vascular damage and death or dysfunction of the neural retina (Kern *et al*, 2009). At all stages of retinopathy, macular edema, characterized by retinal thickening from leaky blood vessels, can develop (Fong *et al.*, 2004). In this diabetic complication, the microvasculature of the retina is damaged, the blood vessels swell and seep out fluid and if not prevented, new vessels start to grow, which eventually lead to the detachment of the retina (Frank, 2004; Aylward, 2005; Kowluru and Chan, 2006).

2.5.3 Nephropathy

Diabetic nephropathy (diabetic kidney disease) is a major microvascular complication (Anjaneyulu and Chopra, 2004). It is categorized into stages: microalbuminuria, the presence of small amounts of albumin in the urine (UAE > 20 μg/min and ≤ 199 μg/min) and macroalbuminuria, the presence of high amounts of albumin in the urine (UAE ≥ 200 μg/min) (Gross *et al.,* 2005). Diabetic nephropathy occurs in ≈ 30% of people with type 1 diabetes and 25-40% of people with type 2 diabetes, often irrespective of glycaemic control (Hall, 2006). The

presence of microalbuminuria is considered to be a manifestation of renal and generalized endothelial injury and strongly predicts progressive diabetic nephropathy and cardiovascular risk and hence, microalbuminuria appearance is used as an important indicator of effective treatment intervention (Hall, 2006). It is the chief cause of chronic kidney disease in patients starting renal replacement therapy and is associated with increased cardiovascular mortality (Gross *et al.*, 2005). Pathophysiological changes associated with diabetic nephropathy include renal and glomerular hypertrophy, mesangial cell hypertrophy and matrix accretion, glomerular basal membrane thickening and functional alterations in glomerular filtration barriers (Djordjevi´c, 2001).

2.5.4 Hepatopathy

Diabetic hepatopathy (disease of the liver) causes lesions to develop in the liver. Diabetic patients have a high prevalence of liver disease and it is an important cause of death in type 2 diabetes (Abolfathi *et al.*, 2011). Increased occurrence of liver disease arises in both type 1 and type 2 diabetic patients, resulting in an increased prevalence of hepatic complications (Albright and Bell, 2003). Liver disease such as abnormal liver enzymes, non-alcoholic fatty liver disease (NAFLD), cirrhosis, hepatocellular carcinoma, and acute liver failure are seen in patients with type 2 diabetes (Abolfathi *et al.*, 2011). There has been a reported increase in the incidence of cirrhosis and cholelithiasis (presence of stones in the gall bladder) in diabetes mellitus and conversely, at least 80% of patients with cirrhosis have glucose intolerance (Levinthal and Tavill, 1999). Excess hepatic glycogen and fat accumulation are also reported to be seen in diabetic complications (Levinthal and Tavill, 1999). In poorly controlled diabetes, glycogen hepatopathy (GH) has been characterized as a pathologic overloading of hepatocytes with glycogen which leads to clinical signs and symptoms such as abdominal discomfort, tender

hepatomegaly and elevated transaminases (Fridell *et al.*, 2007). It is also the rare cause of elevated serum transaminases, mostly confined to type 1 diabetics (van den Brand *et al.*, 2009).

2.5.5 Cardiovascular diseases

Cardiovascular disease (CVD) is a class of diseases which affect the heart and/or blood vessels and it is frequently linked with any disease that affects the cardiovascular system such as atherosclerosis (Oguntibeju *et al.*, 2009b). Diabetic patients have an increased risk of cardiovascular disease (Desouza *et al.*, 2010). There is an increase in the correlation of type 2 diabetes and the death rate from cardiovascular disease which is two-fold to eight-fold higher in diabetics than people without diabetes (Grundy *et al.*, 2002; Lago *et al.*, 2007). The leading cause of death in DM is cardiovascular disease and it has been implicated in more than 80% of the cases of the diabetes disease (Selvaraju *et al.*, 2012). About 80% of all patients with CVD may have diabetes or impaired glucose tolerance (Giugliano *et al.*, 2009). Diabetes mellitus is a major cause of cardiovascular morbidity and mortality in developed countries and atherothrombosis (a condition in which a thrombus originates in an atheromatous blood vessel) is the cause of most deaths among diabetic patients (Stratmann and Tschoepe, 2009). Atherothrombosis comes up as a result of atherosclerosis progression with clinical manifestations such as sudden cardiac death, myocardial infarction (MI), ischaemic stroke, and peripheral arterial ischaemia (Stratmann and Tschoepe, 2009). In diabetic patients, the interaction of auto-antibodies with AGEs is capable of forming AGE-immune complexes which may play a role in atherogenesis (Turk *et al*, 2001). Atherogenesis involves endothelial dysfunction, activation and injury, inflammation, and smooth muscle cell migration and proliferation (Mehta *et al*., 2006). One mechanism by which diabetes promotes atherosclerosis is through abnormal lipid metabolism (Dokken, 2008). Insulin deficiency and insulin resistance

promote dyslipidaemia with an increased oxidation, glycosylation, and triglyceride enrichment of lipoproteins (Dokken, 2008).

2.5.6 Reproductive damage

Erectile dysfunction is a common complication of diabetes (Agostini *et al.,* 2006). Diabetes has been linked with reproductive impairment in both men and women (Baccetti *et al.,* 2002). The occurrence of sexual dysfunction in diabetic men approaches 50% while diabetic women seem to be slightly lower (Amaral *et al.,* 2008). This deleterious effect on male reproductive function is possibly through an increased production of reactive oxygen species and imbalance between antioxidants and oxidants (Amaral *et al.,* 2006). Thakur and Dixit (2008) also reported that oxidative stress is increased in diabetes resulting in impaired sexual dysfunction and impotence in the modern world. Diabetes causes damage to nerves throughout the body which includes the penis (Agostini *et al.,* 2006). In diabetic men, poor semen quality which includes decreased sperm motility and concentration, abnormal morphology, increased seminal plasma abnormalities as well as decreased serum testosterone due to impaired Leydig cell function have been reported (Amaral *et al.,* 2008). The sexual problems in diabetic women include decreased sexual arousal with slow and/or inadequate lubrication and sexual desire (Enzlin *et al.,* 2002) and good glycaemic control would be essential to restore a normal sexual activity in diabetic women (Bultirini *et al.,* 2004). Streptozotocin caused testicular dysfunction and degeneration under situations of experimentally induced diabetes in animals (Shrilatha and Muralidhara, 2007) and alloxan-induced diabetes in male rats was reported to reduce semen parameters and impair distinct phases of spermatogenesis (Arikawe *et al.,* 2006).

2.6 Lycopene

Lycopene is one of the most powerful antioxidants among dietary carotenoids found in foods. It has gained increased attention for its health giving properties Antioxidants protect and repair cells and tissues against the damaging effects of free radicals which cause cell and tissue damage. It is a bright red carotene and a member of the carotenoid family of phytochemicals. Although used as food colourant for many years, the investigation into the health benefits of lycopene did not begin until the last part of the 20th century. However, in a relatively short period of time, an increasing body of evidence based on laboratory, animal, and population-based research supports the role of lycopene in health and disease, and as a potential natural food antioxidant (Rao and Agarwal, 2000).

2.6.1 Bioavailability and Isomerization

Normally, lycopene obtained from plants tends to exist in an all-*trans* configuration, the most thermodynamically stable form. However, the double bonds are subjected to isomerisation (Halloway *et al*., 2000). As a polyene, it undergoes *cis-trans* isomerization induced by light, thermal energy and chemical reactions. Bioavailabilty implies the fraction of carotenoid that is absorbed by the body and available for use in physiological function (Tanumihardjo, 2002). For lycopene, studies have indicated that the bioavailability of the *cis*-isomer of lycopene is greater than the all-*trans* isomer (Boileau *et al*., 1999). This may be because *cis*-isomer are more soluble in bile acid micelles and so preferencially incorporated into chylomicrons (Bramley, 2000). Remaining to be determined, however, is whether or not cis-isomers are biologically more effective than trans-isomers once in the body (Rao and Agarwal, 2000). Not all sources of lycopene are equally bioavailable. Ingested in its natural *trans*-form, such as is prominent in tomatoes, lycopene is poorly absorbed (Rao and Agarwal, 2000). Heat processing of tomatoes and tomato products induces isomerisation of lycopene from all-*trans* to *cis* configuration, which

in turn increases its bioavailability (Stahl and Sies, 1992; Clinton, 1998; Agarwal *et al.*, 2001). More significantly, the chemical form of lycopene is altered by the temperature changes involved in processing. Heat processing improves bioavailability by increasing *cis*- to *trans*-ratio. Unlike other fruits and vegetables, where nutritional content such as vitamin C is diminished upon cooking, heat processing of tomatoes increases the lycopene bioavailability. Lycopene is absorbed better from heat processed foods than unprocessed sources (Bohm and Bitsch, 1999). Thus, lycopene in tomato paste is four times more bioavailable than in fresh tomatoes. For these reasons tomato sauce is a preferable source as opposed to raw tomatoes (Shi and Le Mague, 2000).

In addition to the importance of its conformation at the time of ingestion, bioavailability of lycopene is also affected by factors such as amount of lycopene consumed, food matrix, dietary fibre and fat, interaction with other carotenoids, and interference with proper micelle formation (Castenmiller and West, 1998; Bohm and Bitsch, 1999; van Het Hof *et al.*, 2000; Tanumihardjo, 2002). For instance, the presence of other carotenoids, such as beta-carotene that share a common LDL-mediated absorption pathway, can also influence lycopene absorption (Johnson *et al.*, 1997). Lycopene is absorbed better from lipid–rich diets (Bohm and Bitsch, 1999). Since lycopene is fat-soluble (as are vitamins, A, D, E and beta-carotene), absorption into tissues is improved when oil is added to the diet. Carotenoid bioavailability is increased by presence of fat (De Pee and West, 1996; van Den Berg, 1999; Tanumihardjo, 2002). Carotenoid bioavailability is decreased by dietary fibre (Erdman *et al.*, 1986). While most green leafy vegetables and other sources of lycopene are low in fats and oils, lycopene is tightly bound to vegetable fibre. Generally, lycopene is enclosed in cells of the tissue and only a portion of the lycopene that is present is absorbed. Therefore, processing makes the lycopene more bioavailable by increasing

the surface area available for digestion. For example, processed tomato products such as pasteurised tomato juice, soup, sauce, and ketchup contain the highest concentrations of bioavailable lycopene from tomato-based sources. Cooking and crushing tomatoes (as in the canning process) and serving in oil-rich dishes (such as spaghetti sauce or pizza) greatly increases assimilation from the digestive tract into the bloodstream. Hence, mechanical processing of tomato products help to breakdown cell wall and release lycopene from food matrix, thereby increasing its bioavailability (Hill and Rogers, 1969; Helzsouer *et al.,* 1989).

2.6.2 Pharmacokinetics and Tissue Distribution

After ingestion, lycopene is incorporated into lipid micelles in the small intestine. These micelles are formed from dietary fats and bile acids, and help to dissolve the hydrophobic lycopene and allow it to permeate the intestinal mucosal cells by a passive transport mechanism. When absorbed from the small intestine, lycopene is transported in the blood by various lipoproteins and accumulates in the liver, adrenal glands, and testes. Little is known about the liver metabolism of lycopene, but like other carotenoids, lycopene is incorporated into chylomicrons and released into the lymphatic system. It is then distributed in blood plasma, initially in very-low-density lipoprotein (VLDL) and chylomicrons fractions but later in low-density lipoprotein fractions (LDL) and high-density lipoprotein (HDL) fractions of the serum. The highest levels are found in the LDL (Stahl and Sies, 1996; Bramley, 2000). Lycopene is mainly distributed to fatty tissues and organs such as the adrenal glands, liver, and testes. In human dietary intervention research, serum lycopene levels significantly increase after consuming tomato foods or lycopene supplementation (Rao and Agarwal, 1998; Rao, 2004). However, unlike other carotenoids, lycopene levels in serum or tissues do not correlate well with overall intake of fruits and vegetables. In human plasma, lycopene is the most predominant carotenoid, present naturally

in greater amounts than beta-carotene and other dietary carotenoids. This perhaps indicates its greater biological significance in the human defense system. Its level is affected by several biological and life-style factors. Lycopene is found in most human tissues, but is not deposited uniformly. These differences in tissue distribution suggest that there are specific mechanisms for the preferential deposition of lycopene, particularly in the adrenals and testes (Bramley, 2000).

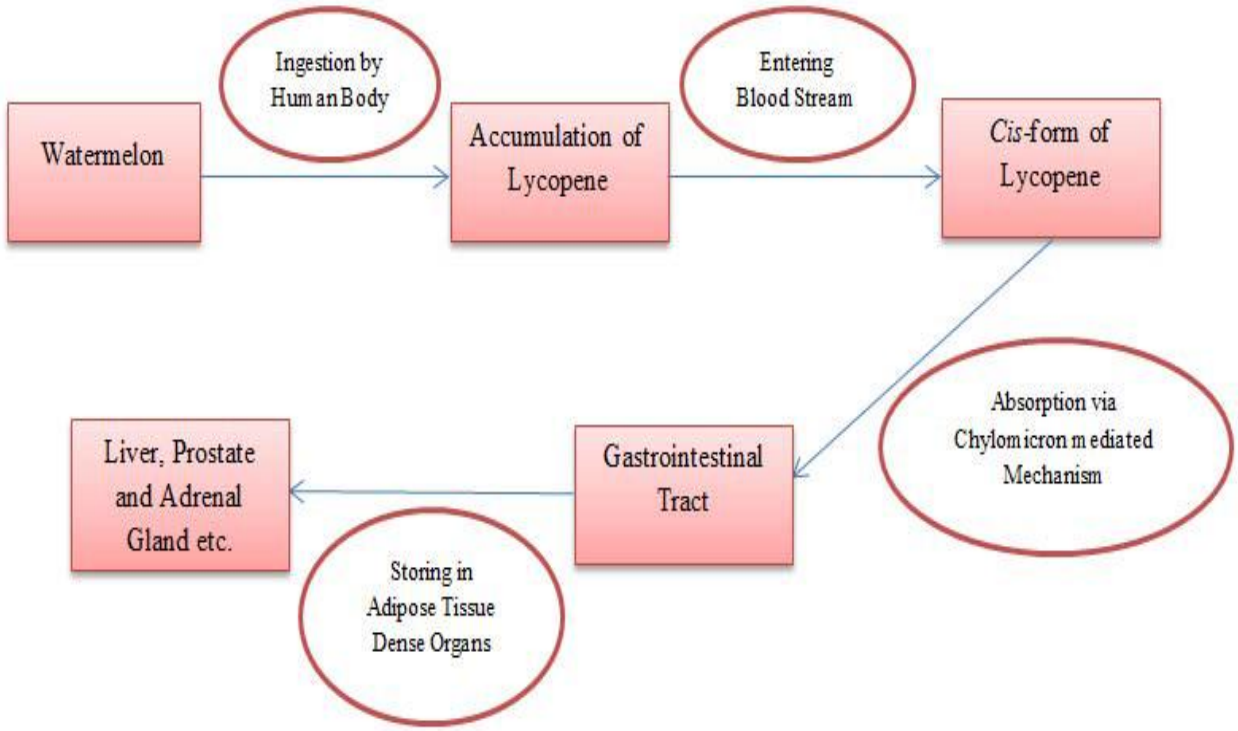


Figure 2.2: Tissue Distribution of Lycopene (Rao and Agarwal, 1998)

2.6.3 Toxicity of Lycopene

This is an important consideration, but so far there are no known adverse effects from taking lycopene other than from excessive intake. Lycopene is non-toxic and is commonly found in the diet. A high intake of foods containing lycopene is not known to cause any harmful side-effects. However, in a middle-aged woman who had prolonged and excessive consumption of tomato juice, the skin and liver were coloured orange-yellow and elevated levels of lycopene in her blood occurred. After three weeks on a lycopene-free diet, the skin colour returned to normal (Stahl and Sies, 1996). This discolouration of the skin is known as lycopenodermia (INFMB, 2000) and is non-toxic. Very little is known about the potential for adverse effects from high doses of supplemental lycopene.

The Institute of Medicine at the National Academy of Sciences did not establish a tolerable upper intake level for carotenoids (including lycopene) when it reviewed these compounds in year 2000. Recommended average daily intake of lycopene ranged from 0.70 to 25.20 mg/day. However, based on human research, recent recommendations for the daily intake of lycopene suggest 7 mg/day (Rao and Shen, 2002). At this level of intake, circulatory lycopene concentration is maintained at a level consistent with that shown to reduce lipid peroxidation and result in other beneficial effects of lycopene. Some research findings indicate that under certain circumstances, lycopene (and other carotenoids) can become oxidized in the body, and may subsequently behave like free radicals and cause cellular damage (Rao and Shen, 2002). Cigarette smoke, for example, may cause lycopene to become oxidized. This may explain, at least in part, the research findings that cigarette smokers who take carotenoid supplements may have an increased risk of cancer or heart disease (Rao and Shen, 2002).

2.6.4 Potential Health Benefits

The evidence in support of the role of lycopene in the prevention of chronic diseases is primarily epidemiological in nature. However, tissue culture, animal experiments and more recently human intervention studies are providing convincing evidence in support of the epidemiological observations. Lycopene is not modified to vitamin A in the body so can be accessible for other benefits such as antioxidation. Dietary supplementation with lycopene results in health benefits due to improvement in antioxidative status and in the prevention of severe chronic disease (Tapiero *et al.,* 2004; Wu, *et al.,* 2004; Bhuvaneswari and Nagini, 2005; Rao *et al.,* 2006; Avci and Durak, 2007). Similarly, several trials in healthy men and women have demonstrated the ability for a variety of doses of dietary lycopene (5 to 150 mg/day) from foods and tomato oleoresin extract to exert a positive significant influence on serum biomarkers considered to relate to atherogenesis and cancer risk (Rao and Agarwal, 1998; Rao *et al.,* 1999). These effects include decreased serum lipid peroxidation (Rao and Agarwal, 1998; Rao and Shen, 2002; Rao, 2004) decreased serum LDL oxidation (Rao and Agarwal, 1998; Rao and Shen, 2002; Rao, 2004) and decreased protein oxidation (Rao, 2004, Rao and Shen, 2002). Human research has also shown lycopene supplementation to result in significantly increased total antioxidant potential (Rao, 2004) and a trend towards decreased serum DNA oxidation (Rao and Agarwal, 1998).

2.6.5 Lycopene and Cancer

Strong scientific evidence suggests that lycopene plays a beneficial role in the reduction of prostate cancer risk. A study by the Harvard School of Public Health found that people who eat seven to ten servings of lycopene-rich foods each week may have a lower risk of developing prostate cancer**.** There have been several studies that analyze the anti-cancer properties of lycopene, although research has been primarily inconclusive Giovannucci (1999). With respect

to lycopene's role in the prevention of cancer, Giovannucci (1999) reviewed 72 epidemiological studies and found the consumption of tomatoes and tomato products or circulating levels of lycopene to be inversely related to risk of cancers, most prominently, cancers of the prostate gland, lung, and stomach. The results from this study (which examined case-control dietary studies, prospective dietary studies and blood specimen-based investigations) also suggested a reduced risk of other cancers, including pancreatic, colorectal, esophageal, oral, breast, and cervical cancers. Other cancers investigated did not show clear relationships to the intake of tomatoes, tomato products or lycopene.

Lycopene may reduce risk of cancer by activating special cancer preventive enzymes such as phase II detoxification enzymes, which remove harmful carcinogens from cells and the body (Giovannucci *et al.,* 2002). It is an inhibitor of human cancer cell proliferation and it was found that, unlike cancer cells, human fibroblasts were less sensitive to lycopene, and the cells gradually escaped growth inhibition over time. Lycopene is also found in lung tissue and is valuable in protecting lymphocytes from NO_2 damage found in lung cancer. In addition to its inhibitory effect on basal endometrial cancer cell proliferation, lycopene also was found to suppress insulin-like growth factor-I stimulated growth. Insulin-like growth factors are major autocrine/paracrine regulators of mammary and endometrial cancer cell growth. Therefore, lycopene interference in this major autocrine/paracrine system may open new avenues for research on the role of lycopene in the regulation of endometrial cancer and other tumours (Levy *et al.*, 1995). In different studies however, lycopene was found to have an inhibitory effect on cataract development (Pollack *et al.,* 1997) and several different kinds of cancer cells including breast and endometrial cancer cells (Nahum *et al.,* 2001), prostate carcinoma cells (Giovannucci *et al.,* 2002), and colon cancer cells (Narisawa *et al.,* 1996). Another meta-analysis of

observational studies examined 11 case-control and 10 cohort studies to determine the effect produced by a daily intake of tomato products (Etminan *et al.*, 2004). It was concluded that tomato products can play a role in the prevention of prostate cancer. However, the beneficial effect of consuming tomato products was found to be modest and restricted to high amounts of intake. In a case-control study that examined prostate cancer, it was found that serum and prostate tissue lycopene levels were significantly lower in prostate cancer patients when compared with their age-matched controls (Rao *et al.*, 1999). The findings suggest that prostate cancer patients either lack the ability to absorb lycopene efficiently or utilise lycopene rapidly due to increased oxidative stress. This case-control study also revealed significantly higher serum prostate specific antigen (PSA) and protein oxidation in prostate cancer patients compared with controls. No significant relationship was observed between serum beta-carotene and PSA in prostate cancer cases compared with controls. Finally, a recent study with prostate cancer patients has also shown a possible prophylactic effect of lycopene in the shrinkage and regression of prostate tumors (Kucuk, *et al.*, 2001; Kucuk, *et al.*, 2002).

2.6.6 Lycopene and Cardiovascular Disease

Another area of research is that of lycopene and cardiovascular disease. In general, epidemiological studies exploring lycopene and cardiovascular disease show significant associations between the intake of tomatoes and serum, plasma, and adipose tissue levels of lycopene. However, some studies have failed to show such associations. In a large multicenter case-control study, the relationship between adipose tissue antioxidant status (alpha- and beta-carotene and lycopene) and acute myocardial infarction were evaluated in 662 cases and 717 controls (Kohlmeir *et al.*, 1997). Adipose antioxidant levels were measured because they are considered to be a better marker of long-term exposure than serum lycopene. After adjusting for

a range of dietary variables, higher lycopene but not alpha- or beta-carotene adipose tissue levels were found to be protective against myocardial infarction risk in non-smokers. Also related to cardiovascular disease, mildly hypercholesterolaemic individuals with grade-1 hypertension taking 15 mg/day of lycopene from tomato oleoresin extract had significantly decreased systolic and diastolic blood pressure compared to placebo (Engelhard *et al.*, 2006).

2.6.7 Lycopene and Bone Health

Recently, research has begun to explore the potential effects of lycopene against the onset of bone disease. Although not fully understood, there is evidence that oxidative stress caused by reactive oxygen species is associated with the pathogenesis of osteoporosis. In a recent *in-vitro* study of bone marrow prepared from rat femurs, it was demonstrated that lycopene, in the absence or presence of parathyroid hormone, inhibited osteoclastic mineral resorption and formation of tartrate-resistant acid phosphatase, positive multinucleated osteoclasts, as well as the reactive oxygen species produced by osteoclasts (Rao *et al.*, 2003). The authors suggested that this finding may be important in the pathogenesis, treatment and prevention of osteoporosis (Rao *et al.*, 2003). Clinical studies are now being conducted to study the role of lycopene in osteoporosis (Rao, 2005). Researchers have studied the relationship between lycopene and bone resorption in postmenopausal women. They found higher lycopene intake and higher serum lycopene to be associated with lower bone resorption (Rao, 2005). Based on the results from this study, the researchers are now conducting a lycopene intervention study with postmenopausal females to evaluate the relationship between lycopene and the risk of osteoporosis (Rao, 2005).

2.6.8 Lycopene and Male Infertility

Several studies have shown the effect of lycopene on male infertility (Martino *et al.,* 2006; Goyal *et al.,* 2007; Turk *et al.,* 2007; Mangiagalli *et al.,* 2010). In one study of 50 volunteers with low active sperm counts, 35 volunteers (70%) experienced an improvement in sperm count, 30 (60%) had improved functional sperm concentrations, 27 (54%) had improved sperm motility, 23 (46%) had improved sperm motility index, and 19 (38%) had improved sperm morphology following consumption of 8 mg/day of lycopene supplementation from tomato oleoresin extract (Mohanty *et al.,* 2001). Further studies are now being undertaken to confirm these preliminary observations and to gain further understanding into the role of lycopene in male infertility.

2.6.9 Lycopene and Coronary Heart Disease

So far, serum cholesterol level has been the main focus as the risk factor for CHD. However, ROS-induced oxidative stress is now recognized as an important etiological factor of CHD (Rao, 2002). Oxidation of the circulating low-density lipoprotein (LDL) to oxidized LDL (LDLox) is thought to play a key role in the pathogenesis of the disease (Heller *et al.,* 1998; Parthasarathy *et al.,* 1992). Lycopene, as a potent antioxidant, is therefore thought to play an important protective role against CHD. *In vitro* studies have shown that lycopene can protect native LDL from oxidation and also inhibit cholesterol synthesis (Dugas *et al.,* 1999). Animal intervention studies have also shown lycopene to increase the resistance of the extracted LDL in vitro to oxidation. However, epidemiological studies provide the main evidence in support of the role of lycopene in the prevention of CHD (Rao and Heber, 2001).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

Materials that were used included: Spectrophotometer (V-16 model, United Kingdom), haematocrit, Centrifuge Hettich (Universal 32, Made in Germany), Automated Analyzer (Selectra XL-model Netherland), haematology analyzer (Systmex model 2X-2N, USA), Automated electrolyte Analyzer, Audicom USA (AC 9900 model), measuring cylinder, beaker, round bottle flask, electronic weighing machine, glucometer and srips (Accu-chek® Advantage, Roche USA), EDTA and plain tubes, sample bottles, cotton wools, dissecting kits, cages and feeders, cannula, syringes and needle, laboratory morta and pestle glibenclamide and rats Insulin Elisa kits.

3.2 Experimental Animals and Care

Wistar rats of both sexes (20 male and ten females) weighing 150-200 g were obtained from the Animal House of the Department of Human Physiology, Ahmadu Bello University, Zaria, Nigeria. The animals were kept and maintained under laboratory condition of ambient temperature, relative humidity and light. The animals were housed five animals per cage. They were fed on standard commercial feeds with water *ad libitum.*

3.3 Chemicals and Lycopene

Streptozotocin was purchased from Sigma Chemicals (St Louis, U.S.A.), while Lycopene (30 mg capsule) was procured from General Nutrition Corporation, Pittsburgh, U.S.A. It was reconstituted in olive oil (*Goya En espana*, S.A.U., Sevilla, Spain) to appropriate working dosage described by Al-Jassabi (2005); Ali *et al.* (2013) and Ogundeji *et al.* (2013) with modifications. All chemicals and solvents used were of analytical grade.

3.4 Induction of Experimental Diabetes Mellitus

Diabetes mellitus was induced by single intraperitoneal injection of 60 mg/kg body weight dose of streptozotocin dissolved in fresh 0.1M cold citrate buffer of pH 4.5 into 18 h-fasted rats. Since STZ is capable of producing fatal hypoglycaemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution orally after 6 h. The rats were kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycemia (Dhandapani *et al*., 2007). Three days after STZ injection, blood was taken from tail artery of the rats. Animals having blood glucose levels ≥ 200 mg/dL were considered diabetic and included in the study. The diabetic animals were randomly divided into different groups.

3.5 Experimental Protocol and Treatment

In the experiment, a total of 30 Wistar rats were used; the animals were randomly divided into six groups of five rats each as follows:

Group 1: Normal control (NC) and administered (0.5 ml/kg body weight) olive oil used in dissolving lycopene

Groups 2: (DC) Diabetic control and administered (0.5 ml/kg body weight) olive oil used in dissolving lycopene

Group 3: (D + LYC 10 mg/kg) Diabetic and treated with 10 mg/kg b w of lycopene

Group 4: (D + 20 mg/kg) Diabetic and treated with 20 mg/kg b w of lycopene

Group 5: (D + 40 mg/kg) Diabetic and received 40 mg/kg b w of lycopene

Group 6: (D + GLB 2 mg/kg) Diabetic and received glibenclamide 2 mg/kg b w

All administrations were given orally, once daily by gavage for four weeks.

3.6 Determination of Blood Glucose Level

Blood glucose level was determined by collection of blood sample from the tail artery of the rats at interval of 0 week, 1st week, 2nd week, 3rd week and 4th week of the treatment period, respectively by glucose-oxidase principle (Beach and Turner, 1958) using digital glucometer (Accu-chek Advantage) and was expressed as mg/dL.

3.7 Blood Sample Collection and Serum Preparation

After the last day of treatment (28 days) all animals from each group was sacrificed using light chloroform after 24 hours and 5 mL of blood was collected through cardiac puncture into heparinized sample bottles for haematological analysis, erythrocyte osmotic fragility test and erythrocyte malondialdehyde concentration. The other portion of blood sample collected into specimen bottles and allowed to clot, and separated by centrifugation at 2,000 *g* for 10 minutes using Centrifuge Hettich (Universal 32, Made in Germany). The supernatant obtained was used for the determination of the physiological parameters.

3.8 Tissue Sample Collection and Homogenates Preparation

Approximately 1.0 g of liver and kidney were dissected out, washed with ice-cold saline, weighed and homogenized immediately in 10 ml ice-cold phosphate-buffer of 0.1 M of pH 7.4. The homogenates were centrifuged at 2,000 *g* for 5 min to remove debris and supernatant was collected for estimation of hepatic glucokinase activity, glycogen content antioxidant enzyme activities, and malondialdehyde (MDA) concentration in the kidney tissue.

3.9 Assessment of Physiological Parameters

3.9.1 Determination of Serum Insulin Level

The estimation of serum insulin levels was done by radio-immunoassay (RIA) using Mercodia Ultrasensitive Rat Insulin ELISA kits (10-1251-01). Briefly, all reagents and samples were brought to room temperature before use. The required amount of enzyme conjugates 1X and wash buffer solution was prepared. The samples, insulin control solutions, and calibrators were also prepared as well as sufficient microplate wells to accommodate calibrators and samples in duplicate. A recommended plate plan which includes: Cal 0-5: calibrator solutions (standards); insulin control low (ICL), Insulin control high (ICH) and sample (S) were prepared. 25 µL each of Calibrators was pipetted into appropriate wells and 100 µL of enzyme conjugate 1X solution added into each well and incubated on a plate shaker 2000 g for 2 hours at room temperature. Each well was washed 6 times with wash buffer 1X solution and reaction volume discarded by inverting the micro plate over a sink. Wash solution of 350 µL was added into each well and the wash solution discarded and tapped firmly several times against absorbent paper to remove excess liquid. This was repeated five times and to avoid prolonged soaking during washing procedure 200 µL substrate TMB was added into each well. This was incubated for 15 minutes at room temperature (18 – 25^0C) and 50 µL Stop solutions were added to each well. The plate was placed on the shaker for approximately 5 seconds to ensure mixing. The absorbance was measured at 450 nm within 30 minutes.

3.9.2 Determination of Hepatic Glucokinase Enzyme Activity

The activity of hepatic glucokinase enzyme activity was determined by the method of Goward (1986) by continuous spectrophotometric rate determination as follows:

Principle:

β-D (+) Glucose + ATP $\xrightarrow{\text{Glucokinase}}$ D-Glucose 6-Phosphate + ADP

D-Glucose 6-Phosphate + β-NADP $\xrightarrow{\text{G-6PDH}}$ 6-Phospho-D-gluconate + β-NADPH

ATP= Adenosine 5'-Triphosphate

ADP= Adenosine 5'-Diphosphate

β-NADP= β-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form

G-6PDH=Glucose-6-Phosphate Dehydrogenase

Conditions: T=30^0C, pH=9.0, $A_{340nm,}$ Light path=1cm

Reagents:

- 75 mM Tris HCl buffer, 100ml was prepared in deionized water using Trizma Base, Sigma Prod. No. T-1503 and adjusted to pH 9.0 at 30°C with 1 M HCl.
- 10 ml of 600 mM Magnesium Chloride Solution ($MgCl_2$), was also prepared in deionized water using Magnesium Chloride, Hexahydrate, Sigma Prod. No. M-0250).
- 10 ml of 120 mM Adenosine Triphosphate Solution (ATP) was prepared in deionized water using Adenosine 5'-Triphosphate, Disodium Salt, Sigma Prod. No. A-5394.
- 10 ml of 360mM β-D(+) Glucose solution (Glucose) was prepared in deionized water using β-D(+) Glucose, Sigma prod.No.G-5250.
- 27 mM β-Nicotinamide Adenine Dinucleotide Phosphate, Oxidized form solution (β-NADP), 30 mg vial of β-Nicotinamide Adenine Dinucleotide Phosphate, sodium salt, Sigma stock No.240-330 in the appropriate volume of deionized water.

- A solution of 100 units/mL of Glucose-6-Phosphate Dehydrogenase (G-6PDH) of Sigma product No.G-6378 in cold deionized water was prepared before use.
- 50 mM Tris HCl buffer, pH 8.5 at 30 ^{0}C (enzyme diluents), was prepared in Trizma base using deionized water, Sigma product No.T-1503, and adjust to pH 8.5 at 30 ^{0}C with 1M HCl.
- Glucokinase Enzyme Solution (GLCK); a solution containing 0.25-0.50 unit/mL of this enzyme was prepared immediately before use in cold enzyme diluents.

Procedure:

A reaction cocktail was prepared by pipetting into a suitable container: 24 mL of Buffer, 1 mLs of $MgCl_2$, ATP, glucose and β-NADP solutions. This content was mixed, swirled and adjusted to PH 9.0 at 30 ^{0}C with 1 M HCl or NaOH, if necessary. Thereafter, 2.8 mL of the reaction cocktail was pippetted into test and blank cuvettes and then 0.1 mL of 6-GPDH solution added into test and blank cuvettes and mixed by inversion and equilibrate to 30 ^{0}C. This was monitored at A_{340nm} using a suitably thermostatted spectrophotometer and 0.1 mL Glucokinase enzyme solution was added into the test cuvette while enzyme diluents were added into the blank cuvette. This was immediately mixed by inversion and the increase in A_{340nm} recorded for approximately 5 minutes. Change in A_{340nm} per minute was also obtained using the maximum linear rate for both the test and blank.

Units/ml enzyme = (ΔA_{340nm}/min Test-$\Delta 340_{nm}$/min Blank) ×3/(6.22)(0.1)

3=Volume (in milliliter) of assay

6.22=Millimolar extinction coefficient of β-NADP at 340 nm

0.1=Volume (in milliliters) of enzyme used.

3.9.3 Determination of serum liver enzymes activities

Activities of serum alanine aminotransaminase was estimated by the method adopted by Tietz (1995), aspartate aminotransaminase was determined by the method of Henderson and Moss (2001) while alkaline phosphatase was determined according to the method of Scherwin (2003). All the above were done using ELITECH Clinical Systems kits.

3.9.4 Determination of Lipid Profile

3.9.4.1 Determination of Serum Total Cholesterol

This was determined spectrophotometrically, using enzymatic colometric assay kits (Randox Laboratories Limited kits, Unite Kingdom) as follows: The serum level of total cholesterol was quantified after enzymatic hydrolysis and oxidation of the sample as described by the method of Stein (1987). 1000 µL of the reagent was added to each of the sample and standard. This was incubated for 10 minutes at 20-25 ^{0}C after mixing and the absorbance of the sample (A_{sample}) and standard ($A_{standard}$) was measured against the reagent blank within 30 minutes at 546 nm. The value was expressed in the unit of (mmol/L).

3.9.4.2 Determination of Serum Triglyceride

The serum triglyceride level was determined after enzymatic hydrolysis of the sample with lipases as described by the method of Tietz (1990). 1000 µL of the reagent was added to each of the sample and standard. This was then incubated for 10 minutes at 20-25 ^{0}C after mixing and the absorbance of the sample (A_{sample}) and standard ($A_{standard}$) was measured against the reagent blank within 30 minutes at 546 nm. The value was expressed in the unit of (mmol/L).

3.9.4.3 Determination of Serum High-density Lipoprotein cholesterol

The serum level of HDL-C was measured by the method of Wacnic and Albers (1978). Low-density lipoproteins (LDL and VLDL) and chylomicron fractions in the sample was precipitated quantitatively by addition of phosphotungstic acid in the presence of magnesium ions. The mixture was allowed to stand for 10 minutes at room temperature and centrifuged for 10 minutes at 2000 g. The supernatant represented the HDL-C fraction. The cholesterol concentration in the HDL fraction, which remained in the supernatant, was determined. The value was expressed in the unit of (mmol/L).

3.9.4.4 Determination of Serum Low-density Lipoprotein cholesterol (LDL-c)

The serum level of (LDL-C) was measured according to the protocol of Friedewald *et al*. (1972) using the relationship below:

$$\text{LDL cholesterol} = \frac{[\text{Total cholesterol}] - [\text{HDL cholesterol}] - \text{Triglyceride}}{2.2}$$

The value was expressed in the unit of (mmol/L).

3.9.3.5 Determination of Atherogenic Risk Predictor Indices

Atherogenic risk predictor indices were calculated as earlier reported by Ikewuchi and Ikewuchi (2009a; 2009b; 2010) and (Owolabi *et al.*, 2010). The percentage protection was calculated based on the method of Dhandapani (2007) using the following formulae:

$$\text{Cardiac Risk Ratio} = \frac{\text{Total cholesterol}}{\text{HDL cholesterol}}$$

$$\text{Atherogenic Index (AI)} = \frac{\text{LDL cholesterol}}{\text{HDLcholesterol}} \quad \text{and} \quad \log\frac{\text{Triglyceride}}{\text{HDL cholesterol}}$$

$$\text{Percentage Protection} = \frac{\text{AI of Diabetic Control} - \text{AI of Diabetic Treated}}{\text{AI of Diabetic Control}} \text{ X } 100$$

3.9.5 Determination of Serum Cortisol, Malondialdehyde Concentration and Antioxidant Enzyme Activities

3.9.5.1 Determination of Serum Cortisol Level

Serum was collected between the hours of 9 am and 12 pm. Cortisol was determined using the standard laboratory of BLK diagnostics kit (BLK Cortisol Enzyme-lnked Immunosorbent assay (ELISA) kit, Badalona, Specificity: 100 %, sensitivity: 6.9 nmol/L), which was based on the principle of competitive binding (Tietz, 1994).

3.9.5.2 Determination of Serum Malondialdehyde Concentration

Malondialdehyde (MDA) is one of many low molecular weight end-products of lipid hydoperoxide decomposition and is the most often measured as an index of lipid peroxidation. Malondialdehyde (MDA) activity was estimated using The NWLSS™ Malondialdehyde assay kits (Northwest Life Sciences Specialies, Product NWK-MDA01, Vancouver WA, Specificity: Malondialdehyde, Sensitivity: 0.08) based on the method adapted by Janero (1990). The test principle is based on the reaction of MDA with thiobarbituric acid (TBA); forming an MDA-TBA2 adduct that absorbs strongly at 532 nm.

3.9.5.3 Determination of Serum Superoxidase Dismutase Activity

Superoxidase Dismutase (SOD) activity was assayed using the North West Life Science Specialties (NWLSS™) SOD assays kit (Product NWK-SOD02, Specificity: Cu/Zn, Mn and Fe Superoxide Dismutase, Sensitivity: 5 U/mL). The method is based on the principle of superoxide inhibition of auto-oxidation rate of hematoxylin as originally described by Martin *et al.,* (1987), with modifications to increase robustness and reliability. Briefly, 920µL of assay buffer was added to each cuvette. This was followed by addition of 40µL of assay buffer (for blank) and 40µL of sample. The mixture was incubated for two (2) minutes. After which 40µL hematoxylin

reagent was added and mixed quickly to start the auto-oxidation reaction. An absorbance was measured at 560 nm for every 10 seconds for 5 minutes.

3.9.5.4 Determination of Serum Catalase Activity

Catalase (CAT) activity was estimated using North West Life Science Specialties (NWLSS™) NWLSS^{TM} Catalase assay kits (Product NWK-CATO1, Specificity: 6.0 U Catalase/mL) based on the method of (Beers and Sizer, 1952) with modifications to increase robustness and convenience. The principle is based on monitoring the consumption of H_2O_2 substrate at 240 nm. Briefly, to a clean cuvette, 1000 µL of sample dilution buffer was added and placed in the reference cuvette holder and the wavelength of spectrophotometer was set to 240 nm. To a clean semi-micro UV cuvette, 950 µL of working assay buffer was added. 50 µL of diluted standard or sample was pippetted to the cuvette and mixed as quickly as possible by repeated pipetting (About 10 times) with the same pipette tip. An absorbance was measured immediately at 240 nm.

3.9.5.5 Determination of Serum Glutathione Peroxidase Activity

Glutathione peroxidase (GPx) activity was assessed using North West Life Science Specialties (NWLSS™) cGPx (GPX1) ELISA kit (Product NWK-GPX02, Specificity: Gluthathione peroxidase, Sensitivity: 12.5pg/ml) based on the method described by Avissar *et al.*, (1994). The assay is based on a sandwich Enzyme-Linked Immunosorbent Assay, where sample GPx concentration was determined by comparing the 450 nm absorbance sample wells to the absorbance of known standards.

3.9.6 Determination of Kidney Tissue Malondialdehyde Concentration and Antioxidant Enzyme Activities

3.9.6.1 Estimation of Kidney Tissue Malondialdehyde Concentration

Kidney tissue MDA levels were measured by the double heating method as described by Placer *et al* (1966). The principle of the method was based on the spectrophotometric measurement of the colour that occured during the reaction to thiobarbituric acid with MDA. Concentration of thiobarbituric acid reactive substance was calculated by the absorbance coefficient of MDA thiobarbituric acid-complex and expressed in nmol/mg protein.

3.9.6.2 Determination of Kidney Tissue Superoxide Dismutase Activity

Total superoxide dismutase (SOD) activity was measured spectrophotometrically using xanthine/xanthine oxidase as an O_2^- generating system and nitroblue tetrazolium (NBT) as a detector as described by Suzuki (2000). Briefly, each sample was diluted 1:10 with phosphate buffer (50 mM, pH 7.5, EDTA 1 mM). Sodium-carbonate working solution (50 mM, xanthine 0.1 mM, NBT 0.025 mM, EDTA 0.1 mM), xanthine oxidase (0.1 U/mL in ammonium sulfate 2 M) and sample or blank (phosphate buffer 50 mM, pH 7.5; EDTA 1 mM) were mixed in a cuvette. The change in absorbance per minute at 560 nm (DA560) was calculated. Enzymatic activity was expressed in units of SOD activity per mg of protein (U/mg protein). One unit of SOD activity is defined as the amount of enzyme needed to inhibit the reaction of O_2^- with NBT by 50%.

3.9.6.3 Determination of Kidney Tissue Catalase Activity

Catalase activity in the kidney tissue was evaluated by measuring the decrease in H_2O_2 concentration at 240 nm as described by Aebi (1984). Briefly, working solution (phosphate buffer 100 mM; H_2O_2 10 mM) and sample were mixed in a cuvette. The change in absorbance per minute at 240 nm (DA240) was calculated. Enzyme activity was expressed in units of CAT

activity per milligram of protein (U/mg protein). One unit of CAT activity was defined as the amount of enzyme needed to reduce 1 µmoL H_2O_2/min.

3.9.6.4 Determination of Kidney Tissue Glutathione Peroxidase Activity

Glutathione Peroxidase activity in the kidney tissue was measured by monitoring the continuous decrease in NADPH concentration using H_2O_2 as a substrate as described by Flohe´and Günzler (1984). Briefly, in a cuvette, potassium phosphate buffer (500 mM), EDTA (50 mM), sodium azide (20 mM), glutathione reductase (15 U/mL), NADPH (1.5 mM), reduced glutathione (250 mM), sample and H_2O_2 (10 mM) were mixed; the absorbance was followed at 340 nm, and the change in absorbance per minute (DA340) was calculated. Two blanks, one without H_2O_2 and another without sample, were run simultaneously. Enzyme activity was expressed in units of GPx activity per mg of protein (U/ mg protein). One unit of GPx activity was defined as the amount of enzyme that oxidizes 1 mole of NADPH per min.

3.9.7 Determination of Serum Total Protein, Albumin, Globulin and Albumin-Globulin Ratio

The serum total protein was determined by the Biuret method of Reinhold (1953) using a commercial kit (Randox Laboratories Ltd., U.K.). The serum albumin which quantitatively binds bromocresol green (BCG) to form an albumin-BCG complex was measured as an endpoint reaction at 596 nm was determined according to the method of Doumas *et al.* (1971). Globulin was obtained by subtracting the albumin from the total protein, while albumin-globulin ratio was obtained according to the method of Coles (1986). Urea was determined according to the method of Kingsley *et al.* (1946). The method was based on the pH increase resulting from the ammonia released by urease hydrolysis of urea. O-Cresolphthalein complexone was used to monitor the pH change colorimetrically.

3.9.8 Determination of Haematological Parameters

The red blood cells (RBC) and white blood cells (WBC) counts were determined by the improved Neubauer haemocytometer method. The haemoglobin (Hb) concentration was determined according to Jain (1986), using the cyanomethaemoglobin method. The packed cell volume (PCV) was determined by the microhaematocrit method according to Dacie and Lewis (1991). Schilling method of differential leucocyte count was used to determine the distribution of the various leucocytes (Mitruka and Rawnsley, 1977). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were computed according to Jain (1986).

3.9.8.1 Determination of Erythrocyte Osmotic Fragility test

Determination of erythrocyte osmotic fragility was based on method described by Faulkner and King (1970) and modified by Oyewale (1992). It is an indirect method of determination of oxidative stress status**.** Briefly, 1 mL of freshly obtained blood was collected into heparinized sample bottles from each rat was pipette into a set of test tubes containing 0.0, 0.1, 0.3, 0.5, 0.7, 0.9 g/dL of sodium chloride stock solution (pH 7.4). This was followed by careful mixing and incubation for 30 min at room temperature (18 – 25^0C). Thereafter, the test tubes were centrifuged at 800 g for 10 min using a centrifuge IEC HNSH (Damon/IEC Division, UK). The supernatant was transferred into a glass cuvette. The concentration of haemoglobin in the supernatant was measured colorimetrically by reading the absorbance at a wavelength of 540 nm using a spectrophotometer (Jenwey, 6405, Japan). The percentage haemolysis was calculated using the following formula:

$$\text{Percentage haemolysis} = \frac{\text{Optical density of test solution}}{\text{Optical density of standard water}} \times 100$$

Erythrocytes malondialdehyde concentration: Heparinized blood samples (2 ml) obtained from each rat was centrifuged at 3000 g for 5 minutes, and the plasma discarded. By washing erythrocytes three times in cold isotonic saline (0.9%, w/v), erythrocyte packets were prepared and used to assay for MDA concentrations using the double-heating method of Draper and Hadley (1990), as modified by Yavuz *et al.* (2004). The principle of the method was based on spectrophotometric measurement of the colour produced during the reaction to thiobarbituric acid (TBA) with MDA. The concentration of MDA was calculated by the absorbance coefficient of MDA–TBA complex, $1.56 \times 105\ cm^{-1}M^{-1}$, and expressed in nanomoles per gram of haemoglobin. The method of Dacie and Lewis (1991) was used to evaluate the haemoglobin concentration in the washed erythrocytes.

3.9.9 Evaluation of Renal Function

The level of serum urea was determined using the method of Tietz *et al.* (1994). Serum sodium and potassium ions were measured by the flame photometry method of Vogel (1960), and bicarbonate ion was determined using the titration method of Segal (1955). Chloride ion was analyzed using the method of Schales and Schales (1941).

3.10 Determination of Hepatic Glycogen Content

This was determined spectrophotometrically using enzymatic colometric assay kits, as described by method of Iwabuchi and Kawahara (2011). Immediately after incision from the animal 1.0 g of liver was homogenized in 10 ml of 0.1 M of cold citrate buffer of pH 7.4. The homogenate

was then centrifuged 14,000 *g* for 5 min to remove debris and 10 µL clear supernatant was then used for the assay. All components were equilibrated to room temperature and thawed enzymes kept in a refrigerator during the experiment. A calibration curve was then prepared to cover concentration of 0 - 200 µg/mL. A total of 10 µL of the standard and sample was transferred into separate wells of a clear flat-bottom microplate. For each reaction well, 90 µL assay buffer, 1 µL enzyme A, 1 µL enzyme B and 1 µL dye reagent was mixed in a clean tube. 90 µL working reagent was transferred into each reaction well. The plate was tapped to mix well and incubated for 30 mins at room temperature (18 – 25^{0}C). The optical density was read at 570 nm.

3.11 Histological Preparation of Liver and Kidney Tissues

At the end of four weeks of lycopene and drug treatment, all animals from each group were euthanized and liver tissues dissected out and fixed immediately in 70% ethanol fixative solution for histological studies. After fixation, tissues were embedded in paraffin, solid sections were cut at 5µm and various sections were stained with periodic acidic Schiff (PAS) as described by Bancroft and Gamble (2008) for determination of hepatic glycogen content. Briefly, the section was washed with water after which the section was treated with 1% periodic acid for 5 minutes. The section was then washed in water and treated with Schiff reagent for 15 minutes. Thereafter the section was then washed in water and stained with haematoxylin for 3 minutes. The section was washed in water again and was differentiated with 1% acid alcohol for 30 seconds. The section was washed in water and was stained blued in scott tap water for 60 seconds. The section was washed in water after which it was dehydrated, cleared and coverslip. The slides were viewed at the magnification of X 250 and photomicrographs taken. In addition, the kidney tissues were also dissected out and washed with ice-cold saline immediately. A portion of each tissue was fixed in 10% neutral formal-saline fixative solution for general histological studies

with Haematoxylin and Eosin. The slides were viewed at the magnification of X 250 and photomicrographs taken.

3.12 Statistical Analysis

Data obtained from each group were expressed as mean ± SEM. The data were statistically analyzed using (ANOVA) with Tukey's *post-hoc test* to compare the levels of significant between the control and experimental groups. All statistical analysis was evaluated using SPSS version 17.0 software and Microsoft Excel (2007). The values of $P \leq 0.05$ were considered as significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Changes on Fasting Blood Glucose Level in Streptozotocin-induced Diabetic Wistar Rats

The blood glucose concentration was significantly increased ($P < 0.05$) (from 91.0 ± 5.74 to 364.4 ± 44.50 mg/dl) after STZ injection on week 0. There were no significant differences (P >

0.05) between and within the treated groups on week 0. However, treatment of diabetic animals with the graded doses of lycopene (10, 20 and 40 mg/kg) and glibenclamide (2 mg/kg) resulted to a significant ($P < 0.05$) steady decrease on blood glucose concentration especially from the second week of treatment (from 465.2 ± 39.81 to 216.4 ±19.55, 240.2 ± 21.60 216.0 ± 28.51 and 188.0 ± 10.06 mg/dL) and the week 3 (from 487.0 ± 25.64 to 186.2 ±9.20, 183.0 ± 10.57, 164.4 ± 21.19 and 150.2 ± 20.28 mg/dL) and week 4 (from 431.4 ± 48.84 to 171.1 ± 7.65, 118.4 ± 1.97, 100.8 ± 6.89 and 108.8 ± 16.74 mg/dL) when compared with corresponding diabetic control group (Figure 4.1).

4.2 Effect of Lycopene on Serum Insulin Level in Streptozotocin-induced Diabetic Wistar Rats

The serum insulin levels decreased significantly ($P < 0.05$) in the diabetic untreated animals to (3.02 ± 0.24 µIU/mL) following STZ treatment from (12.04 ± 0.93 µIU/mL) in normal control (NC) when compared. There were no significant ($P > 0.05$) differences between and within groups in the diabetic lycopene treated groups. However, administration of various doses (10, 20 and 40 mg/kg) of lycopene to diabetic rats did not produce any significant ($P > 0.05$) increase on serum insulin levels (4.02 ± 0.70, 3.96 ± 1.41 and 5.06 ± 0.96 µIU/mL), when compared with diabetic control group. Conversely, treatment of diabetic animals with the standard drug (glibenclamide 2 mg/kg) produced a significantly ($P < 0.05$) elevated serum insulin level (7.76 ± 0.42 µIU/mL), when compared with the diabetic (3.02 ± 0.24 µIU/mL) control group (Figure 4.2).

4.3 Effect of Lycopene on Hepatic Glucokinase Enzyme Activity in Streptozotocin-induced Diabetic Wistar Rats

The activity of hepatic glucokinase was significantly ($P < 0.05$) decreased from 16.62 ± 0.63 ng/mL in the normal control animals to 8.78 ± 1.11 ng/mL in diabetic control group when

compared. The administration of lycopene to diabetic rats of (10, 20 and 40 mg/kg) significantly ($P < 0.05$) increased activity of hepatic glucokinase to 11.96 ± 0.54, 14.23 ± 0.88 and 15.78 ± 0.27 ng/mL respectively in a dose dependent manner, with (glibenclamide 2 mg/kg) producing the highest effect (17.68 ± 0.75 ng/mL) when compared with the diabetic control group (Figure 4.3).

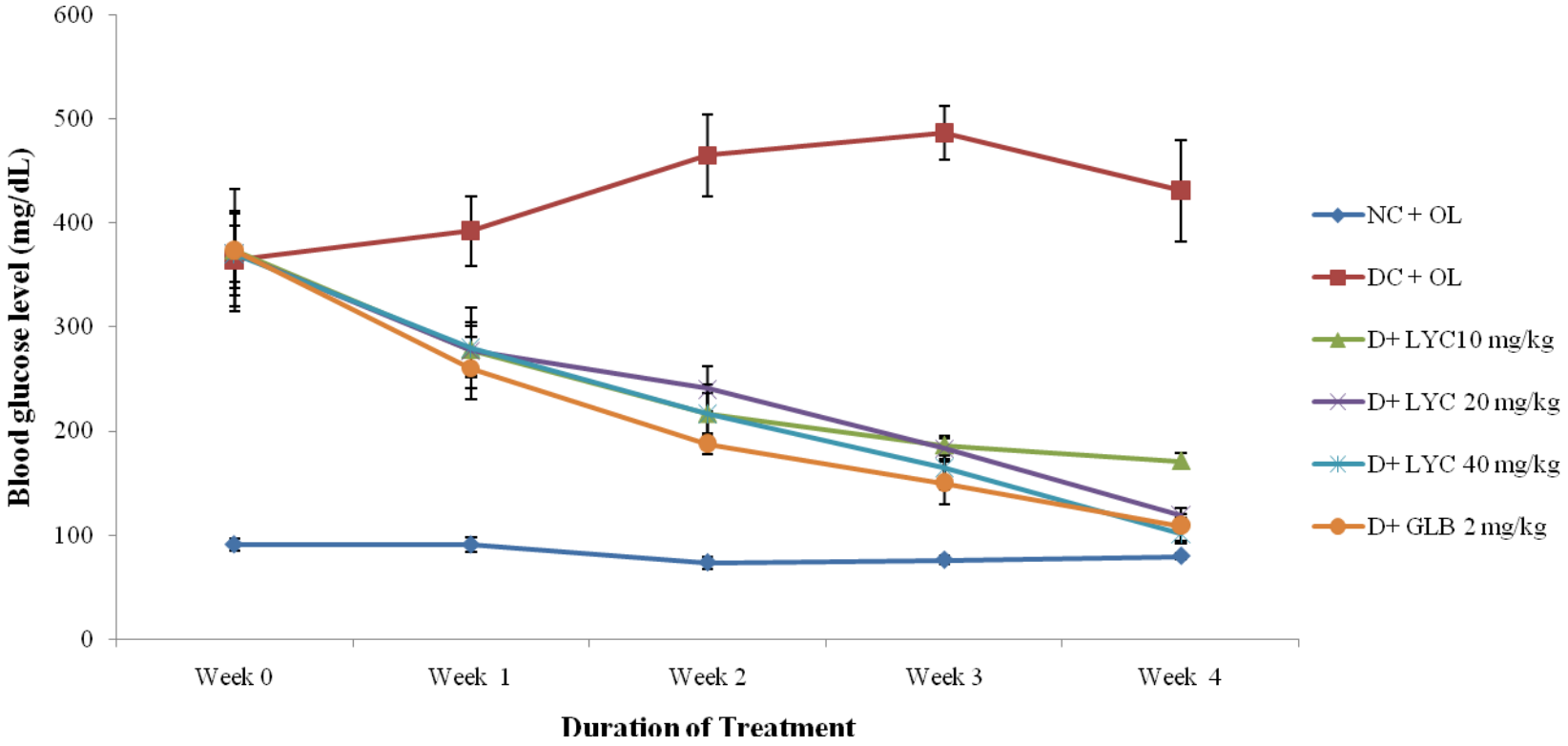


Figure 4.1 Changes on fasing blood glucose level in streptozotocin-induced diabetic Wistar rats

DC+OL = Diabetic rats treated with olive oil, NC+OL = Normal (Non-diabetic) rats treated with olive oil, D+LYC10 mg/kg = Diabetic rats treated with 10 mg/kg of lycopene, D+LYC 20 mg/kg = Diabetic rats treated with 20 mg/kg of lycopene, D+LYC 40 mg/kg = Diabetic rats treated with 40 mg/kg of lycopene and D+GLB 2 mg/kg = Diabetic rats treated with glibenclamide 2 mg/kg

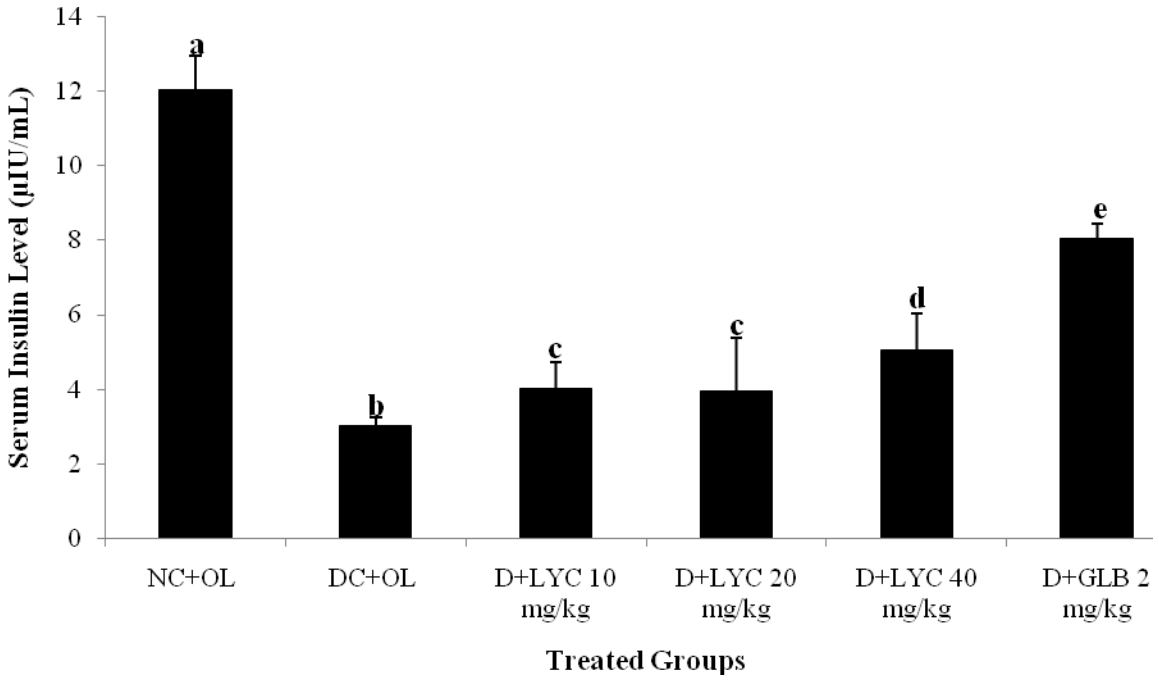


Figure 4.2: Effects of lycopene on serum insulin level in streptozotocin-induced diabetic Wistar rats

Each bar represents mean of five animals

Bars with different superscript letters (a, b, c, d, e) differ significantly ($P < 0.05$) compared with the control groups, while bars with the same superscript letters are not significantly different ($P > 0.05$) compared with the control groups

DC+OL = Diabetic rats treated with olive oil, NC+OL = Normal (Non-diabetic) rats treated with olive oil, D+LYC10 mg/kg = Diabetic rats treated with 10 mg/kg of lycopene, D+LYC 20 mg/kg = Diabetic rats treated with 20 mg/kg of lycopene, D+LYC 40 mg/kg = Diabetic rats treated with 40 mg/kg of lycopene and D + GLB 2 mg/kg = Diabetic rats treated with glibenclamide 2 mg/kg

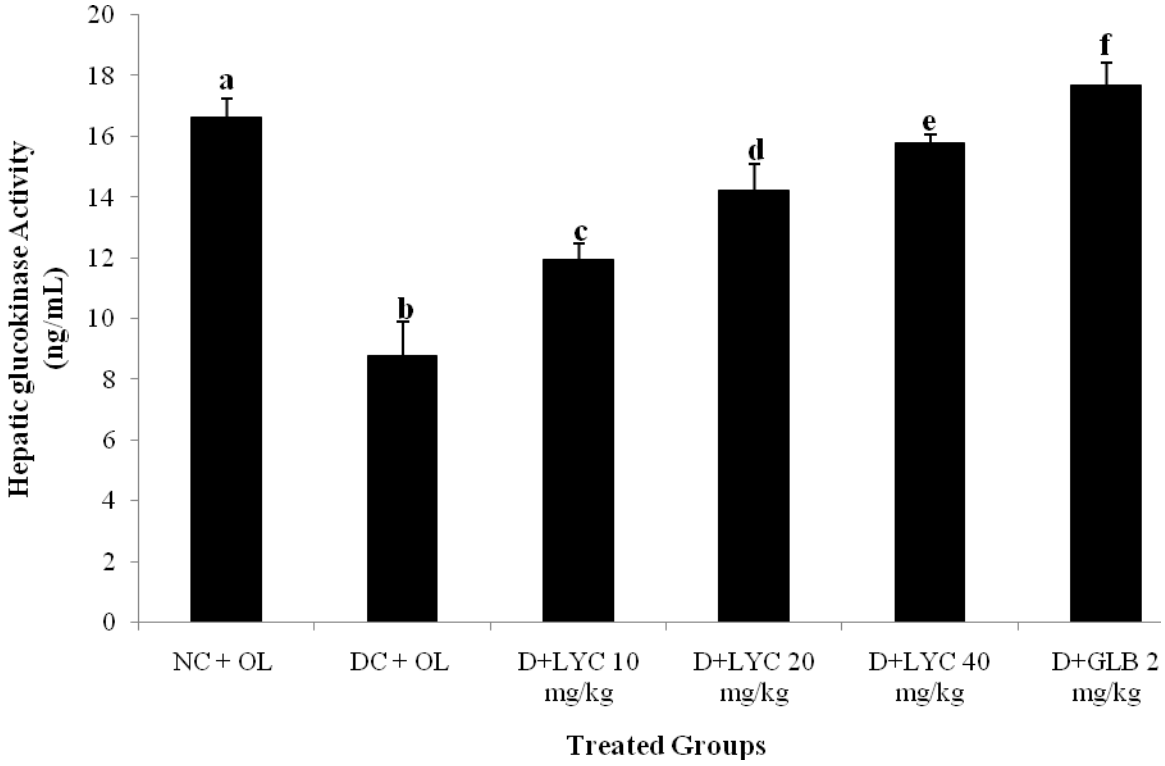


Figure 4.3: Effects of lycopene on hepatic glucokinse acitivity in streptozotocin-induced diabetic Wistar rats

Each bar represent mean of five animals

Bars with different superscript letters (a, b, c, d, e, f) differ significantly ($P < 0.05$) compared with control groups

DC+OL = Diabetic rats treated with olive oil, NC+OL = Normal (Non-diabetic) rats treated with olive oil, D+LYC10 mg/kg = Diabetic rats treated with 10 mg/kg of lycopene, D+LYC 20 mg/kg = Diabetic rats treated with 20 mg/kg of lycopene, D+LYC 40 mg/kg = Diabetic rats treated with 40 mg/kg of lycopene and D+GLB 2 mg/kg = Diabetic rats treated with glibenclamide 2 mg/kg

4.4 Effects of Lycopene on Liver Glycogen Content, Its Percentage Increase and Activities of Serum Liver Enzymes in Streptozotocin-induced Diabetic Wistar Rats

4.4.1 Effect on Liver Glycogen and Its percentage increase

Results in Table 4.4 showed that the diabetic control group that received no treatment expressed significantly ($P < 0.05$) decreased hepatic glycogen content of (4.10 ± 0.09 mg/g tissue) when comapared with those obtained in the normal control group (7.66 ± 0.52 mg/g tissue). Treatment of diabetic animals with lycopene and glibenclamide significantly ($P < 0.05$) restored the depleted hepatic glycogen level to (6.00 ± 0.18, 6.28 ± 0.19, 7.18 ± 0.17 mg/g tissue) and (5.50 ± 0.09 mg/g tissue) when compared with the diabetic control group. The percentage increase in liver glycogen level was also in a dose dependent fashion (44.40 ± 4.81, 53.61 ± 6.63, 78.24 ± 4.88 % respectively) in the diabetic-lycopene and glibenclamide treated groups, respectively when compared with the diabetic control group. However, the diabetic group treated with 40 mg/kg of lycopene recorded the highest percentage increase, while glibenclamide (2 mg/kg) produced the lowest percentage increase in hepatic glycogen level (34.47 ± 4.27 %).

4.4.2 Effect on Activities of Serum Liver Enzymes

Significant ($P < 0.05$) increase in the activity of serum AST of (91.20 ± 2.06 IU/L) was recorded in the diabetic control, when compared with the normal control group that had 41.80 ± 1.69 IU/L. Treatment of diabetic animals with 10, 20 amd 40 mg/kg of lycopene as well as (glibenclamide 2 mg/kg) significantly ($P < 0.05$) reduced the activity of serum AST to (47.40 ± 1.81, 38.20 ± 0.86 and 36.20 ± 0.86 IU/L) and (40.40 ± 2.58 IU/L) for the standad drug when compared with the diabetic control group (Table 4.4) .

The diabetic control animals had significantly ($P < 0.05$) elevated serum ALT activity of (93.60 ± 3.25 IU/L) when compared with the normal control animals group that had ALT activity of

(45.60 ± 1.54 IU/L). The administration of graded doses of lycopene (10, 20 and 40 mg/kg) to diabetic resulted to significantly ($P < 0.05$) decreased serum ALT activity to (51.00 ± 1.73, 40.80 ± 1.59 and 39.00 ± 3.15 IU/L) and (46.40 ± 1.86 IU/L) for the glibenclamide (2 mg/kg) diabetic treated group, when compared with diabetic control group. The effect of lycopene was found to be dose dependent (Table 4.4).

There was a significant ($P < 0.05$) increase in serum ALP activity (134.80 ± 3.14 IU/L) in the diabetic untreated animals, when compared with the normal control rats that had (66.20 ± 2.20 IU/L). Administration of 10, 20 and 40 mg/kg of lycopene to diabetic animals produced a significantly ($P < 0.05$) reduced serum ALP activity of (78.40 ± 2.38, 59.20 ± 7.33 and 72.80 ± 2.65 IU/L) and (70.40 ± 2.87 IU/L) for the standard drug (glibenclamide 2 mg/kg) when compared with diabetic control group (Table 4.4).

4.5 Microscopic Findings of Effects of Lycopene on the Histology Liver Tissue of Streptozotocin-Induced Diabetic Wistar Rats

The histological findings of liver section stained with Period acid Schiff (PAS) of control groups and diabetic groups treated with graded doses (10, 20 and 40 mg/kg) of lycopene and glibenclamide (2 mg/kg) are shown in (Plate I to Plate VI). The histological results indicated significant depletion in cytoplasmic glycogen in hepatocytes of diabetic untreated animals (Plate II), when compared with the normal control group (Plate I). Treatement of diabetic animals with lycopene at rates (10, 20 and 40 mg/kg) and glibenclamide (2 mg/kg) recorded significantly improved hepatic glycogen stores, when compared with the corresponding diabetic control group (Plate III - VI). The observed effect was in a dose-dependent manner. However, diabetic group treated with (40 mg/kg) of lycopene recorded the highest amount of hepatic glycogen restoration than those obtained with 10 and 20 mg/kg of lycopene and standard drug (glibenclamide).

Table 4.4: Effects of lycopene on liver glycogen, percentage increase in liver glycogen content and liver enzyme activities in streptozotocin-induced diabetic Wistar rats

Treatment Groups	Liver Glycogen Content (mg/g tissue)	% Increase in Liver Glycogen Content	Aspartate amino transferase (IU/L)	Alanine amino transferase (IU/ L)	Alkaline phosphatase (IU/ L)
NC+OL	7.66±0.52[a]	87.39±13.69	41.80 ± 1.69[a]	45.60 ± 1.54[a]	66.20 ± 2.20[a]
DC+OL	4.10±0.09 [b]	0.00 ±0.00	91.20 ± 2.06[b]	93.60 ± 3.25[b]	134.80 ± 3.14[b]
D+ LYC10 mg/kg	6.00±0.18 [c]	44.40±4.8[a]	47.40 ± 1.81[c]	51.00 ± 1.73[c]	78.40 ± 2.38[b]
D+ LYC 20 mg/kg	6.28±0.19 [c]	53.61±6.63[b]	38.20 ± 0.86[d]	40.80 ± 1.59[c]	59.20 ± 7.33[c]
D+ LYC 40 mg/kg	7.18±0.17[d]	78.24±4.88[d]	36.20 ± 0.86[d]	39.00 ± 3.15[d]	72.80 ± 2.65[b]
D+ GLB 2 mg/kg	5.50±0.09 [c]	34.47±4.27[a]	40.40 ± 2.58[c]	46.40 ± 1.86[b]	70.40 ± 2.87[b]

[a,b,c,d] = Means on the same column with different superscript letters differ significantly ($P < 0.05$) compared with the control groups

While means with the same superscript letters are not significantly different ($P > 0.05$) compared with the control groups

DC+OL = Diabetic rats treated with olive oil, NC+OL = Normal (Non-diabetic) rats treated with olive oil, D+LYC10 mg/kg b w = Diabetic rats treated with 10 mg/kg b w of lycopene, D+LYC 20 mg/kg b w = Diabetic rats treated with 20 mg/kg b w of lycopene, D+LYC 40 mg/kg b w = Daibetic rats treated with 40 mg/kg of lycopene and D+GLB 2 mg/kg b w = Diabetic rats treated with gilbenclamide 2 mg/kg b w.

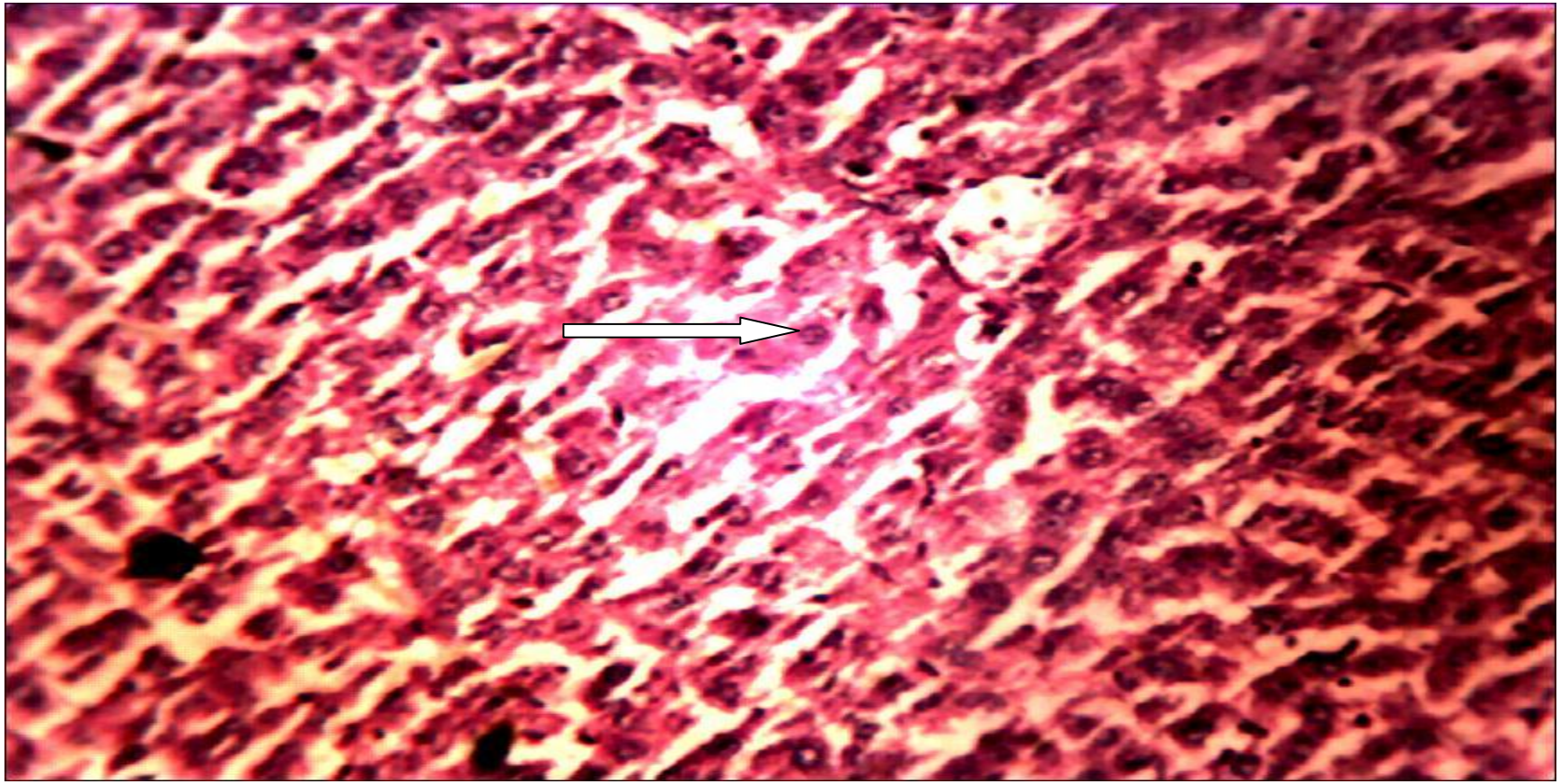


Plate I: Photomicrograph of a section of liver of normal control rat that received olive oil (0.5 ml) orally, showing normal cytoplasmic glycogen deposits in hepatocytes. PAS Stained X 250.

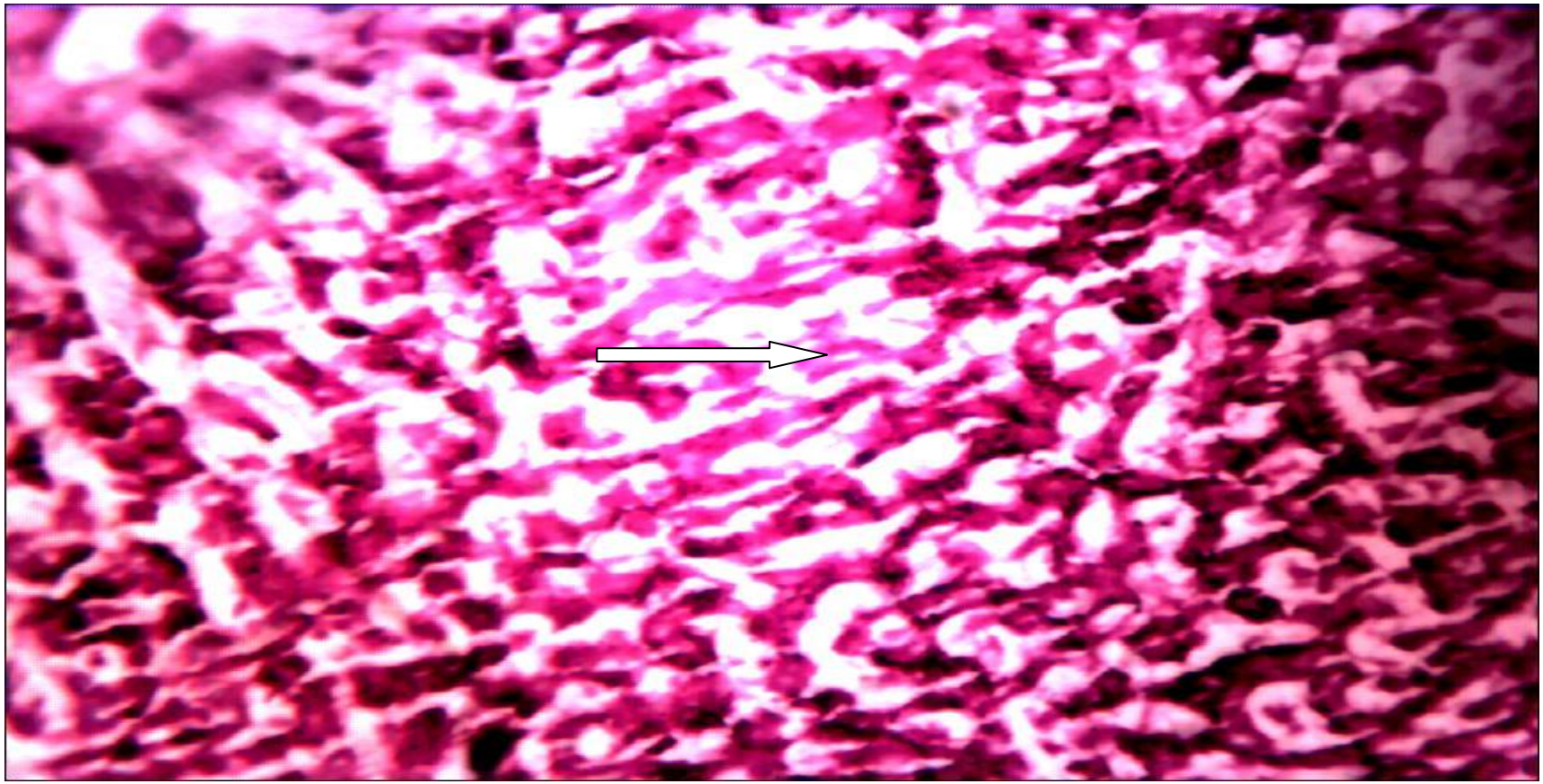


Plate II: Photomicrograph of a section of liver of diabetic control rat treated with olive oil (0.5 ml) orally, showing significantly depleted cytoplasmic glycogen stores. PAS Stained X 250.

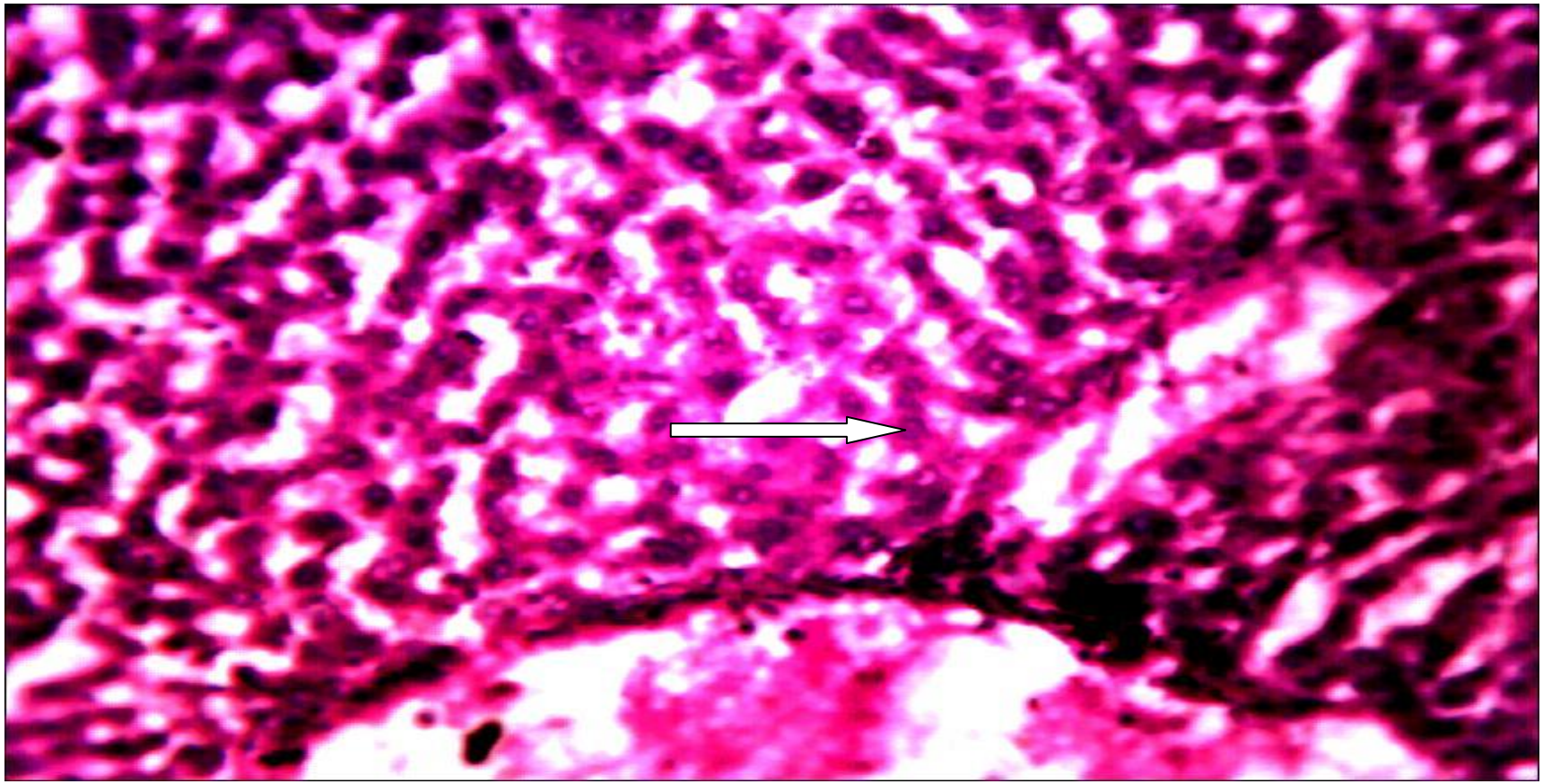


Plate III: Photomicrograph of a section of liver of diabetic rat treated with 10 mg/kg b w orally, showing more improved cytoplasmic glycogen stores. PAS stained X 250.

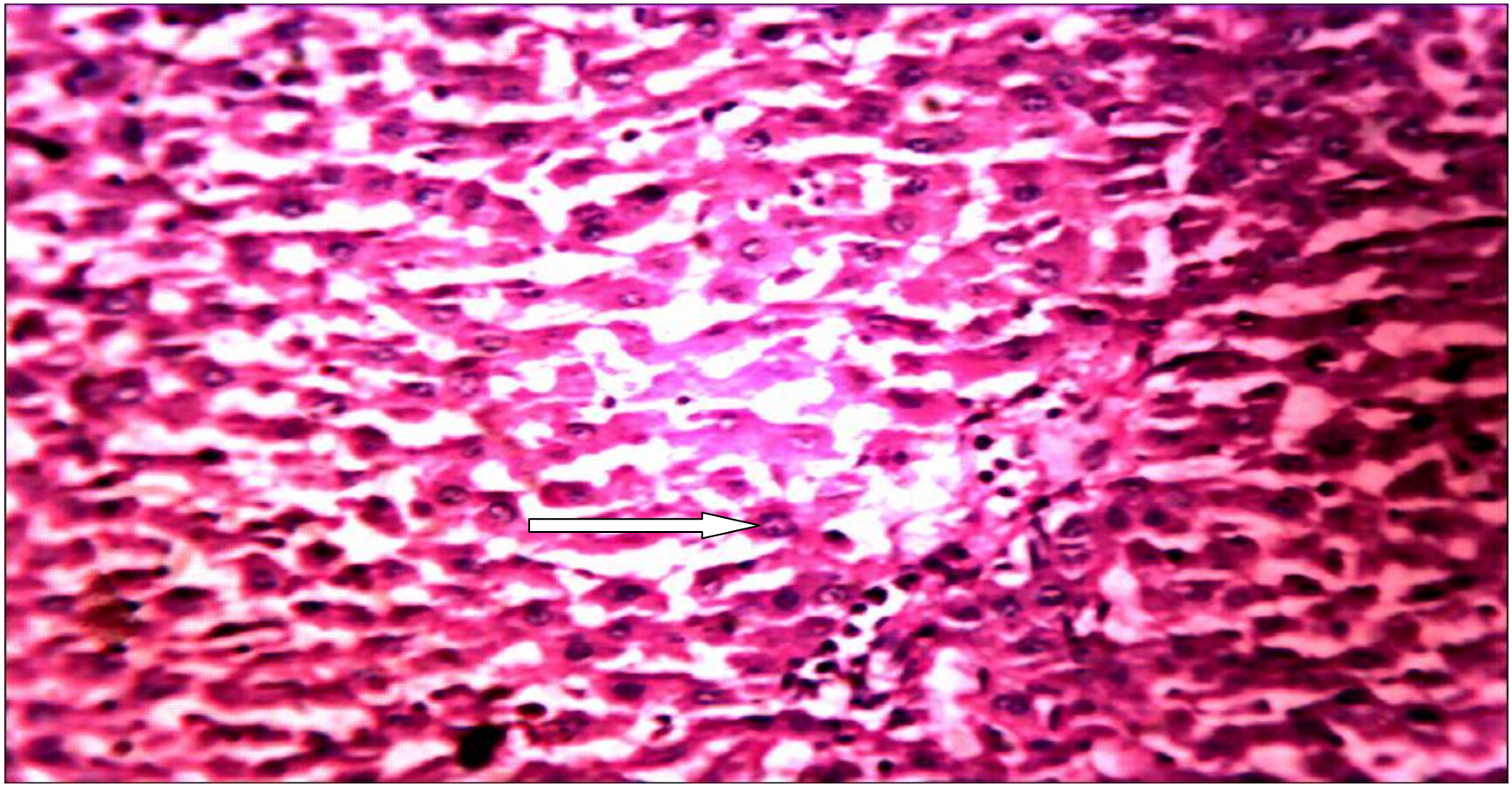


Plate IV: Photomicrograph of a section of liver of diabetic rat treated with 20 mg/kg b w orally, showing relatively improved cytoplamic glycogen deposits in hepatocytes. PAS stained X 250.

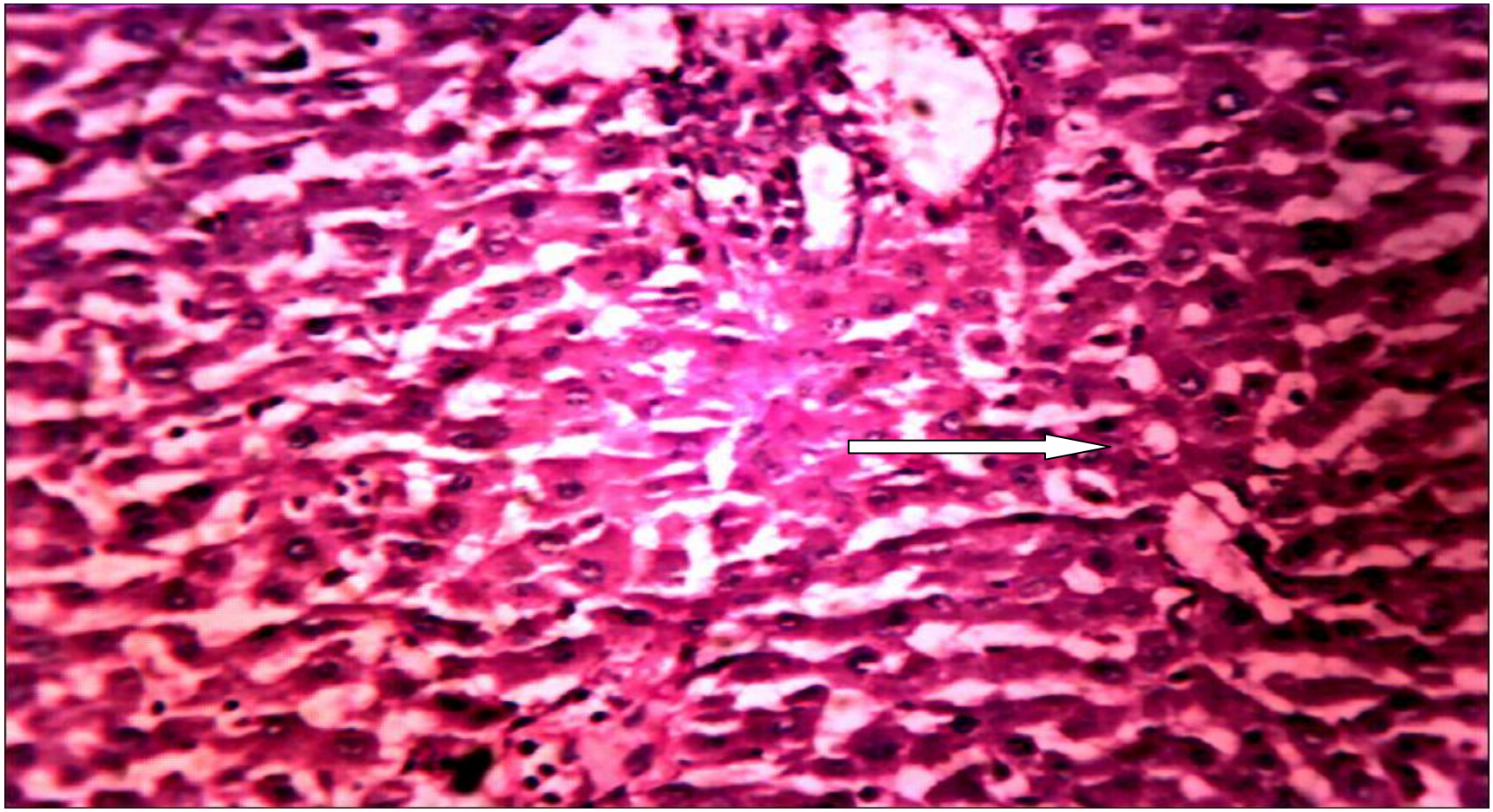


Plate V: Photomicrograph of a section of liver of diabetic rat treated with 40 mg/kg b w orally, showing highest cytoplasmic glycogen stores in hepatocytes. PAS stained X 250.

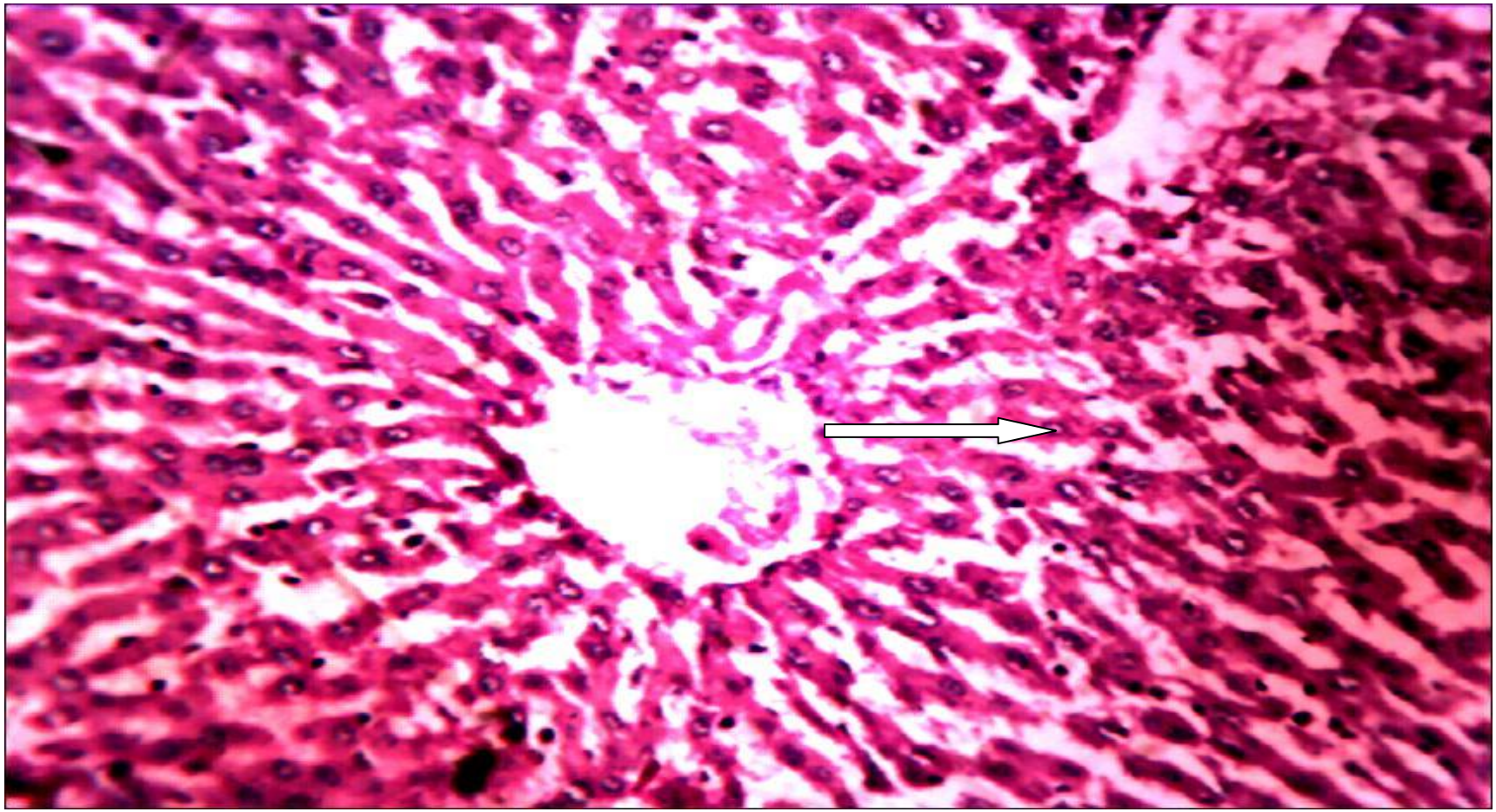


Plate VI: Photomicrograph of a section of liver of diabetic rat treated with glibenclamide 2 mg/kg b w orally, showing improved cytoplasmic glycogen deposits in hepatocytes. PAS stained X 250.

4.6 Effect of Lycopene on Serum Lipid Profile of Streptozotocin-induced Diabetic Wistar Rats

4.6.1 Effect on Serum Total Cholesterol Concentration

The study showed that levels of serum total cholesterol was significantly ($P < 0.05$) increased to (2.70 ± 0.09 mmol/L) in the diabetic untreated group, when compared to the normal control group that recorded (2.30 ± 0.06 mmol/L). Oral administration of lycopene at doses especially at 20 and 40 mg/kg, and (glibenclamide 2 mg/kg) significantly ($P < 0.05$) decreased the serum total cholesterol level to (2.30 ± 0.09 and 2.30 ± 0.08 mmol/L respectively), when compared with diabetic untreated animals that recorded 2.70 ± 0.09 mmol/L (Figure 4.6.1).

4.6.2 Effect on Serum Triglyceride Level

The serum triglyceride level showed significant ($P < 0.05$) increase in the level of serum triglyceride to (1.36 ± 0.09 mmol/L) in diabetic control animals, when compared with the normal control group that recorded (0.58 ± 0.08 mmol/L). Administration of graded doses of lycopene to diabetic rats produced a significant ($P < 0.05$) reduction of serum triglyceride level to (1.00 ± 0.07, 0.72 ± 0.06 and 0.52 ± 0.04 mmol/L) in a dose dependent manner, when compared with the diabetic control group. The effect of lycopene on serum triglyceride reduction was to similar to glibenclamide (2 mg/kg) which recorded (0.54 ± 0.07 mmol/L), when compared with the diabetic conrol group (Figure 4.6.2).

4.6.3 Effect on Serum High-Density Lipoprotein-cholesterol Level

The mean serum high-density lipoprotein level of diabetic control group recorded a significantly ($P < 0.05$) depleted serum level of HDL-c to (0.86 ± 0.06 mmol/L), when compared with the normal control animals (1.26 ± 0.05 mmol/L). However, treatment of diabetic animals with 10, 20 and 40 mg/kg of lycopene significantly ($P < 0.05$) elevated level of serum HDL-c (to 1.18 ±

0.05, 1.26 ± 0.16 and 1.44 ± 0.17 mmol/L) and glibenclamide (2 mg/kg), which produced non significant (P > 0.05) change on serum HDL-c level, when compared with the diabetic untreated animals (Figure 4.6.3).

4.6.4 Effect on Serum Low-density Lipoprotein-cholesterol Level (LDL-c)

Figure 4.6.4 shows that the mean serum LDL-c level was significantly (P < 0.05) increased in the diabetic untreated animals (to 1.57 ± 0.10 mmol/L), when compared with normal control animals that had 0.92 ± 0.07 mmol/L. Treatment of diabetic animals with various doses of lycopene (10, 20 and 40 mg/kg) and glibenclamide (2 mg/kg) significantly (P < 0.05) reduced the serum level of LDL-c (to 1.16 ± 0.06, 0.89 ± 0.20 0.53 ± 0.22, 1.11 ± 0.05 mmol/L respectively) and (1.11 ± 0.05 mmol/L) when compared with the diabetic control group. The effect of lycopene was in a dose-dependent fashion.

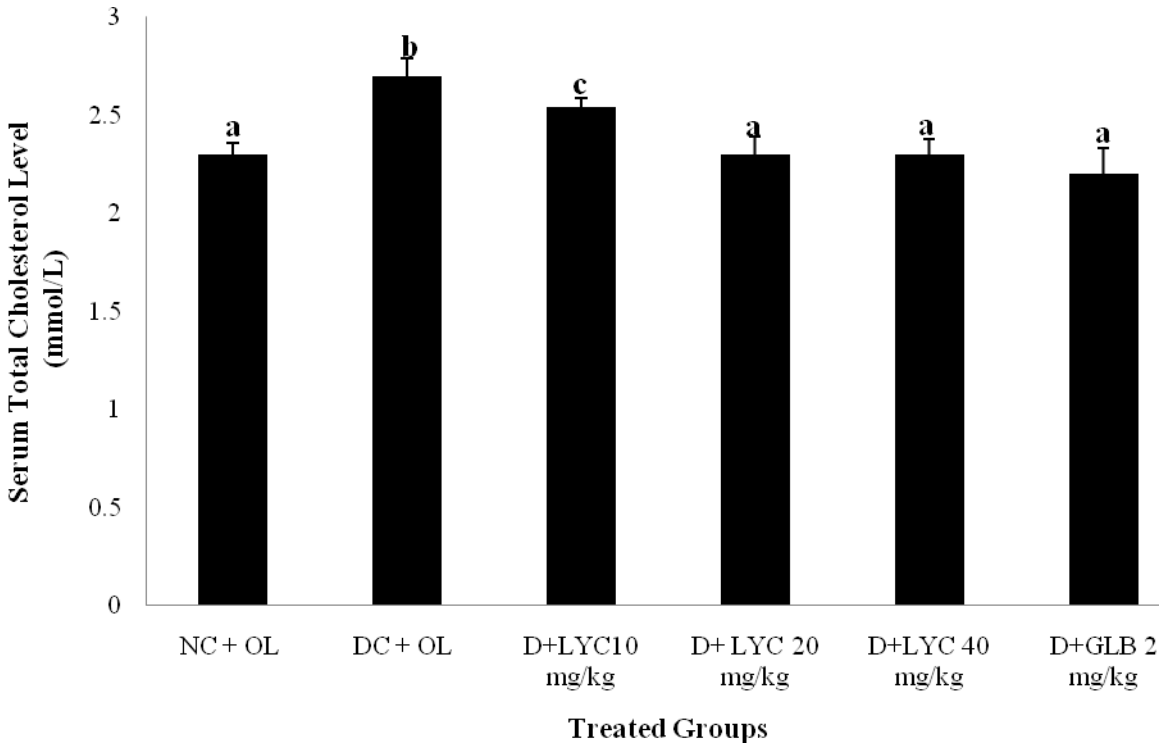


Figure 4.6.1: Effects of lycopene on serum total cholesterol level in streptozotocin-induced diabetic Wistar rats

Each bar represents mean of five animals

Bars with different superscript letters (a, b, c, d) differ significantly ($P < 0.05$) compared with the control groups, while bars with the same superscript letters are not significantly different ($P > 0.05$) compared with the control groups

DC+OL = Diabetic rats treated with olive oil, NC+OL = Normal (Non-diabetic) rats treated with olive oil, D+LYC10 mg/kg = Diabetic rats treated with 10 mg/kg of lycopene, D+LYC 20 mg/kg = Diabetic rats treated with 20 mg/kg of lycopene, D+LYC 40 mg/kg = Diabetic rats treated with 40 mg/kg of lycopene and D+GLB 2 mg/kg = Diabetic rats treated with glibenclamide 2 mg/kg

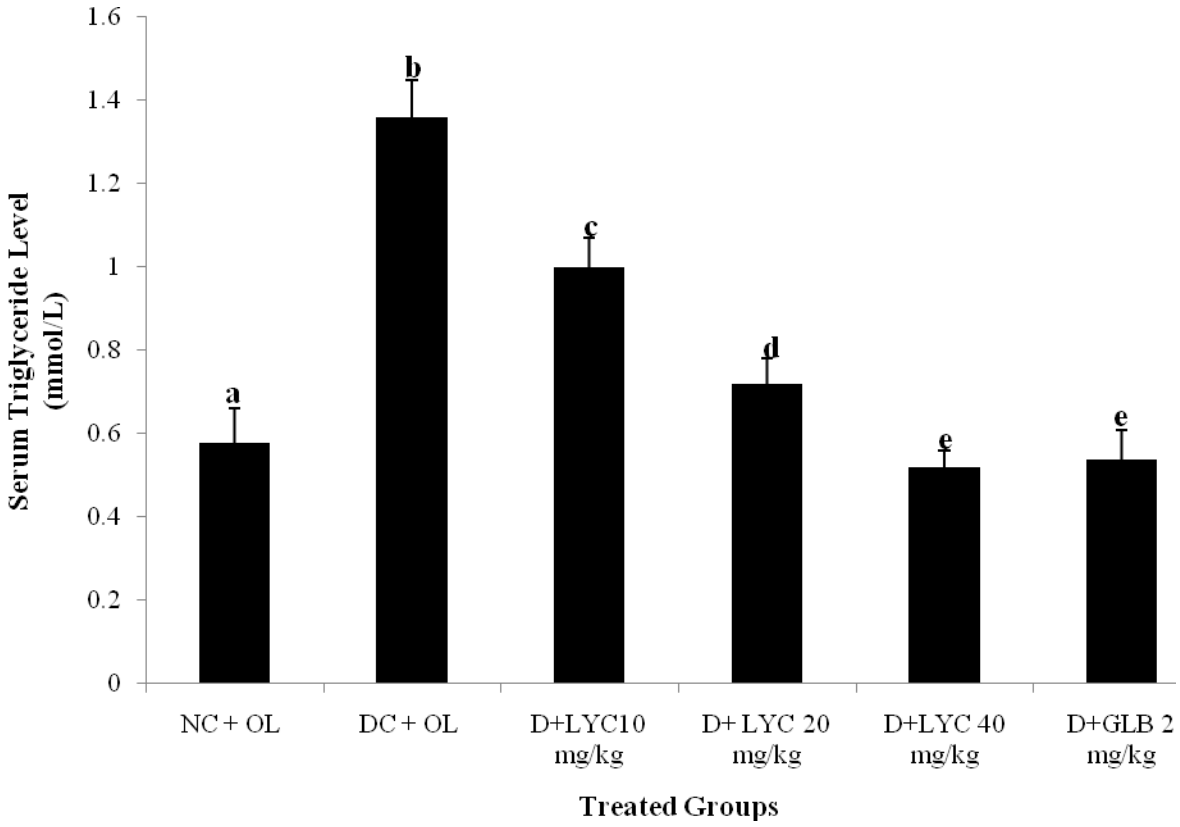


Figure 4.6.2: Effects of lycopene on serum triglyceride level in streptozotocin-induced diabetic Wistar rats

Each bar represents mean of five animals

Bars with different superscript letters (a, b, c, d, e) differ significantly ($P < 0.05$) compared with the control groups, while bars with thesame superscript letters are not significantly different ($P > 0.05$) compared with the control groups

DC+OL = Diabetic rats treated with olive oil, NC+OL = Normal (Non-diabetic) rats treated with olive oil, D+LYC10 mg/kg = Diabetic rats treated with 10 mg/kg of lycopene, D+LYC 20 mg/kg = Diabetic rats treated with 20 mg/kg of lycopene, D+LYC 40 mg/kg = Diabetic rats treated with 40 mg/kg of lycopene and D+GLB 2 mg/kg = Diabetic rats treated with glibenclamide 2 mg/kg.

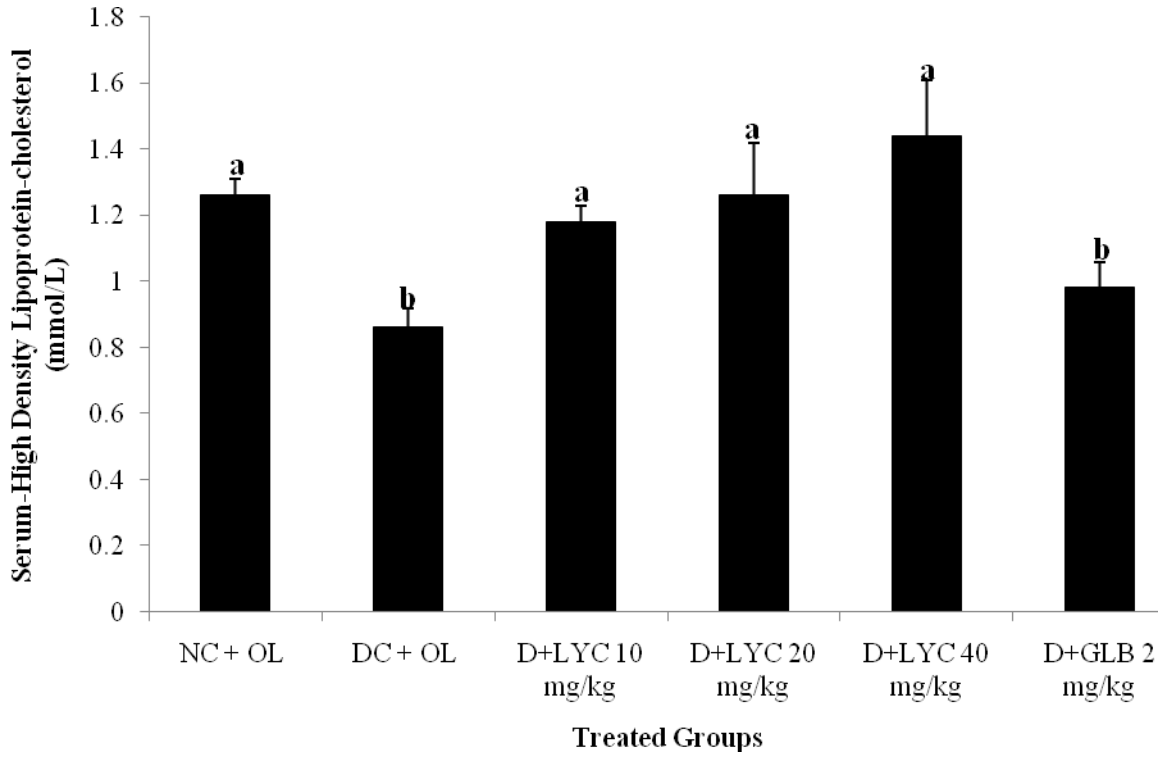


Figure 4.6.3: Effects of lycopene on serum high-density lipoprotein cholesterol level in streptozotocin-induced diabetic Wistar rats

Each bar represents mean of five animals

Bars with different superscript letters (a, b, c, d) differ significantly ($P < 0.05$) compared with the control groups, while bars with the same superscript letters are not significantly different ($P > 0.05$) compared with the control groups

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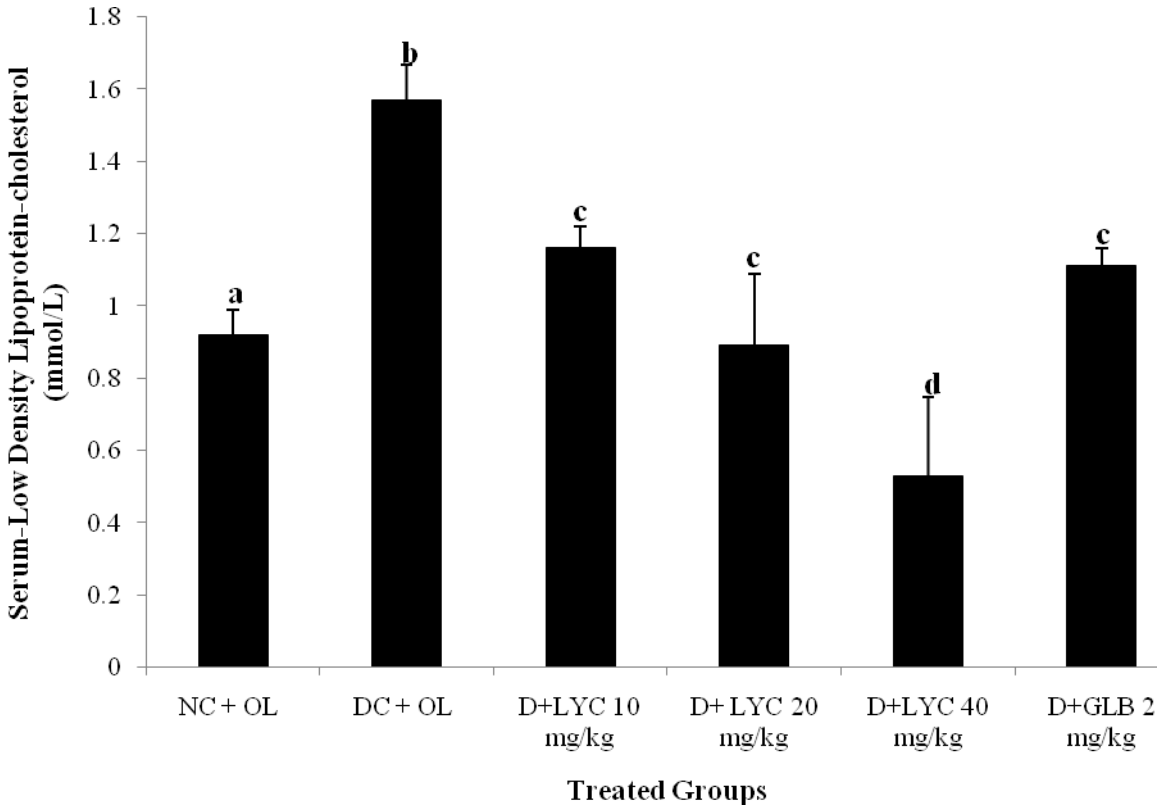


Figure 4.6.4: Effects of lycopene on serum low-density lipoprotein cholesterol level in streptozotocin-induced diabetic Wistar rats

Each bar represents mean of five animals

Bars with different superscript letters (a, b, c, d, e) differ significantly ($P < 0.05$) compared with the control groups, while bars with the same superscript letters are not significantly different ($P > 0.05$) compared with the control groups

DC+OL = Diabetic rats treated with olive oil, NC+OL = Normal (Non-diabetic) rats treated with olive oil, D+LYC10 mg/kg = Diabetic rats treated with 10 mg/kg of lycopene, D+LYC 20 mg/kg = Diabetic rats treated with 20 mg/kg of lycopene, D+LYC 40 mg/kg = Daibetic rats treated with 40 mg/kg of lycopene and D+GLB 2 mg/kg = Diabetic rats treated with glibenclamide 2 mg

4.7 Effect of Lycopene on Serum Atherogenic Risk Predictor Indices in Streptozotocin-induced Diabetic Wistar Rats

4.7.1 Effect on Serum Atherogenic Risk Predictor Indices (LDL-cholesterol/HDL-cholesterol)

The mean values of atherogenic risk predictor indices (LDL-cholesterol/HDL-cholesterol) significantly ($P < 0.05$) increased in the diabetic control group (to 1.88 ± 0.25 mmol/L), when compared with the normal control group, which recorded (0.74 ± 0.82 mmol/L). However, there was a significant ($P < 0.05$) reduction in the serum level of (LDL-cholesterol/HDL-cholesterol) to (1.00 ± 0.10, 0.81 ± 0.22, 0.45 ± 0.21 mmol/L) and (1.15 ± 0.08 mmol/L) in the diabetic groups that received (10, 20 and 40 mg/kg) of lycopene and (glibenclamide 2 mg/kg), when compared with the diabetic untreated animals (Table 4.7).

4.7.2 Effect on Cardiac Risk Ratio (TC/HDL-cholesterol)

A significant ($P < 0.05$) increase in cardiac risk ratio (3.21 ± 0.28 mmol/L) was recorded in the diabetic control animals, when compared with those in the normal group (1.83 ± 0.08 mmol/L). Oral administration of 10, 20 and 40 mg/kg of lycopene and glibenclamide 2 mg/kg produced a dose dependent decrease in CRR (2.17 ± 0.11, 1.94 ± 0.24 and 1.70 ± 0.23 mmol/L) and (2.26 ± 0.09 mmol/L) when compared with animals in diabetic control group (Table 4.7).

4.7.3 Effect on Serum Atherogenic Risk Predictor Indices (HDL-cholesterol/TC)

Table 4.7 shows that there was a significant ($P < 0.05$) decrease in the serum level of HDL-cholesterol/TC (0.32 ± 0.03 mmol/L) in the diabetic untreated animals, when compared with the normal control rats that had a mean serum level of HDL-cholesterol/TC (0.56 ± 0.02 mmol/L). However, the mean serum level of HDL-cholesterol/TC increased significantly ($P < 0.05$) to (0.46 ± 0.02, 0.56 ± 0.08, 0.63 ± 0.09 mmol/L) and (0.45 ± 0.02 mmol/L) in all lycopene and (glibenclamide 2 mg/kg) treated group, when compared with the diabetic control group.

4.7.4 Effect on Serum Atherogenic Risk Predictor Indices log (TRIG/HDL-cholesterol)

The mean serum level of log (TRIG/HDL-cholesterol) in diabetic control animals significantly ($P < 0.05$) increased (1.62 ± 0.17 mmol/L), when compared with the normal control rats (0.47 ± 0.06 mmol/L). Oral administration of graded doses of lycopene (10, 20, 40 mgkg) and glibenclamide (2 mg/kg) resulted to significant ($P < 0.05$) decrease in the serum level of TRIG/HDL-cholesterol (0.89 ± 0.09, 0.62 ± 0.11, 0.39 ± 0.07 mmol/L) and 0.56 ± 0.08 mmol/L), when compared with diabetic untreated (Table 4.7).

4.7.5 Effect on Percentage Protection of Diabetic Treated Groups

Rats in the diabetic group were not protected (0.00 ± 0.00 %) when compared with animals in the normal control group (40.95 ± 6.37 %). However, all the concentrations of lycopene and glibenclamide tested in diabetic animals produced a significant ($P < 0.05$) increase in percentage protection (31.75 ± 7.05, 37.97 ± 9.11, 44.04 ± 9.92 %) and (27.14 ± 7.32 %), when compared with diabetic control group (0.00 ± 0.00 %)(Table 4.7).

Table 4.7: Effects of lycopene administration on atherogenic risk predictor indices in streptozotocin-induced diabetic Wistar rats

Treatment Groups (n=5)	Atherogenic Risk Predictor Indices (LDL-c/HDL-c) (mmol/L)	Cardiac Risk Ratio (TC/HDL)	Atherogenic Risk Predictor Indices HDL/TC Ratio (mmol/L)	Atherogenic Risk Predictor Indices TRIG/HDL Ratio (mmol/L)	% Protection
NC + OL	0.74 ± 0.82^{a}	1.83 ± 0.08^{a}	0.56 ± 0.02^{a}	0.47 ± 0.06^{a}	40.95 ± 6.37^{a}
DC + OL	1.88 ± 0.25^{b}	3.21 ± 0.28^{b}	0.32 ± 0.03^{b}	1.62 ± 0.17^{b}	0.00 ± 0.00^{b}
D+ LYC10 mg/kg	1.00 ± 0.10^{c}	2.17 ± 0.11^{c}	0.46 ± 0.02^{c}	0.89 ± 0.09^{c}	31.75 ± 7.05^{c}
D+ LYC 20 mg/kg	0.81 ± 0.22^{d}	1.94 ± 0.24^{d}	0.56 ± 0.08^{d}	0.62 ± 0.11^{d}	37.97 ± 9.11^{c}
D+LYC 40 mgkg	0.45 ± 0.2^{e}	1.70 ± 0.23^{e}	0.63 ± 0.09^{e}	0.39 ± 0.07^{e}	44.04 ± 9.92^{c}
D+ GLB 2 mg/kg	1.15 ± 0.08^{c}	2.26 ± 0.09^{c}	0.45 ± 0.02^{c}	0.56 ± 0.08^{c}	27.14 ± 7.32^{d}

^{a,b,c,d,e} = Means on the same column with different superscript letters differ significantly ($P < 0.05$) compared with the control groups

While means with the same superscript letters are not significantly different ($P > 0.05$) compared with the control groups

DC+OL = Diabetic rats treated with olive oil, NC+ OL = Normal (Non-diabetic) rats treated with olive oil, D+LYC10 mg/kg b w = Diabetic rats treated with 10 mg/kg b w of lycopene, D+LYC 20 mg/kg b w = Diabetic rats treated with 20 mg/kg b w of lycopene, D+LYC 40 mg/kg b w = Daibetic rats treated with 40 mg/kg of lycopene and D+GLB 2 mg/kg b w = Diabetic rats treated with gilbenclamide 2 mg/kg b w.

4.8 Effect of Lycopene on Serum Cortisol, Malondialdehyde Concentration and Antioxidant Enzymes Activities in Streptozotocin-induced Diabetic Wistar Rats

4.8.1 Effect on Serum Cortisol Level

The serum cortisol level was significantly ($P < 0.05$) higher in diabetic untreated animals (12.48 ± 0.33 µg/dL) than that obtained from normal control animals (8.58 ± 1.05 µg/dL). Lycopene (10, 20 40 mg/kg) and glibenclamide (2 mg/kg) administration to diabetic animals significantly ($P < 0.05$) reduced the serum cortisol level (to 5.36 ± 0.45, 6.42 ± 0.48 and 8.86 ± 0.44 µg/dL respectively) and (7.12 ± 0.96 µg/dL) in glibenclamide treated rats when compared with the diabetic control group (Figure 4.8.1).

4.8.2 Effect on Serum Malondialdehyde Concentration

The effect of treatments on serum MDA concentration is shown in Figure 4.8.2. The MDA concentration, an index of lipid peroxidation was higher ($P < 0.05$) in diabetic control rats (2.80 ± 0.07 nmol/ml), compared to those obtained in the normal control animals (1.84 ± 0.08 nmol/ml). Oral administration of lycopene (10, 20, 40 mg/kg) and glibenclamide (2 mg/kg) respectively significantly ($P < 0.05$) reduced the serum MDA concentration to (2.22 ± 0.07, 1.76 ± 0.20, 1.52 ± 0.19 nmol/ml) and (1.80 ± 0.07 nmol/ml) when compared with the diabetic control group. The highest effect was obtained with 40 mg/kg of lycopene.

4.8.3 Effect on Serum Superoxide Dismutase Activity

The superoxide dismutase activity significantly ($P < 0.05$) decreased (1.64 ± 0.08 IU/L) in animals of diabetic control group, when compared with the normal rats. However, treatment of diabetic animals with lycopene (10, 20 and 40 mg/kg) and glibencalmide (2 mg/kg) produced a significantly ($P < 0.05$) increased activity (1.90 ± 0.05, 2.14 ± 0.09, 2.40 ± 0.14 IU/L) and (2.28

± 0.12 IU/L), when compared with the diabetic untreated animals. The increase in the activity of SOD was in dose-dependent manner (Figure 4.8.3).

4.8.4 Effect on Serum Catalase Activity

A significant ($P < 0.05$) decrease in the activity of serum CAT (43.20 ± 1.16 IU/L) in diabetic untreated group was obtained when compared to those obtained in rats in normal control group (51.60 ± 0.81 IU/L). There was a significant ($P < 0.05$) increase in CAT activity (46.60 ± 1.503, 50.40 ± 1.75, 55.20 ± 1.43 IU/L) and (52.40 ± 0.87 IU/L), when the diabetic animals were administered with 10, 20 and 40 mg/kg of lycopene and 2 mg/kg of glibenclamide respectively compared with diabetic control group. The highest activity of SOD was obtained in the group, treated with 40 mg/kg of lycopene (Figure 4.8.4).

4.8.5 Effect on Serum Glutathione Peroxidase Activity

The activity of serum GPx was significantly ($P < 0.05$) reduced in the diabetic control group (37.20 ± 1.11 IU/L) compared with the animals in the normal group (45.20 ± 1.16 IU/L). Oral administration of graded doses of lycopene produced a significant ($P < 0.05$) increase in GPx activity (40.00 ± 2.09, 43.80 ± 2.04 and 47.60 ± 0.68 IU/L) in a dose-dependent manner, when compared with diabetic untreated group (Figure 4.8.5).

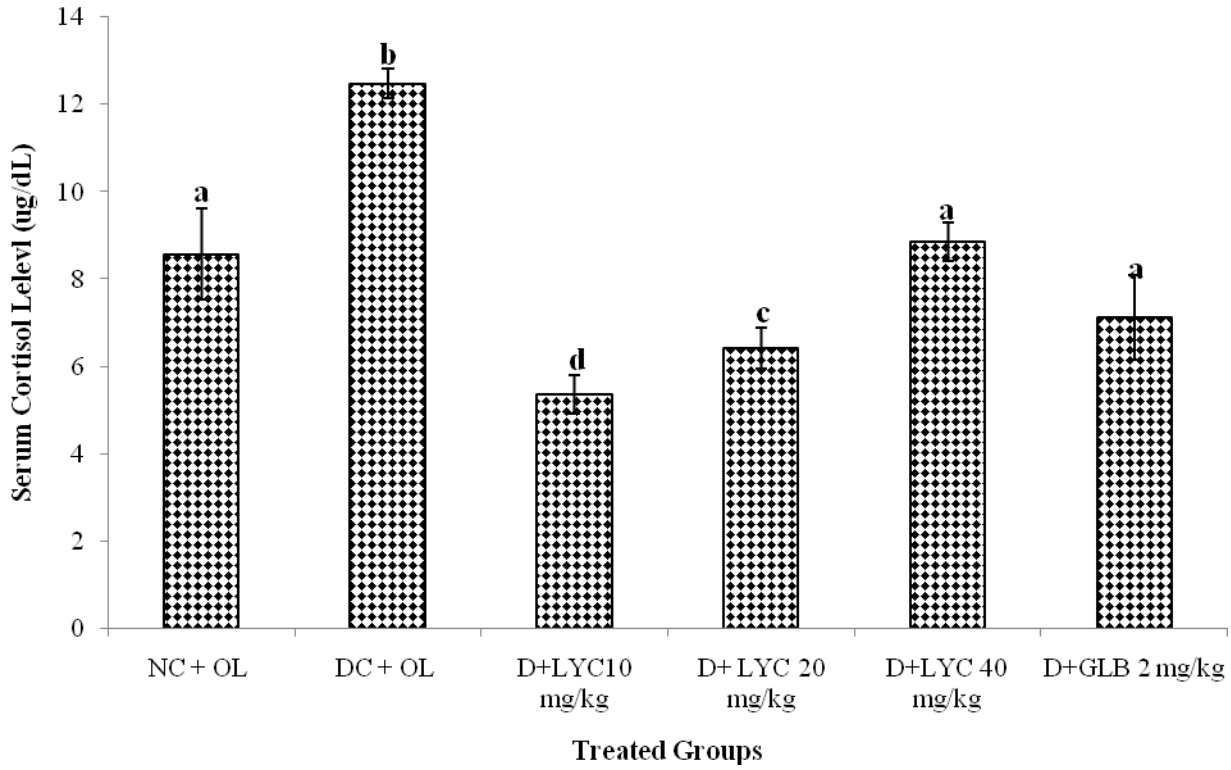


Figure 4.8.1: Effects of lycopene on serum cortisol level in streptozotocin-induced diabetic Wistar rats

Each bar represents mean of five animals

Bars with different superscript letters (a, b, c, d) differ significantly ($P < 0.05$) compared with the control groups, while bars with the same superscript letters are not significantly different ($P > 0.05$) compared with the control groups

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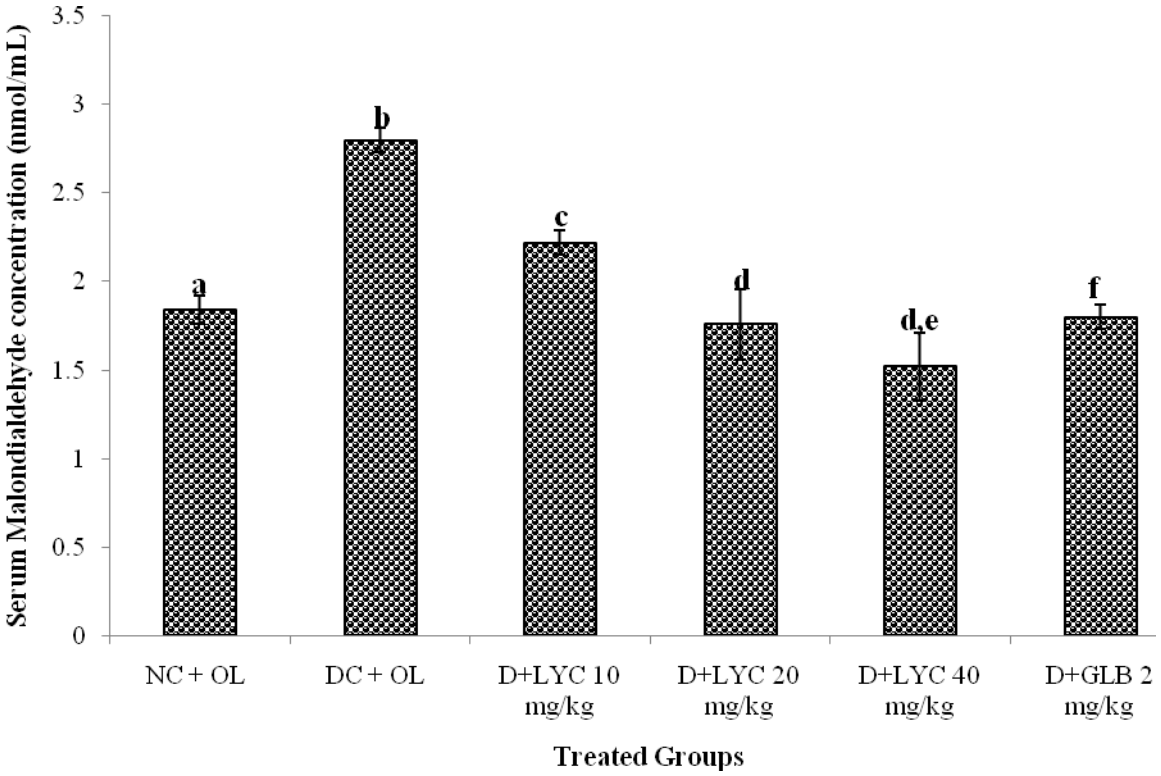


Figure 4.8.2: Effects of lycopene on serum malondialdehyde concentration (MDA) in streptozotocin-induced diabetic Wistar rats

Each bar represents mean of five animals

Bars with different superscript letters (a, b, c, d,e) differ significantly ($P < 0.05$) compared with the control groups, while bars with the same superscript letters are not significantly different ($P > 0.05$) compared with the control groups

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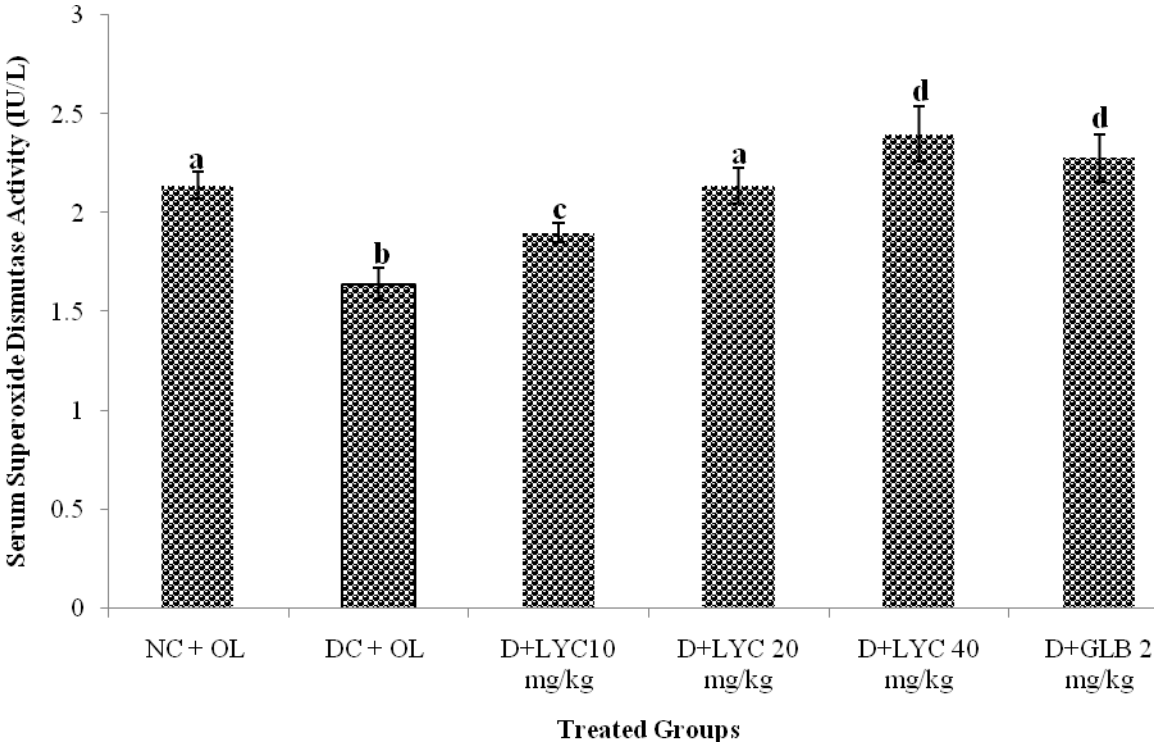


Figure 4.8.3: Effects of lycopene on serum superoxide dismutase activity (SOD) in streptozotocin-induced diabetic Wistar rats

Each bar represents mean of five animals

Bars with different superscript letters (a, b, c, d,e) differ significantly ($P < 0.05$) compared with the control groups, while bars with the same superscript letters are not significantly different ($P > 0.05$) compared with the control groups

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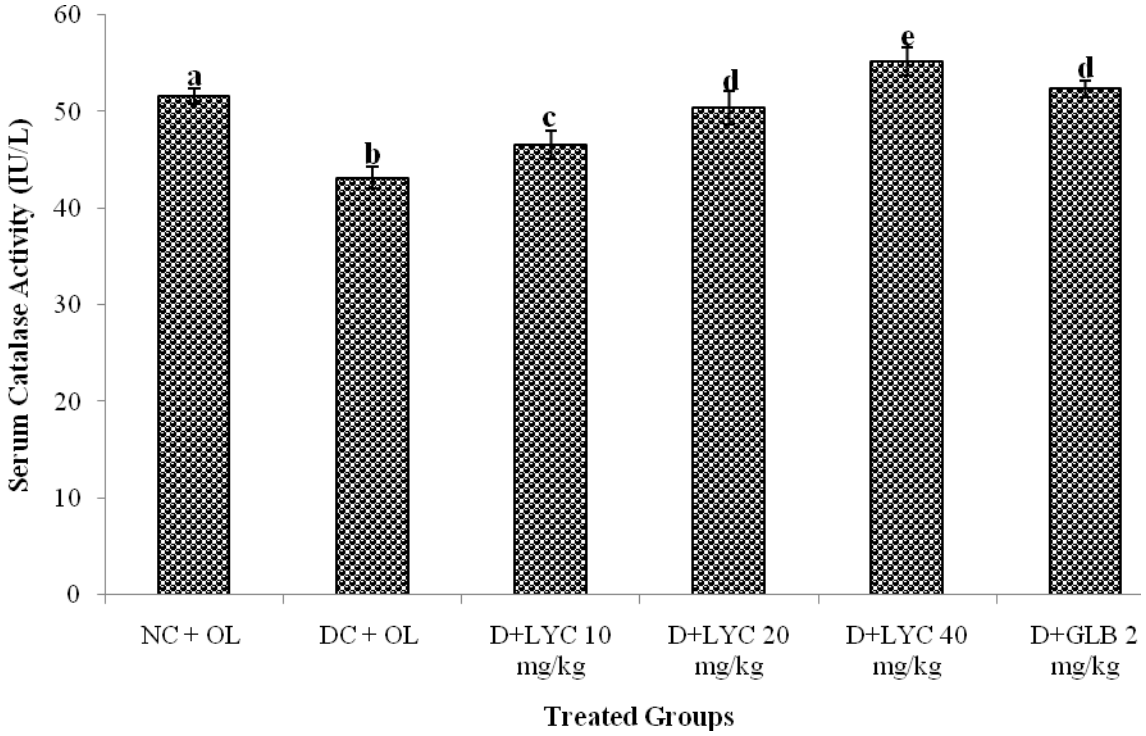


Figure 4.8.4: Effects of lycopene on serum catalase activity (CAT) in streptozotocin induced diabetic Wistar rats

Each bar represents mean of five animals

Bars with different superscript letters (a, b, c, d,e) differ significantly ($P < 0.05$) compared with the control groups, while bars with the same superscript letters are not significantly different ($P > 0.05$) compared with the control groups

DC+OL = Diabetic rats treated with olive oil, NC+OL = Normal (Non-diabetic) rats treated with olive oil, D+LYC10 mg/kg = Diabetic rats treated with 10 mg/kg of lycopene, D+LYC 20 mg/kg = Diabetic rats treated with 20 mg/kg of lycopene, D+LYC 40 mg/kg = Diabetic rats treated with 40 mg/kg of lycopene and D+GLB 2 mg/kg = Diabetic rats treated with glibenclamide 2 mg/kg

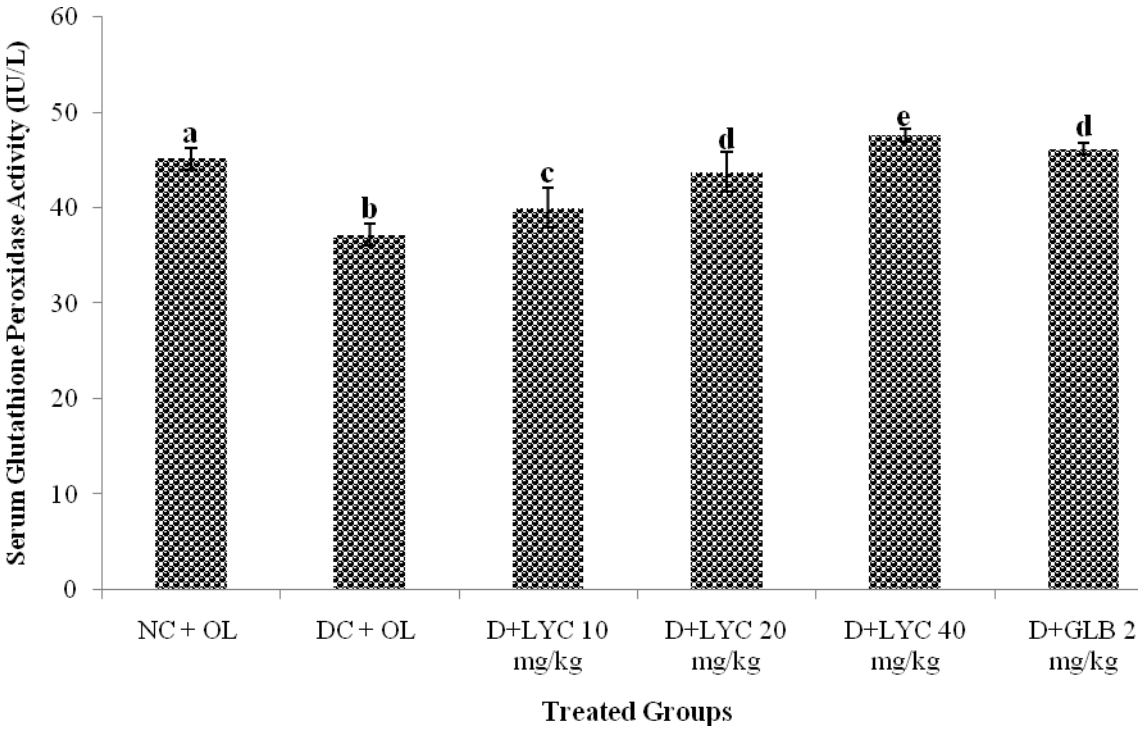


Figure 4.8.5: Effects of lycopene on serum glutathione peroxidase activity (GPx) in streptozotocin-induced diabetic Wistar rats.

Each bar represents mean of five animals

Bars with different superscript letters (a, b, c, d,e) differ significantly ($P < 0.05$) compared with the control groups, while bars with the same superscript letters are not significantly different ($P > 0.05$) compared with the control groups

DC+OL = Diabetic rats treated with olive oil, NC+OL = Normal (Non-diabetic) rats treated with olive oil, D+LYC10 mg/kg = Diabetic rats treated with 10 mg/kg of lycopene, D+LYC 20 mg/kg = Diabetic rats treated with 20 mg/kg of lycopene, D+LYC 40 mg/kg = Diabetic rats treated with 40 mg/kg of lycopene and D+GLB 2 mg/kg = Diabetic rats treated with glibenclamide 2 mg/kg

4.9 Effect of Lycopene on Malondialdehyde Concentration and Activities of Antioxidant Enzymes in Kidneys of Streptozotocin-induced Diabetic Wistar Rats

4.9.1 Effect on Kidney Malondialdehyde Concentration

The concentration of MDA, a maker of lipid peroxidation was significantly ($P < 0.05$) higher in the kidney of diabetic control rats (2.45 ± 0.10 nmol/mg protein) when compared with those obtained in normal control animals (1.60 ± 0.15). However, lyopene treatment at all doses and glibenclamide 2 mg/kg lowered the level of kidney MDA to (1.80 ± 0.19, 1.36 ± 0.18, 1.42 ± 0.12 nmol/mg protein) and (1.44 ± 0.11 nmol/mg protein), when compared to diabetic untreated animals (Figure 4.9.1).

4.9.2 Effect on Kidney Superoxide Dismutase Activity

The activity of SOD was significantly ($P < 0.05$) depleted in the kidney of diabetic untreated rats (DC) (1.66 ± 0.05 U/mg protein) as compared to the animals in the normal control group. Decreased activity of SOD was elevated to (1.80 ± 0.08, 2.08 ± 0.21, 2.32 ± 0.12 U/mg protein) and (2.22 ± 0.06 U/mg protein) when the diabetic animals was treated with (10, 20 and 40 mg/kg) of lycopene and glibenclamide (2 mg/kg), when compared with diabetic control animals (Figure 4.9.2).

4.9.3 Effect on Kidney Catalase Activity

There was a significant ($P < 0.05$) decrease in catalase activity in kidney of diabetic untreated group (43.20 ± 1.16 U/mg protein) in comparison to that obtained in the normal control group (52.20 ± 1.39 U/mg protein). Administration of lycopene (10, 20 and 40 mg/kg) and glibenclamide (2 mg/kg) signififcantly ($P < 0.05$) increased the level of CAT to (44.80 ± 0.90, 47.80 ± 0.90, 52.60 ± 1.86 U/mg protein) and (52.00 ± 0.95 U/mg protein), when compared wth the diabetic control group (Figure 4.9.3).

4.9.4 Effect on Kidney Glutathione Peroxidase Activity

The kidney GPx activity was significantly ($P < 0.05$) lower in diabetic control animals (36.00 ± 0.84 U/mg protein) when compared with those obtained from normal control animals (46.62 ± 0.66 U/mg protein). Lycopene and glibenclamide administration (at dose rates of 10, 20 and 40 mg/kg and 2 mg/kg, respectively) to diabetic animals, significantly ($P < 0.05$) increase the activity of GPx to (41.20 ± 0.50, 46.00 ± 1.79, 49.20 ± 1.80 U/mg protein) and 46.80 ± 0.97 U/mg protein) when compared with the diabetic control group (Figure 4.9.4).

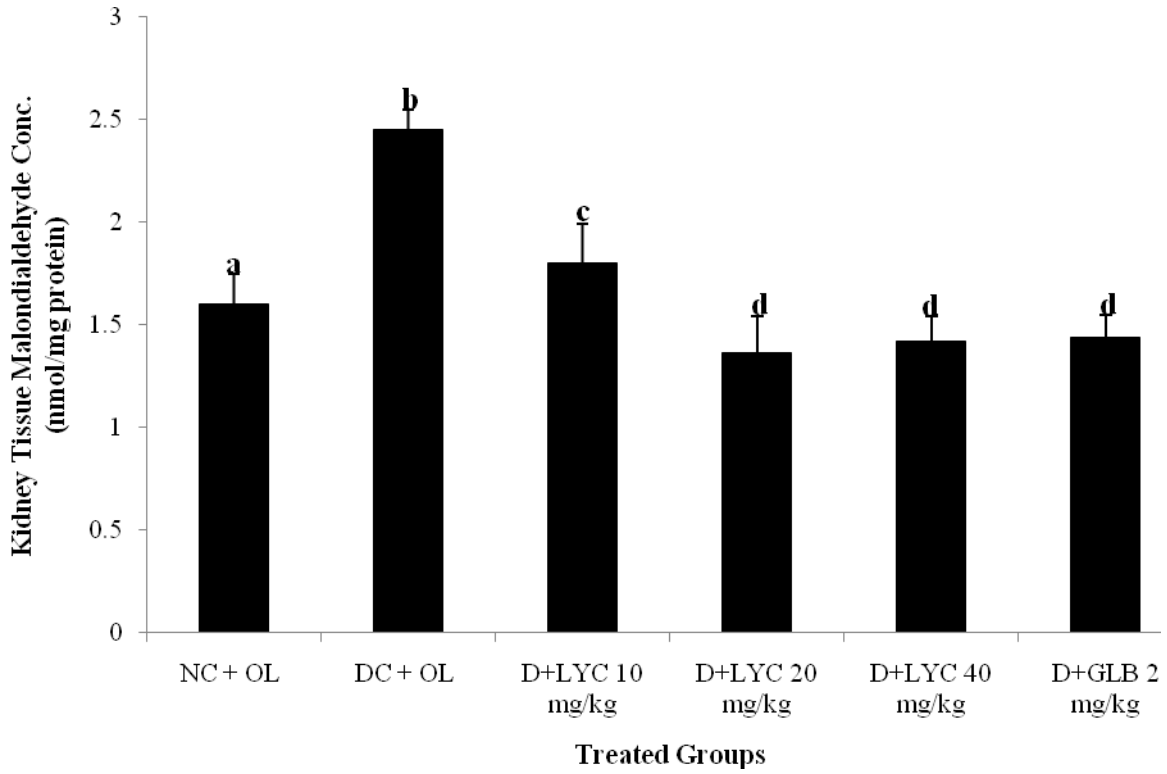


Figure 4.9.1: Effects of lycopene on kidney tissue malondialdehyde concentration in streptozotocin-induced diabetic Wistar rats

Each bar represents mean of five animals

Bars with different superscript letters (a, b, c, d) differ significantly ($P < 0.05$) compared with the control groups, while bars with the same superscript letters are not significantly different ($P > 0.05$) compared with the control groups

DC+OL = Diabetic rats treated with olive oil, NC+OL = Normal (Non-diabetic) rats treated with olive oil, D+LYC10 mg/kg = Diabetic rats treated with 10 mg/kg of lycopene, D+LYC 20 mg/kg = Diabetic rats treated with 20 mg/kg of lycopene, D+LYC 40 mg/kg = Diabetic rats treated with 40 mg/kg of lycopene and D+GLB 2 mg/kg = Diabetic rats treated with glibenclamide 2 mg/kg

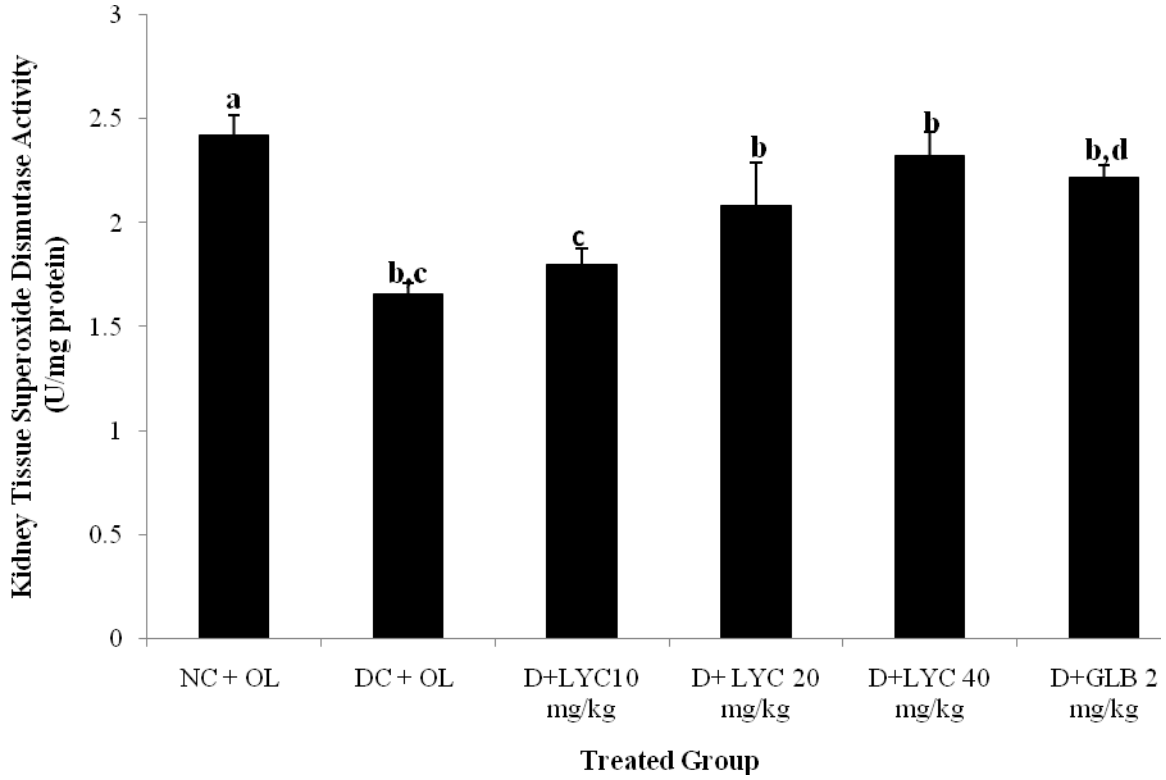


Figure 4.9.2: Effects of lycopene on kidney tissue superoxide dismutase activity in streptozotocin-induced diabetic wistar rats.

Each bar represents mean of five animals

Bars with different superscript letters (a, b, c, d) differ significantly ($P < 0.05$) compared with the control groups, while bars with the same superscript letters are not significantly different ($P > 0.05$) compared with the control groups

DC+OL = Diabetic rats treated with olive oil, NC+OL = Normal (Non-diabetic) rats treated with olive oil, D+LYC10 mg/kg = Diabetic rats treated with 10 mg/kg of lycopene, D+LYC 20 mg/kg = Diabetic rats treated with 20 mg/kg of lycopene, D+LYC 40 mg/kg = Diabetic rats treated with 40 mg/kg of lycopene and D+GLB 2 mg/kg = Diabetic rats treated with glibenclamide 2 mg/kg

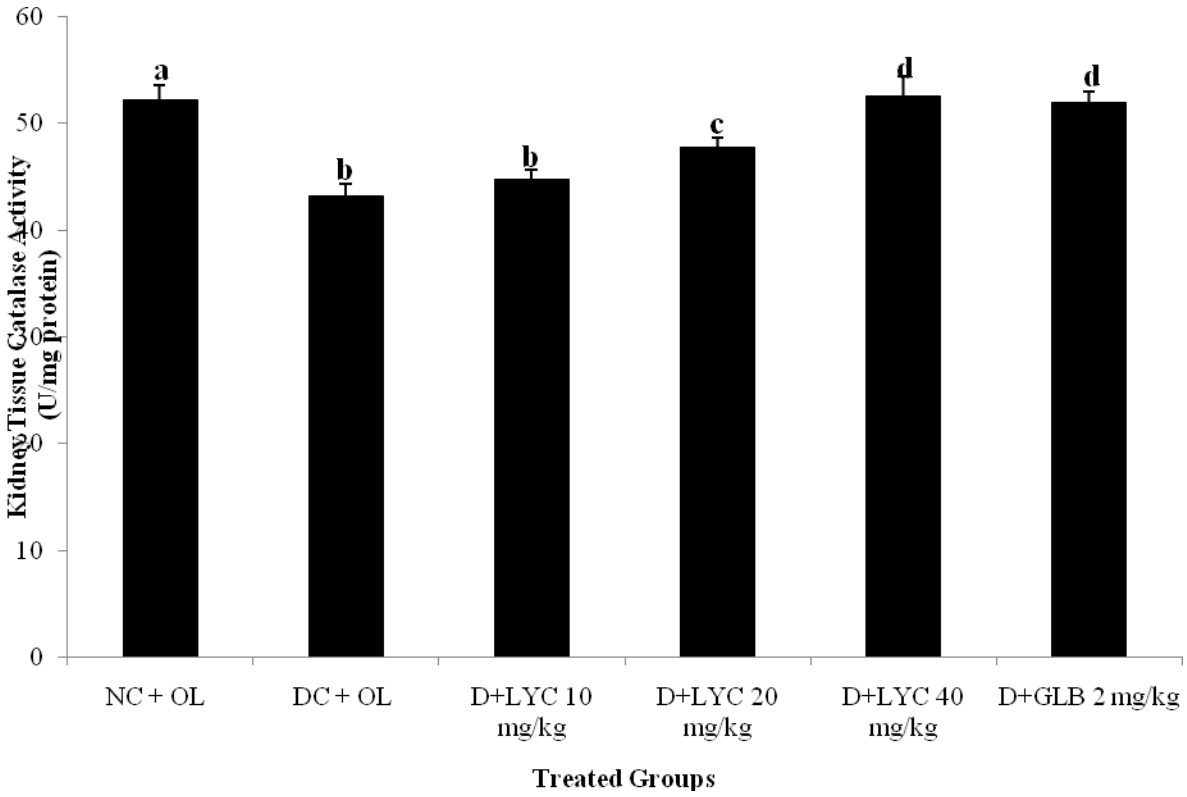


Figure 4.9.3: Effects of lycopene on kidney tissue catalase activity in streptozotocin-induced diabetic Wistar rats

Each bar represents mean of five animals

Bars with different superscript letters (a, b, c, d) differ significantly ($P < 0.05$) compared with the control groups, while bars with the same superscript letters are not significantly different ($P > 0.05$) compared with the control groups

DC+OL = Diabetic rats treated with olive oil, NC+OL = Normal (Non-diabetic) rats treated with olive oil, D+LYC10 mg/kg = Diabetic rats treated with 10 mg/kg of lycopene, D+LYC 20 mg/kg = Diabetic rats treated with 20 mg/kg of lycopene, D+LYC 40 mg/kg = Diabetic rats treated with 40 mg/kg of lycopene and D+GLB 2 mg/kg = Diabetic rats treated with glibenclamide 2 mg/kg

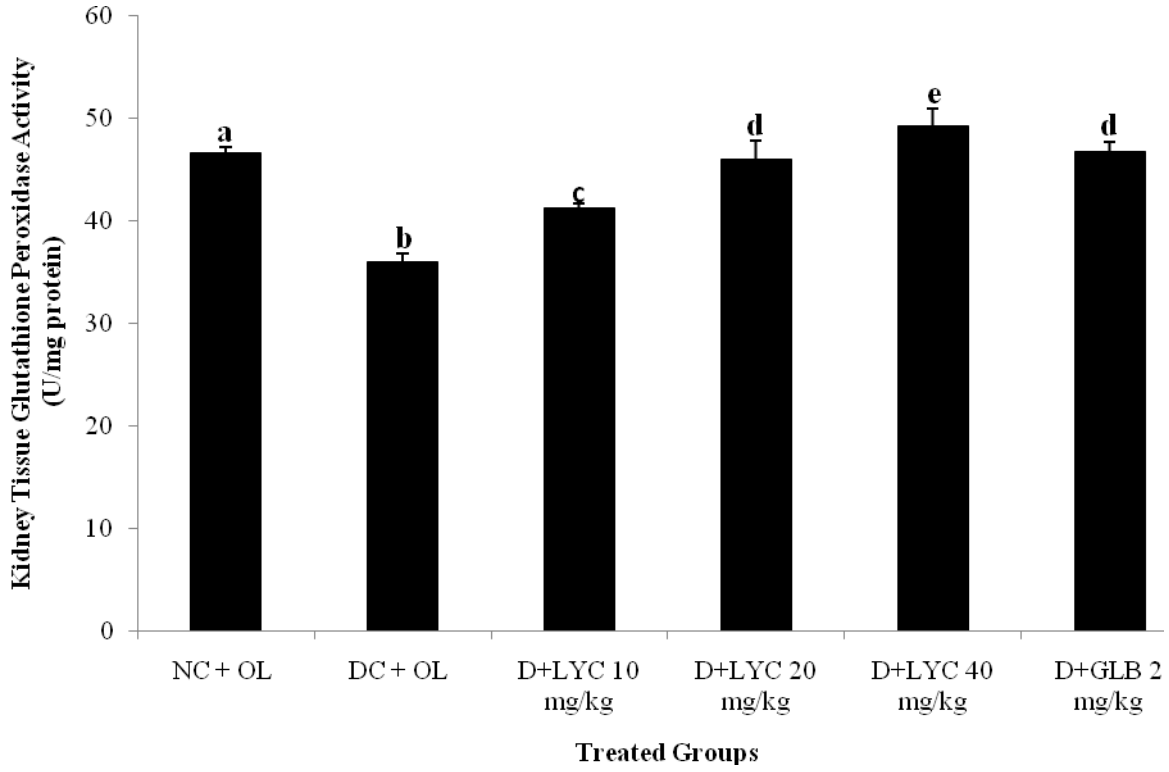


Figure 4.9.4: Effects of lycopene on kidney tissue glutathione peroxidase activity in streptozotocin-induced diabetic Wistar rats

Each bar represents mean of five animals

Bars with different superscript letters (a, b, c, d,e) differ significantly ($P < 0.05$) compared with the control groups, while bars with the same superscript letters are not significantly different ($P > 0.05$) compared with the control groups

DC+OL = Diabetic rats treated with olive oil, NC+OL = Normal (Non-diabetic) rats treated with olive oil, D+LYC10 mg/kg = Diabetic rats treated with 10 mg/kg of lycopene, D+LYC 20 mg/kg = Diabetic rats treated with 20 mg/kg of lycopene, D+LYC 40 mg/kg = Diabetic rats treated with 40 mg/kg of lycopene and D+GLB 2 mg/kg = Diabetic rats treated with glibenclamide 2 mg/kg

4.10 Histological Findings on Kidney Tissues of Streptozotocin-Induced Diabetic Wistar Rats Treated With Lycopene

The histological changes of kidney tissues of control and experimental groups treated with graded doses of lycopene and glibenclamide are shown in (Plates I - VI). The microscopic findings revealed intact and normal architecture of glomeruli, renal tubules and epithelium in kidney tissue of normal control rats treated with olive oil (Plate I). However, the histological finding of kidney tissue of streptozotocin-induced diabetic untreated group showed severe glomerular necrosis with lymphocyte hyperplasia when compared (Plate II). Administration of lycopene and glibenclamide to diabetic rats improved renal damage as reflected by reduce glomerular and tubular necrosis (Plate III - VI).

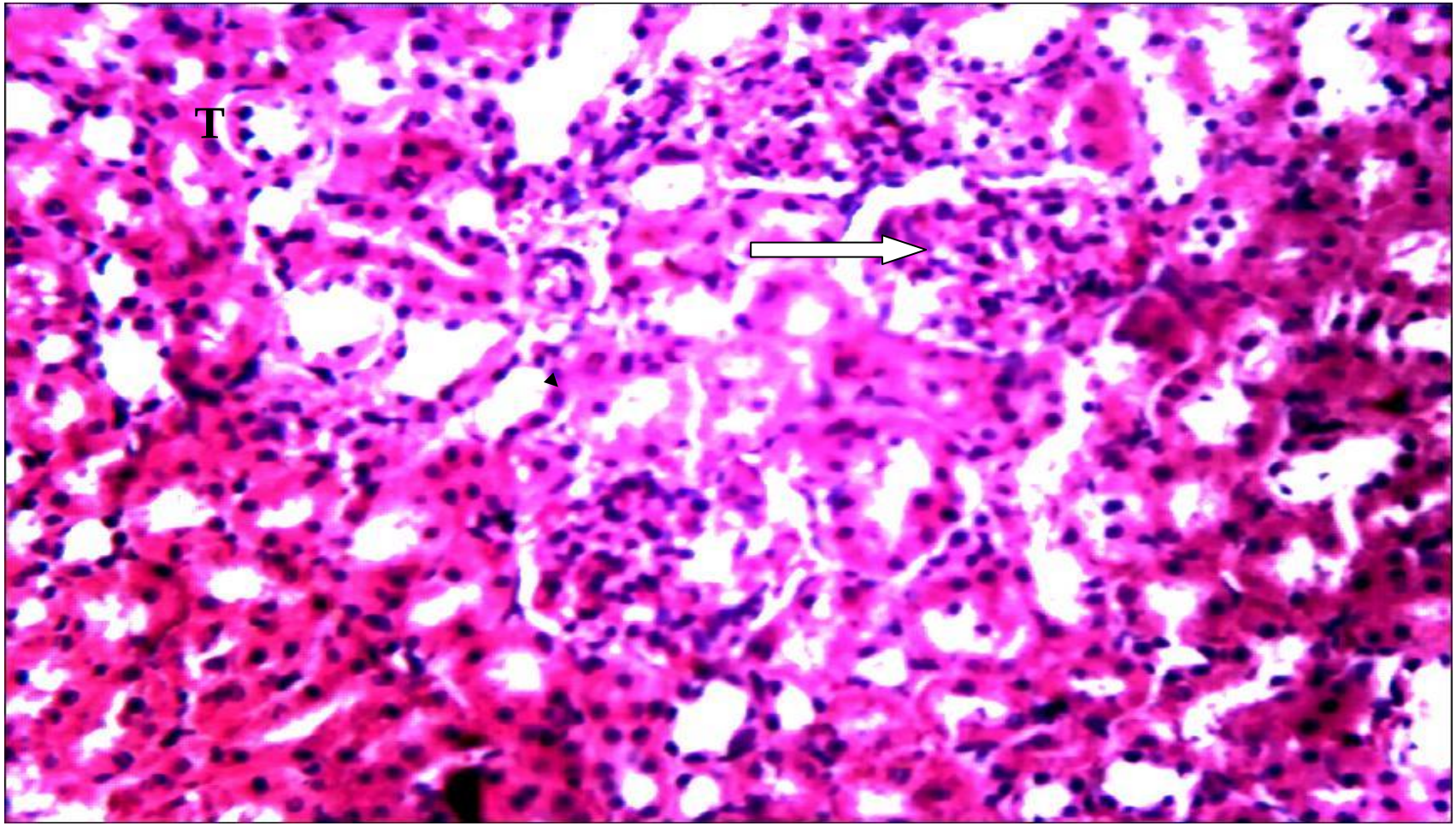


Plate I: Photomicrograph of kidney of normal control rat that received olive oil (0.5 ml) orally, showing normal glomeruli and convulated tubules. Stained in H & E X 250.

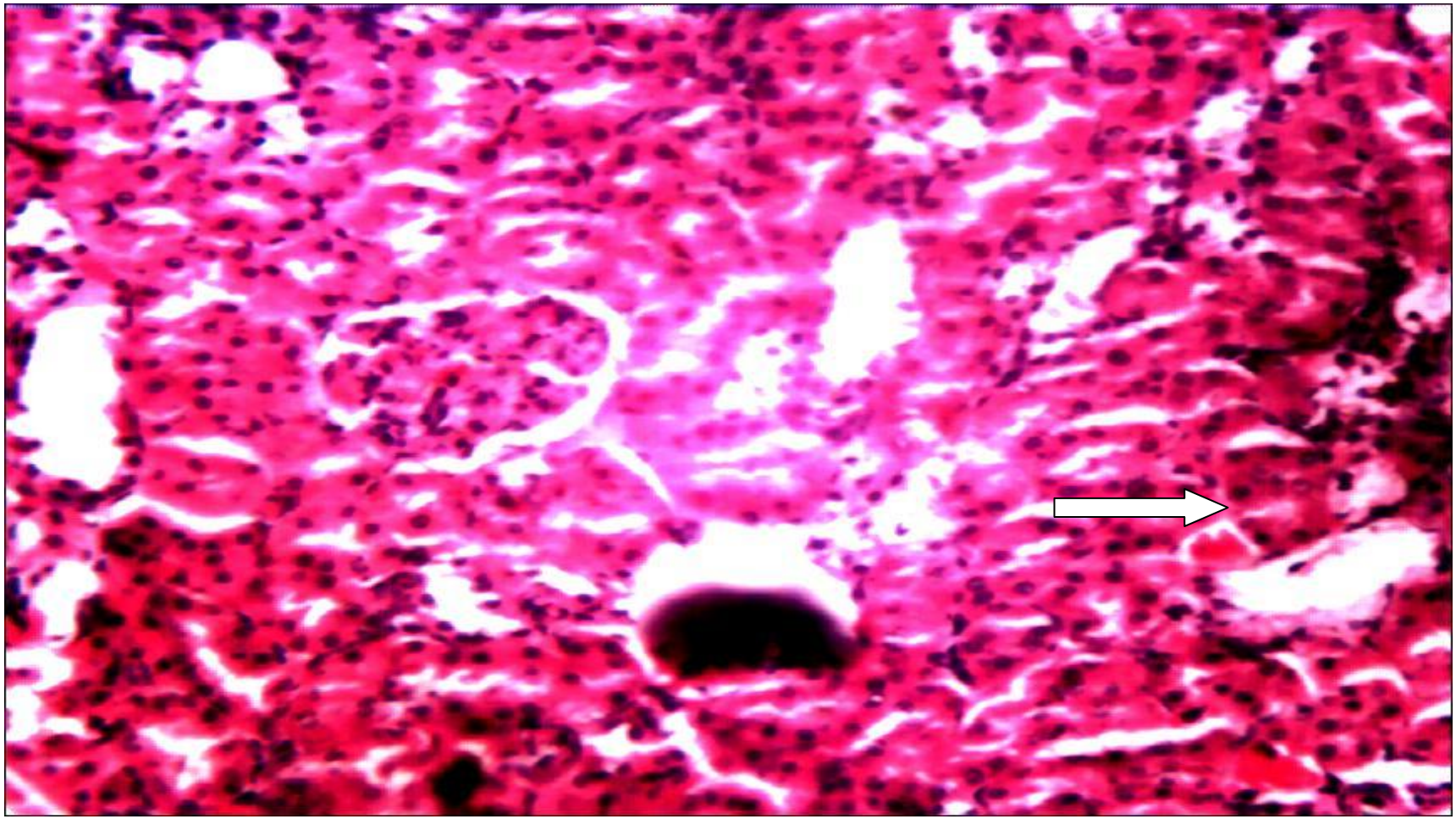


Plate II: Photomicrograph of kidney of diabetic control rat that received olive oil (0.5 ml) orally, showing severe glomerular necrosis with lymphocyte hyperplasia. Stained in H & E X 250.

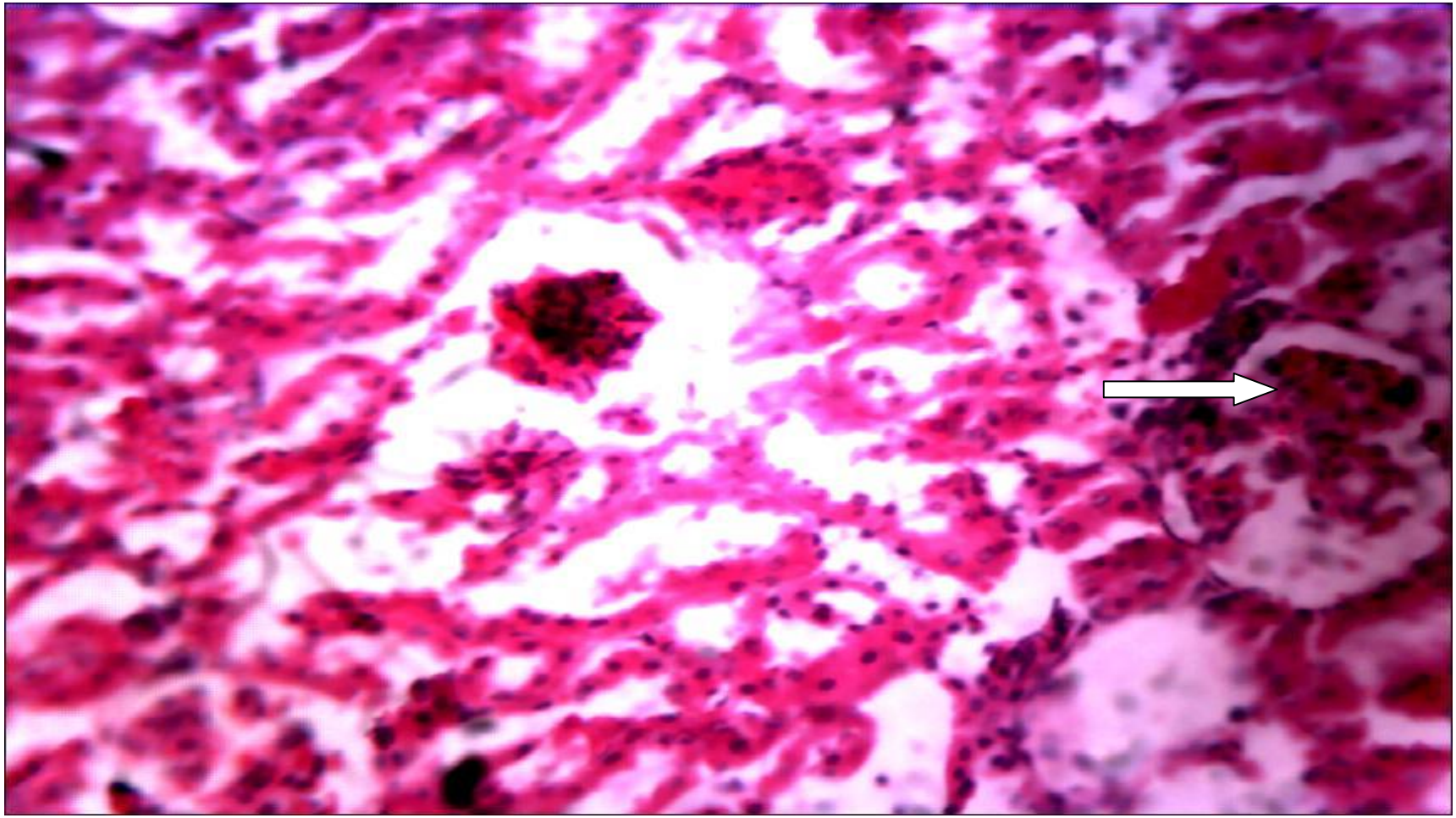


Plate III: Photomicrograph of kidney of diabetic rat administered with 10 mg/kg of lycopene orally, showing moderate glomerular and tubular necrosis. Stained in H & E X 250.

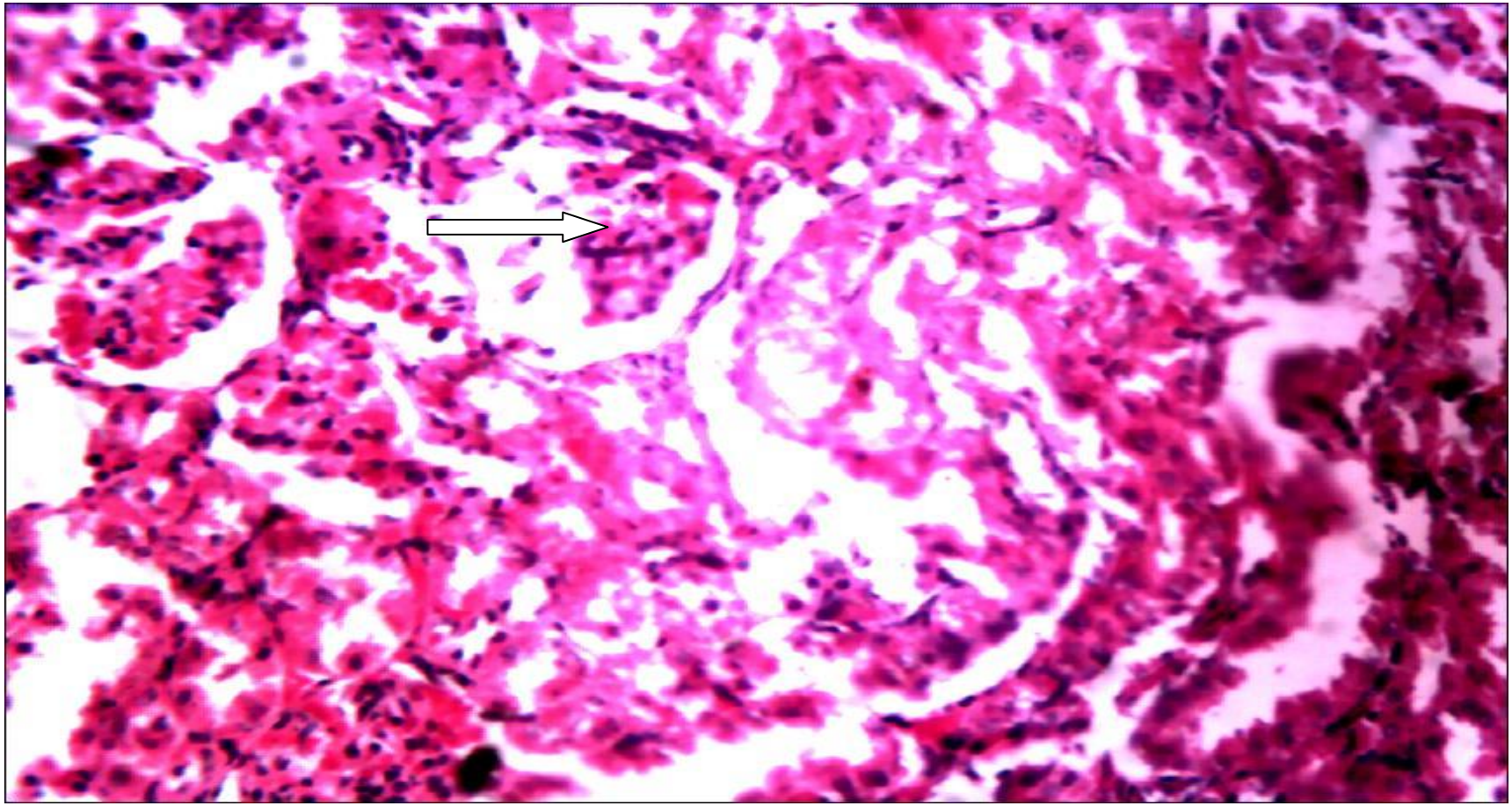


Plate IV: Photomicrograph of kidney of diabetic rat administered with 20 mg/kg of lycopene orally, showing moderate glomerular and tubular necrosis. Stained in H & E X 250.

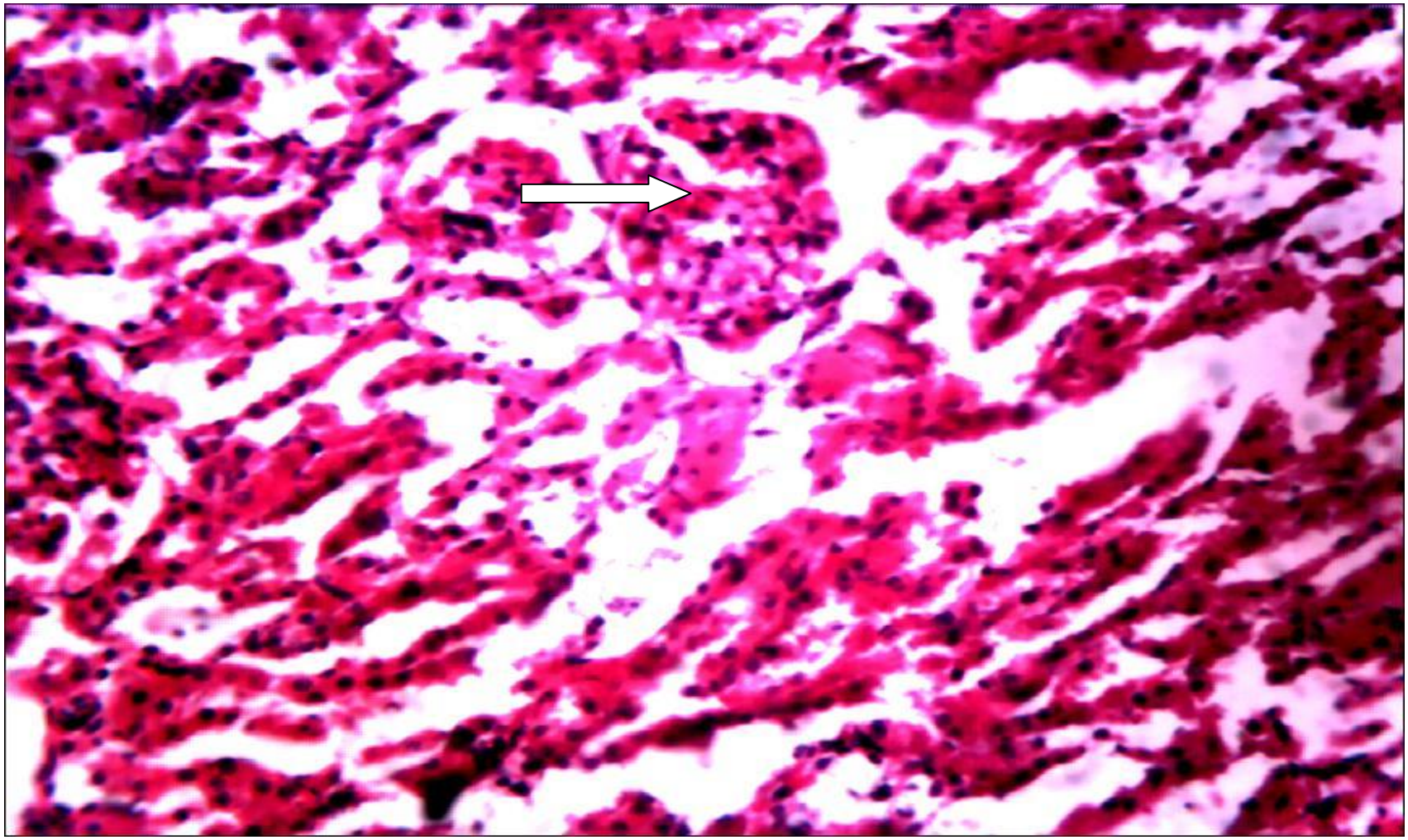


Plate V: Photomicrograph of kidney of diabetic rat administered with 40 mg/kg of lycopene orally, showing mild glomerular and tubular necrosis. Stained in H & E X 250.

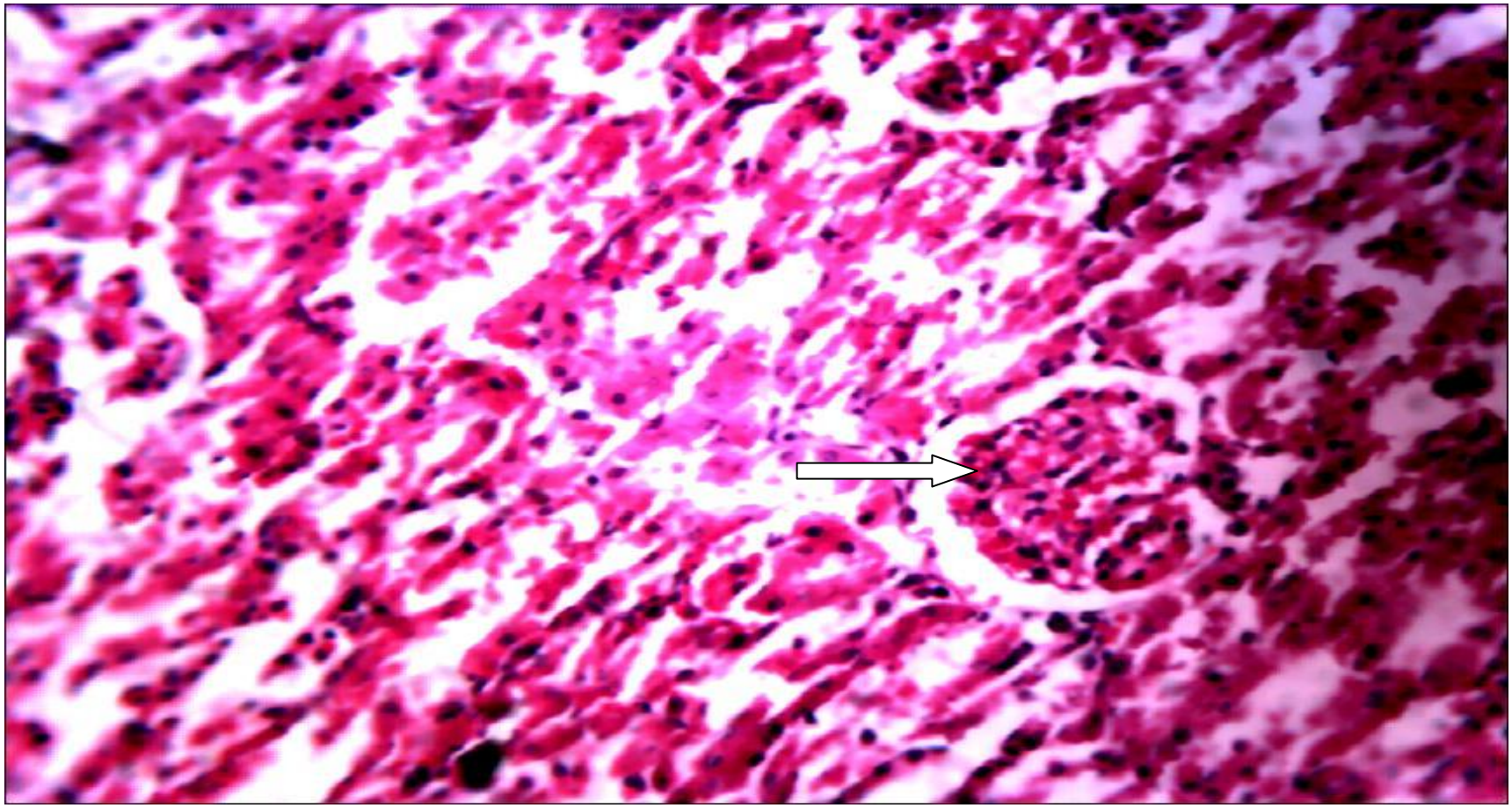


Plate VI: Photomicrograph of kidney of diabetic rat administered with 2 mg/kg of glibencalmide orally, showing mild glomerular and tubular necrosis. Stained in H & E X 250.

4.11 Effect of Lycopene on Serum Proteins in Streptozotocin-induced Diabetic Wistar Rats

4.11.1 Effect on Serum Total Protein Level

The results of the study recorded a significant ($P < 0.05$) decrease of (54.20 ± 1.32 g/L) in the levels of total serum protein of diabetic untreated animals when compared with the animals in the normal control group that had (63.60 ± 12.2 g/L). Treatment of diabetic animals with graded doses of lycopene (10, 20 and 40 mg/kg) and glibenclamide (2mg/kg), resulted to a significant ($P < 0.05$) elevation of serum total protein level to (65.80 ± 1.28, 65.60 ± 1.33, 66.80 ± 1.39 g/L) and (68.40 ± 1.50 g/L) when compared with the diabetic control group (Table 4.11).

4.11.2 Effect on Serum Albumin Level

The results showed that streptozotocin administration caused a significant ($P < 0.05$) decrease in the level of serum albumin in the diabetic untreated animals (33.20 ± 1.11 g/L), when compared with normal control rats. However, administration various doses of lycopene (10, 20 40 mg/kg) and glibenclamide (2 mg/kg), significantly ($P < 0.05$) increase the serum albumin levels to (37.80 ± 1.39, 36.00 ± 1.58, 38.20 ± 1.39 g/L) and (43.20 ± 2.88 g/L) respectively when compared with the corresponding diabetic untreated animals (Table 4.11).

4.11.3 Effect on Serum Globulin Level

The results obtained showed that the diabetic untreated rats had a significantly ($P < 0.05$) decreased level of serum globulin (21.00 ± 1.64 g/L), when compared with the animals in the normal control group (28.20 ± 0.66 g/L). Oral administration of various doses of lycopene (10, 20, 40 mg/kg) and glibenclamide (2 mg/kg) produced a significant ($P < 0.05$) increase of (28.00 ± 0.32, 29.60 ± 1.03, 27.60 ± 0.51 g/L) and (25.20 ± 2.85 g/L) on the level of serum globulin when compared with animals in the diabetic control group (Table 4.11)

4.11.4 Effect on Serum Albumin/Globulin Ratio

Table 4.11 showed a significantly ($P < 0.05$) increased serum albumin/globulin ratio of (1.62 ± 0.14 g/L) in diabetic control, when compared with the animals in the normal control group (1.26 ± 0.05 g/L). However, albumin/globulin ratio was significantly ($P < 0.05$) reduced to (1.35 ± 0.06, 1.23 ± 0.08, 1.39 ± 0.06 g/L) and (1.37 ± 0.35 g/L) following lycopene and glibenclamide administration to diabetic animals when compared to the diabetic control rats.

Table 4.11: Effects of lycopene on serum total proteins, albumin, globulin and albumin/globulin ratio in streptozotocin-induced diabetic Wistar rats

Treatment Groups	Serum Total Protein (g/L)	Serum Albumin (g/L)	Serum Globulin (g/L)	Albumin/Globulin Ratio
NC+ OL	63.60 ± 12.2^{a}	35.40 ± 0.98^{a}	28.20 ± 0.66^{a}	1.26 ± 0.05^{a}
DC+ OL	54.20 ± 1.32^{b}	33.20 ± 1.11^{b}	21.00 ± 1.64^{b}	1.62 ± 0.14^{b}
D+LYC10 mg/kg	65.80 ± 1.28^{c}	37.80 ± 1.39^{b}	28.00 ± 0.32^{b}	1.35 ± 0.06^{c}
D+LYC 20 mg/kg	65.60 ± 1.33^{c}	36.00 ± 1.58^{b}	29.60 ± 1.03^{b}	1.23 ± 0.08^{c}
D+LYC 40 mg/kg	66.80 ± 1.39^{c}	38.20 ± 1.39^{b}	27.60 ± 0.51^{b}	1.39 ± 0.06^{c}
D+GLB 2 mg/kg	68.40 ± 1.50^{c}	43.20 ± 2.88^{c}	25.20 ± 2.85^{b}	1.37 ± 0.35^{c}

^{a,b,c,d} = Means on the same column with different superscript letters differ significantly ($P < 0.05$) compared with the control groups

DC+OL = Diabetic rats treated with olive oil, NC+OL = Normal (Non-diabetic) rats treated with olive oil, D+LYC10 mg/kg b w = Diabetic rats treated with 10 mg/kg b w of lycopene, D+LYC 20 mg/kg b w = Diabetic rats treated with 20 mg/kg b w of lycopene, D+LYC 40 mg/kg b w = Diabetic rats treated with 40 mg/kg of lycopene and D+GLB 2 mg/kg b w = Diabetic rats treated with gilbenclamide 2 mg/kg.

4.12 Effect of Lycopene on Concentration of Serum Electrolytes in Streptozotocin-induced Diabetic Wistar Rats

(Table 4.12) showed the results of changes in some kidney functional indices in the streptozotocin- induced diabetic animals administered with graded doses of lycopene. There was a significant ($P < 0.05$) depleted serum sodium ion concentration with a concomitant elevated serum urea level in diabetic untreated group (128.60 ± 0.60 and 5.04 ± 0.18 mmol/L), when compared with animals in the normal control group (139.00 ± 0.84 and 2.68 ± 0.16 mmol/L). However, oral administration of (10, 20 and 40 mg/kg) of lycopene and (2 mg/kg) glibenclamide, recorded a significant ($P < 0.05$) increase of (138.80 ± 0.97, 135.20 ± 3.93, 130.40 ± 4.69 mmol/L) and (139.80 ± 1.20 mmol/L) in the serum sodium ion level and reduction of (3.14 ± 0.12, 2.20 ± 0.31, 2.40 ± 0.18 mmol/L) and (2.84 ± 0.24 mmol/L) in the serum urea level when compared with the diabetic control group. No significant ($P > 0.05$) difference was recorded in the serum potassium, chloride and bicarbonate ions concentrations of all diabetic animals treated various doses of lycopene when compared with the diabetic control group.

Table 4.12: Effect of lycopene on concentration of serum electrolytes in streptozotocin-induced diabetic Wistar rats

Treatment Groups	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	HCO_3^- (mmol/L)	Urea (mmol/L)
NC+ OL	139.00 ± 0.84^a	4.14 ± 0.14^a	97.60 ± 1.47^a	22.80 ± 1.36^a	2.68 ± 0.16^a
DC + OL	128.60 ± 0.60^b	4.26 ± 0.13^a	98.40 ± 0.93^a	24.60 ± 0.87^a	5.04 ± 0.18^b
D + LYC10 mg/kg	138.80 ± 0.97^c	4.08 ± 0.17^a	96.40 ± 1.17^a	20.80 ± 1.02^a	3.14 ± 0.12^c
D + LYC 20 mg/kg	135.20 ± 3.93^c	4.06 ± 0.51^a	97.40 ± 3.40^a	22.80 ± 1.02^a	2.20 ± 0.31^e
D + LYC 40 mg/kg	130.40 ± 4.69^c	3.28 ± 0.49^a	92.00 ± 3.74^a	21.40 ± 2.44^a	2.40 ± 0.18^{dd}
D + GLB 2 mg/kg	139.80 ± 1.20^d	4.08 ± 0.13^a	98.80 ± 1.24^a	24.00 ± 0.63^a	2.84 ± 0.24^c

^{a,b,c,d,e} = Means on the same column with different superscript letters differ significantly ($P < 0.05$) compared with the control groups

DC+OL = Diabetic rats treated with olive oil, NC+OL = Normal (Non-diabetic) rats treated with olive oil, D+LYC10 mg/kg b w = Diabetic rats treated with 10 mg/kg b w of lycopene, D+LYC 20 mg/kg b w = Diabetic rats treated with 20 mg/kg b w of lycopene, D+LYC 40 mg/kg b w = Diabetic rats treated with 40 mg/kg of lycopene and D+GLB 2 mg/kg b w = Diabetic rats treated with gilbenclamide 2 mg/kg.

4.13 Effect of Lycopene on Haematological Parameters in Streptozotocin-induced Diabetic Wistar Rats

4.13.1 Effect on Erythrocyte Lipoperoxidative Changes

The erythrocyte MDA concentrations was significantly increased ($P < 0.05$) in the diabetic untreated group (2.10 ± 0.14 µmol/g Hb), when compared to those obtained in the normal control animals (1.10 ± 0.09 µmol/g Hb). The erythrocytes MDA concentration of all lycopene and glibenclamide treated diabetic animals was significantly ($P < 0.01$) decreased (1.48 ± 0.07, 1.26 ± 0.08, 1.02 ± 0.09 µmol/g Hb) and (1.44 ± 0.11 µmol/g Hb), when compared with diabetic control group that recorded (2.10 ± 0.14 µmol/g Hb) (Figure 4.13.1).

4.13.2 Effect on Erythrocyte Osmotic Fragility

The percentage erythrocyte osmotic fragility decreased significantly with increasing NaCl concentration. There was complete (100%) haemolysis at 0.0 and 0.1 % of NaCl. And there was also no significant changes in erythrocyte osmotic fragility were observed at 0.0 and 0.1 % of Na Cl (distilled water) in all control and experimental groups when compared. However, significant ($P < 0.05$) changes in percentage erythrocyte fragility were recorded at 0.3, 0.5, 0.7 and 0.9 % of Nacl concentrations, when compared with the corresponding diabetic control group (Figure 4.11.2). At 0.3 % NaCl concentration, the erythrocyte osmotic fragility of the diabetic control animals was significantly ($P < 0.05$) higher (98.40 ± 0.51 %) than those obtained in the normal control group (89.60 ± 1.17 %). Following oral treatment with graded doses of lycopene and glibenclamide, the study recorded a significantly ($P < 0.05$) dose dependent decrease in erythrocyte osmotic fragility of (97.60 ± 1.17, 94.00 ± 1.82, 86.20 ± 1.49 %) and (80.40 ± 6.52 %) at 0.3 % NaCl concentration, when compared with the diabetic control group. However, glibenclamide appeared to have a better effect than the lycopene treated groups especially at 0.3 % NaCl concentration. Similarly, at 0.5, 0.7 and 0.9 % NaCl concentrations, a significant ($P <$

0.05) increase in the erythrocyte osmotic fragility of (32.00 ±1.00, 11.80 ± 0.66 and 7.60 ± 0.51 %) was recorded in the diabetic control animals when compared with those obtained in the normal control rats (8.20 ± 0.74, 2.00 ± 0.32 and 1.20 ± 0.20 %). Treatment of diabetic animals with lycopene and glibencalmide produced a significantly ($P < 0.05$) lower erythrocyte osmotic fragility of (12.20 ± 0.66, 8.80 ± 0.37, 8.40 ± 0.75 and 10.6 ± 1.25 %), (3.00 ± 0.45, 2.00 ± 0.32, 1.60 ± 0.25 and 3.40 ± 0.75 %) and (1.60 ± 0.25, 1.80 ± 0.49, 1.40 ± 0.25 and 1.40 ± 0.25 %), when compared with the diabetic untreated group (Figure 4.13.2).

4.13.3 Effect on Erythrocyte Indices

The Hb concentration, RBC count and PCV of the untreated diabetic animals were significantly ($P < 0.05$) lower (8.36± 0.86 g/dL, 4.74 ± 0.20 and 25.24 ± 2.50 %) than those of normal control group (12.84 ± 0.23 g/dL, 6.98 ± 0.25 and 37.74 ± 1.03 %) when compared. Similarly, the MCV, MCH and MCHC significantly ($P < 0.05$) decreased in the diabetic control rats to (50.11 ± 6.04 fl, 16.75 ± 1.95 Pg and 30.92 ± 1.14 g/dL) when compared with rats of the normal control group (64.58 ± 3.51 fl, 21.78 ± 1.14 Pg and 35.20 ± 0.49 g/dL). Administration of lycopene of (10, 20 and 40 mg/kg) and glibenclamide (2 mg/kg) to diabetic animals significantly ($P < 0.05$) elevated Hb concentration (12.56 ± 0.32, 12.68 ± 0.36, 13.30 ± 0.35 g/dL) and (11.52 ± 0.51 g/dL), RBC (6.65 ± 0.28, 6.88 ± 0.31, 6.77 ± 0 .26) and (5.77 ± 0.29), PCV (35.62 ± 0.83, 35.84 ± 1.10, 37.46 ± 1.03 %) and (37.14 ± 2.80 %). In addition, MCV (53.92 ± 2.15, 55.82 ± 4.09, 64.58 ± 3.51 fl) and (61.64 ± 2.48 fl), MCH (18.90 ± 0.48, 18.96 ± 0.52, 19.78 ± 0.35 Pg) and (18.84 ± 0.21 Pg) and MCHC (33.09±0.13, 34.64 ± 0.25, 36.42 ± 1.59 g/dL) and (34.06 ± 0.63 g/dL) were significantly ($P < 0.05$) higher in diabetic lycopene and glibenclamide treated animals, when compared with the diabetic control animals. There was however no significant difference ($P > 0.05$) in the Hb, RBC and PCV as well as MCV, MCH and MCHC was observed between and within the diabetic lycopene and glibencalmide treated groups when compared (Table 4.13.3).

4.13.4 Effect on Leucocytes Indices

The total white blood cell (WBC) count, lymphocytes and neutrophils obtained in diabetic control rats were signifciantly lower ($P < 0.05$) (6.70 ± 0.26, 4.26 ± 0.27 and 1.50 ± 0.23) than that of the normal control group that had (14.26 ± 2.68, 11.14 ± 2.21 and 3.44 ± 0.79) when compared. Contrary to the observed trend in the other parameters, the nuetrophil/lymphocyte ratio and platelets count in the diabetic untreated animals were significantly ($P < 0.05$) (0.50 ± 0.04 and 892.60 ± 20.10) higher than those obtained in animals of the normal control group (0.20 ± 0.04 and 391.20 ± 51.21) when compared. However, no significant ($P > 0.05$) difference between and wthin among groups of the diabetic rats administered with lycopene and glibencalmide were obtained. Treatment of diabetic animals with graded doses of lycopene and glibenclamide produced a significantly ($P < 0.05$) increased total WBC count from (6.70 ± 0.26) to (13.12 ± 1.65, 11.08 ±1.58, 10.88 ± 0.59) and (10.72 ± 1.14), lymphocytes count from (4.26 ± 0.27) to (8.08 ± 0.69, 7.64 ± 1.33, 7.54 ± 0.53) and (7.92 ± 1.21) and neutrophils count from (1.50 ± 0.23) to (2.09 ± 0.10, 2.42 ± 0.34, 2.40 ± 0.16) and (2.30 ± 0.29) when compared with diabetic control animals (Figure 4.12.4a to 4.12.4c). Conversely, treatment of diabetic animals with lycopene and glibenclamide produced a significant ($P < 0.05$) decrease in neutrophli/lymphocyte ratio from (0.50 ± 0.04) to (0.32 ± 0.04, 0.28 ± 0.03, 0.32 ± 0.02) and (0.22 ± 0.03) and platelets counts from (892.60 ± 20.10) to (483.00 ± 52.92, 568.80 ± 80.20, 586.20 ± 40.65) and (509.20 ± 58.14) when compared with the diabetic control group (Table 4.13.4).

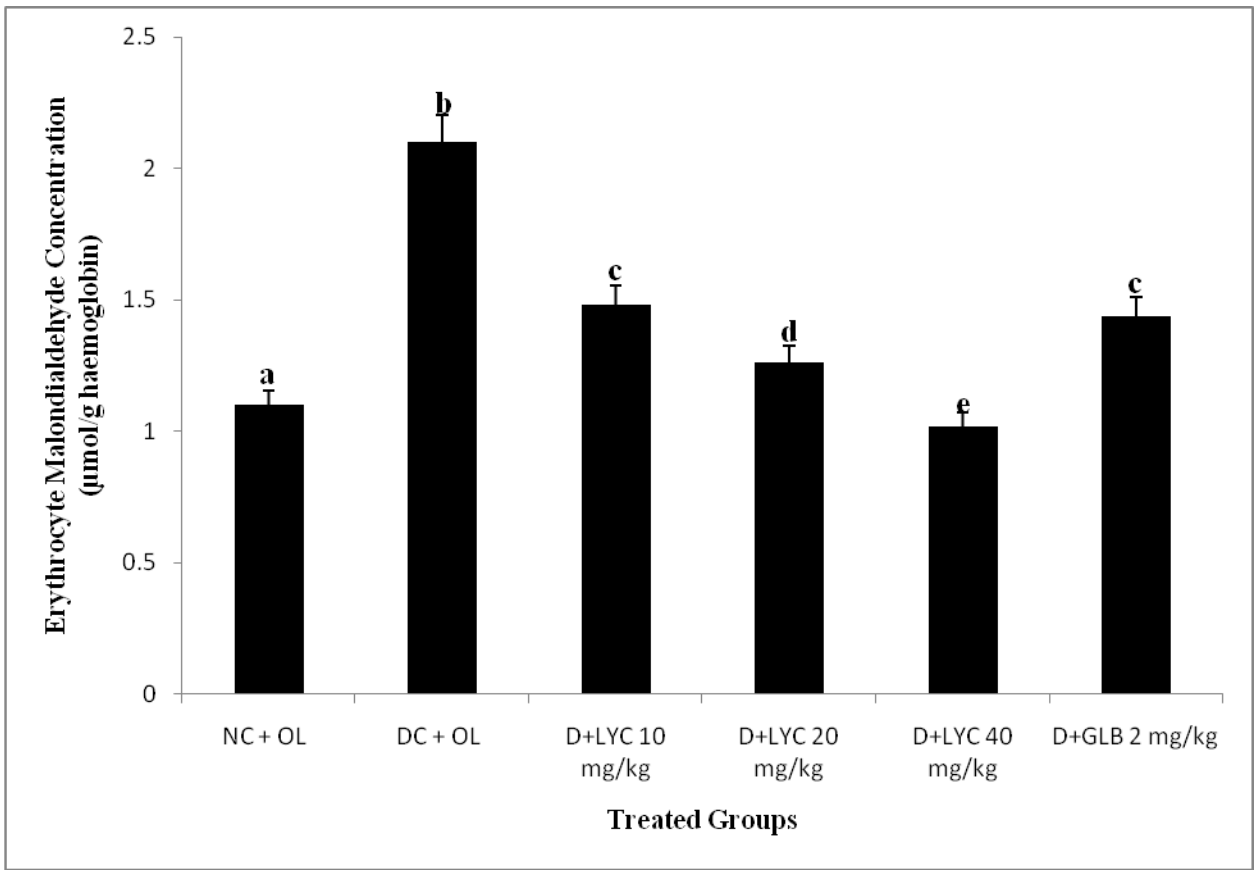


Figure 4.13.1: Effects of lycopene on erythrocyte malondialdehyde concentration in streptozotocin-induced diabetic Wistar rats

Each bar represents mean of five animals

Bars with different superscript letters (a, b, c, d,e) differ significantly ($P < 0.05$) compared with the control groups, while bars with the same superscript letters are not significantly different ($P > 0.05$) compared with the control groups

DC+OL = Diabetic rats treated with olive oil, NC+OL = Normal (Non-diabetic) rats treated with olive oil, D+LYC10 mg/kg = Diabetic rats treated with 10 mg/kg of lycopene, D+LYC 20 mg/kg = Diabetic rats treated with 20 mg/kg of lycopene, D+LYC 40 mg/kg = Diabetic rats treated with 40 mg/kg of lycopene and D+GLB 2 mg/kg = Diabetic rats treated with glibenclamide 2 mg/kg

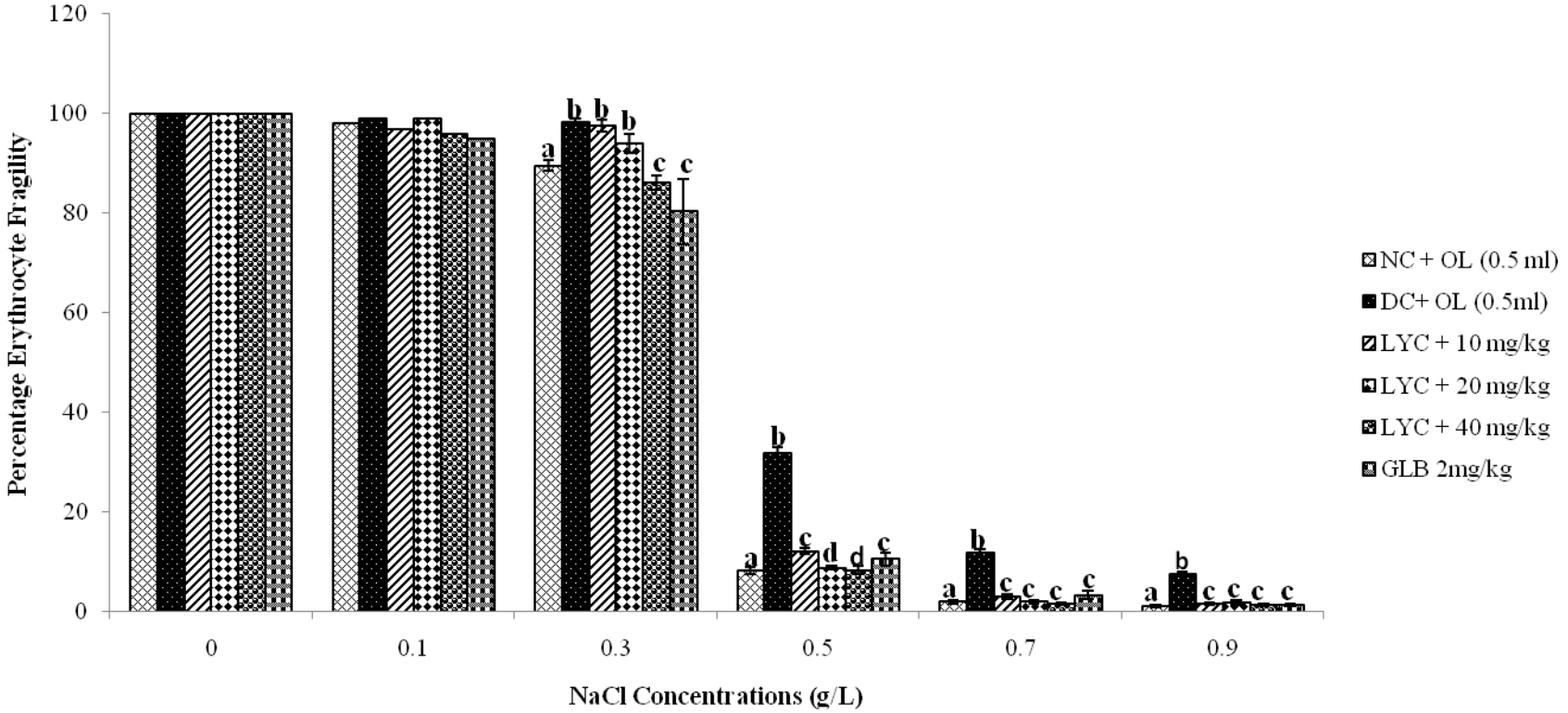


Figure 4.13.2: Effects of lycopene on erythrocyte osmotic fragility in streptozotocin-induced diabetic Wistar rats

Each bar represent mean of five animals

Bars with different superscript letters (a, b, c, d) differ significantly ($P < 0.05$) compared with the control groups, while bars with the same superscript letters are not significantly different ($P > 0.05$) compared with the control groups

DC+OL = Diabetic rats treated with olive oil, NC+OL = Normal (Non-diabetic) rats treated with olive oil, D+LYC10 mg/kg = Diabetic rats treated with 10 mg/kg of lycopene, D+LYC 20 mg/kg = Diabetic rats treated with 20 mg/kg of lycopene, D+LYC 40 mg/kg = Diabetic rats treated with 40 mg/kg of lycopene and D+GLB 2 mg/kg = Diabetic rats treated with glibenclamide 2 mg/kg.

Table 4.13.3.: Effects of Lycopene on Erythrocyte Indices in Streptozotocin-induced Diabetic Wistar Rats

Treatment Groups	Hb (g/dL)	RBC ($\times 10^{12}$/L)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (g/dl)
NC + OL	12.84±0.23[a]	6.98±0.25 [a]	37.74±1.03 [a]	64.58±3.51 [a]	21.78±1.14 [b]	35.20±0.49 [b]
DC+ OL	8.36± 0.86 [b]	4.74±0.20 [b]	25.24±2.50 [b]	50.11±6.04 [b]	16.75±1.95 [b]	30.92±1.14 [b]
D+LYC10 mg/kg	12.56±0.32 [c]	6.65±0.28 [c]	35.62±0.83 [c]	53.92±2.15 [b]	18.90±0.48[b]	33.09±0.13 [b]
D+LYC 20 mg/kg	12.68±0.36 [c]	6.88±0.31[c]	35.84±1.10 [c]	55.82±4.09 [b]	18.96±0.52 [b]	34.64±0.25 [c]
D+LYC 40 mg/kg	13.30±0.35[c]	6.77±0.26 [c]	37.46± 1.03 [c]	64.58±3.51 [c]	19.78±0.35 [b]	36.42±1.59 [c]
D+GLB 2mg/kg	11.52±0.51 [c]	5.77±0.29 [c]	37.14±2.80 [c]	61.64±2.48 [c]	18.84±0.21 [b]	34.06±0.63 [c]

[a,b,c,d] = Means on the same column with different superscript letters differ significantly ($P < 0.05$) compared with the control groups

DC+OL = Diabetic rats treated with olive oil, NC+OL = Normal (Non-diabetic) rats treated with olive oil, D+LYC10 mg/kg = Diabetic rats treated with 10 mg/kg of lycopene, D+LYC 20 mg/kg = Diabetic rats treated with 20 mg/kg of lycopene, D+LYC 40 mg/kg = Diabetic rats treated with 40 mg/kg of lycopene and D+GLB 2 mg/kg = Diabetic rats treated with glibenclamide 2 mg/kg.

Hb = Haemoglobin concentration, RBC = Red blood cell, PCV = Packed cell volume, MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration

Table 4.13.4: Effects of Lycopene on Leucocyte Indices in Streptozotocin-induced Diabetic Wistar Rats

Treatment Groups	WBC ($\times 10^9$/L)	Neutrophils ($\times 10^9$)	Lymphocytes ($\times 10^9$)	Neutrophils/ Lymphocytes ratio	Platelets Count ($\times 10^9$)
NC+ OL	14.26±2.68[a]	3.44±0.79 [a]	11.14±2.21 [a]	0.20±0.04[a]	391.20±51.21[a]
DC+ OL	6.70±0.26 [b]	1.50±0.23[b]	4.26±0.27[b]	0.50±0.04[b]	892.60±20.10[b]
D+LYC 10 mg/kg	13.12±1.65[c]	2.09±0.10[c]	8.08±0.69[c]	0.32±0.04[c]	483.00±52.92[c]
D+LYC 20 mg/kg	11.08±1.58[c]	2.42±0.34[c]	7.64±1.33[c]	0.28±0.03[c]	568.80±80.20 [d]
D+LYC 40 mg/kg	10.88±0.59[c]	2.40±0.16[c]	7.54±0.53[c]	0.32±0.02[c]	586.20±40.65 [d]
D+GLB 2mg/kg	10.72±1.14[c]	2.30±0.29[c]	7.92±1.21[c]	0.22±0.03[c]	509.20±58.14 [d]

[a,b,c,d] = Means on the same column with different superscript letters differ significantly ($P < 0.05$) compared with the control groups

DC+OL = Diabetic rats treated with olive oil, NC+OL = Normal (Non-diabetic) rats treated with olive oil, D+LYC10 mg/kg = Diabetic rats treated with 10 mg/kg of lycopene, D+LYC 20 mg/kg = Diabetic rats treated with 20 mg/kg of lycopene, D+LYC 40 mg/kg = Daibetic rats treated with 40 mg/kg of lycopene and D+GLB 2 mg/kg = Diabetic rats treated with gilbenclamide 2 mg/kg

CHAPTER FIVE

5.0 DISCUSSION

It has been suggested that experimental animal models are one of the best ways to understand the pathophysiology of any disease (Rees and Alcolado, 2005; Chatzigeorgiou *et al.,* 2009; Ali *et al.,* 2011). In this study, the intra-peritoneal administration of streptozotocin (STZ) effectively induced diabetes mellitus in rats, which was confirmed by elevated levels of fasting blood glucose, three days after STZ injection. This agrees with the reports of Mohammed *et al.* (2007) and (2008); Tanko *et al.* (2008) and Krishna *et al.* (2012) that blood glucose level increased significantly after three days STZ injection to rats. STZ induces diabetes which resembles human hyperglycaemic non-ketotic diabetes mellitus in animal models (Weir *et al.,* 1981). STZ selectively destroys the insulin producing β-cells which is accompanied by characteristic alterations in blood insulin and glucose concentrations (Szkudelski, 2001).

Results obtained in our present study also indicated that the serum insulin levels decreased significantly in the diabetic untreated animals following streptozotocin (STZ) treatment when compared normal control rats. This finding has been substantiated by other researchers (Mallick *et al.,* 2006; Daisy *et al.,* 2012). Streptozotocin has been reported to induce insulin-dependent dependent diabetes mellitus in animal models (Bedoya *et al*., 1996). After administration, STZ is taken up by pancreatic B cells *via* glucose transporter GLUT2 (Elsner *et al.,* 2000). Intracellular action of STZ results in changes of DNA in pancreatic β-cells comprising its fragmentation (Morgan *et al*. 1994). This results to impaired glucose oxidation (Bedoya *et al*. 1996) and decreases insulin biosynthesis and secretion (Nukatsuka *et al*. 1990a, 1990b). However, treatment of diabetic animals with the graded doses of lycopene and glibenclamide significantly decreased the blood glucose concentration, with better effect recorded after week 3 and week 4

respectively when compared with corresponding diabetic untreated animals. This finding agrees with the report of Aydin and Celik (2012) that showed that five weeks of lycopene administration significantly reduced blood glucose levels in diabetic rats. Duzguner *et al.* (2008) had also demonstrated that elevated blood glucose concentration in streptozotocin-induced was significantly decreased following three weeks of lycopene treatment. Sevim *et al.* (2013) also reported a significantly decreased blood glucose level in diabetic rats treated with lycopene for four weeks. Similar findings were also observed in the study of Kuhad *et al*. (2008) and Ali and Agha (2009), who reported that lycopene has significant, dose-dependent antidiabetic action in streptozotocin-induced diabetic rats.

The possible mechanism involved in the hypoglycaemic action of lycopene may be stimulation of insulin secretion by the pancreas or/and enhance insulin sensitivity in various organs especially the muscles by promoting glucose uptake and metabolism inhibiting hepatic gluconeogenesis. However, the results of our present finding in this study revealed that lycopene administartion to diabetic rats did not produce any significant increase on serum insulin level when compared with diabetic control group. This finding does not corroborate the previous reports of Aydin and Celik (2012) and Duzguner *et al.* (2008) who showed that the depleted serum insulin level in diabetic rats was reversed following lycopene administration. Based on our present findings, it may be suggested that insulin secretion may not be part of the observed hypoglycaemic property of lycopene. Unlike glibenclamide which produced a significantly elevated serum insulin level when compared with the diabetic control group. Glibenclamide have been reported to stimulate insulin secretion from pancreatic β-cells and also reduces hepatic glucose production resulting in reduced blood glucose level (Rendell, 2004; Erejuwa *et al.,* 2011). Furthermore, the improvement with glibenclamide administration in diabetic animals was

evident by significant increase in the serum insulin levels as observed in the present study. Mulder *et al.* (1991) and Sayed *et al.* (2011) have demonstrated that glibenclamide is able to maintain prolonged increase in serum insulin. Glibenclamide binds to receptors on the surface of pancreatic β-cells; as a result, the cell membrane creates an influx of calcium ions and a subsequent release of insulin (Martha and Karam, 2001).

The comparable effect of lycopene with glibenclamide in this study may suggest similar mechanism of action. Oxidative stress induced by reactive oxygen species (ROS) which are generated due to hyperglycaemia has been implicated in the onset and progression of diabetes mellitus and its related complications (Jay et *al.*, 2006; Wei et *al.*, 2009; Giacco and Brownlee, 2010). Hyperglycemia in diabetes mellitus causes a depletion of the cellular antioxidant defenses and increases the levels of free radicals (Sharma *et al.*, 2010; Tsuruta *et al.*, 2010). Lycopene which is one of the potent antioxidants, shown to have good free radical scavenging capacity because of its unique structure (high number of conjugated double bonds) (Bose and Agrawal, 2006). Therefore, hypoglycaemic effect of lycopene may also be attributed to its strong antioxidant property (Atessahin *et al.*, 2005; Kumar and Kumar, 2009). Bose and Agrawal (2006) reported that lycopene have the ability to quench the superoxide and other free radical anions which are released in diabetes due to abnormal glucose metabolism, hence resulting to decreased blood glucose concentration in diabetic animals as was observed in the present study.

The result obtained revealed that the activity of hepatic glucokinase enzyme was significantly decreased in diabetic untreated animals when compared with those obtained from the normal control animals. These findings have been substantiated by other investigators (Hikino *et al.*, 1989; Kalaivanan and Pugalendi, 2011), who reported that deficiency of insulin leads to

derangement in carbohydrate metabolism and reduces the activities of a number of key enzymes, including glucokinase. Since insulin has been reported to increase hepatic glucokinase activity in liver (Nirav *et al.,* 2012), it may, therefore, be suggested that the decrease in the hepatic glucokinase activity may be due the reduced serum insulin level in the diabetic untreated animals that was observed in the present study. The findings is in consonance with the report of other investigators (Shukla *et al.,* 2007; Matschinsky, 2009) have reported impaired glucokinase activity, a critical enzyme that regulates glucose uptake in the liver and which has an exceptionally high impact on glucose homeostasis and serving as a glucose rate-controlling enzyme for hepatic glucose clearance and glycogen synthesis. In addition, Iynedjian (2009) reported that hepatic glucokinase is the most sensitive indicator of the glycolytic pathway in diabetes and decreased levels could decrease the utilization of blood glucose for glycogen storage in the liver.

Furthermore, in order to enter the lipogenic pathway, glucose must be metabolized. The first and rate limiting step is the phosphorylation of glucose at the 6^{th} carbon to obtain glucose-6-phosphate (Schuit *et al.,* 2001). This reaction is catalyzed by hepatic glucokinase, a member of the hexokinase family. Hence, hepatic glucokinase can be considered an intracellular glucose sensor. Consequently, apart from hepatocytes, GK is expressed in glucosensitive cells of the pancreas, hypothalamus, anterior pituitary gland, and entero-endocrine K and L cells of the gut (Schuit, 2001; Zelent, 2006; Vieira, 2007; Iynedjian, 2009), all of them crucial in the control of the whole-body glucose homeostasis. Liver contains 99.9% of the body hepatic glucokinase. Moreso, glucokinase reaction has also been shown to control the flux of glucose through several metabolic pathways such as: glycolysis, glucose oxidization, glycogenesis, triglyceride synthesis, phospholipids and cholesterol synthesis, glycogenolysis as well as gluconeogenesis (Iynedjian,

2009). The results from this study showed that administration of lycopene and glibenclamide to diabetic rats produced a significant increased activity of hepatic glucokinase. These observations imply that entry of glucose into the cells is facilitated by lycopene treatment, which in turn stimulates the activity of this enzyme, with a consequent decrease in the blood glucose level observed in the current study. Franz (1990) reported that liver glucokinase is sufficiently active to allow effective nearly quantitative hepatic removal of alimentary glucose from the portal vein and that this enzyme constitutes the first rate limiting step in the pathway that results in the storage of glucose in the form of liver glycogen. This finding is also substantiated by the significant elevated hepatic glycogen level of diabetic animals that were treated with lycopene in the present study.

Serum enzyme measurements are valuable tool in clinical diagnosis that provides information on the effect and nature of pathological damage to any tissue (Daisy and Saipriya, 2012). AST, ALT and ALP are biomarkers of damage to the plasma membrane and endoplasmic reticulum and are often used to assess the integrity of the plasma membrane and tissues after being exposed to certain pharmacological agents (Rathod *et al.,* 2009). Result obtained in the present study showed that the activities of serum liver enzymes; aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were significantly increased in the diabetic untreated animals in comparison with non-diabetic untreated rats. This is consistent with the studies of Daisy and Saipriya (2012), who reported increased transaminase levels in streptozotocin-induced diabetes. Increased gluconeogenesis and ketogenesis might be due to an elevated activity of transaminase (Ghosh and Suryawansi, 2001; Gandhi *et al.,* 2011). Abolfathi *et al.* (2012) reported that the elevation in markers of liver injury such as ALT, AST and ALP indicate hepatocyte damage in experimental diabetes. And this increase in the levels of these

enzymes in diabetes may be as a result of leaking out of these enzymes from the tissue into the blood stream (Concepción *et al.,* 1993). AST and and ALT are released when injury involves organelles such as the mitochondria (Kumar *et al.,* 2003). Transaminases mediate the catalysis of amino transfer reactions, and are vital markers of liver injury in clinical diagnostics (Li *et al.*, 2007), alkaline phosphatase is a hydrolase enzyme located in the cytoplasm (Han *et al.,* 2006) and is responsible for removing phosphate from nucleotides and proteins released due to hepatic cellular damage. The ability of lycopene administration to diabetic animals to significantly decrease the ALT, AST and ALP serum levels sugges their hepato-cellular protective function and this can be attributed to its antioxidant effects.

Lipid profile is a group of blood tests which are carried out to determine the risk of coronary artery diseases (CAD). Results of lipid profile are considered as good indicators of whether someone is prone to develop stroke or heart attack, caused by atherosclerosis. Tests included in lipid profile are total cholesterol (TC), triglyceride (TRIG), high-density lipoprotein-cholesterol (HDL-c) and low-density lipoprotein-cholesterol (LDL-c) (Sembulingam and Sembulingam, 2013). Alterations in lipid metabolism and increased mobilization of free fatty acids from muscle and fat deposition occur in tissues such as liver and heart in diabetes mellitus (Bloomgarden, 2003; Shukla *et al.,* 2012). Hyperlipidaemia, a risk factor of cardiovascular diseases is frequently seen among diabetic patients (Mengesha, 2006). Serum lipid levels are commonly increased in diabetes mellitus and such an elevation represents a risk factor for coronary heart disease (Al-Shamaony *et al.*, 1994, Muthulingam, 2010). The present study showed an increase in the concentration of TC, TRIG, LDL-c and a decrease in HDL-C in diabetic control animals. This is in agreement with the reports of Fernandes *et al.* (2010); Mironova et *al*. (2000); Odetola *et al*., (2006) and Iweala and Oludare, (2011) who demonstrated increased serum lipids in diabetes in

animals. Diabetic-induced hyperlipidemia is attributable to excess mobilization fat from the adipose due to under utilization of glucose (Krishnakumar *et al*., 2000; Nimenibo-uadia, 2003). The lack of insulin and elevations of the counter-regulatory hormones (e.g glucagon) lead to activation of enzymes (hormone-sensitive lipase) that stimulate lipolysis and enhanced release of free fatty acids from adipose tissue (Subbiah *et al*., 2006;Rotimi *et al*., 2011; Matsinkou *et al.*, 2012). The fatty acids from adipose tissues are mobilized for energy purpose and excess fatty acids are accumulated in the liver, which are converted to triglyceride (Shih *et al.*, 1997; Suryawanshi *et al*., 2006).

The marked hyperlipidemia that characterizes the diabetic state may therefore be regarded as a consequence of unlimited actions of lipolytic hormones on the fat depots (Ayeleso *et al.*, 2012). Lowering of serum lipid levels through dietary or drugs therapy seems to be associated with a decrease in the risk of vascular disease in diabetes (Ayeleso *et al.*, 2012). The results of the present investigation showed that all doses of the lycopene administered to diabetic rats produced a significant beneficial effect on serum lipid profile in STZ-induced diabetic rats. The findings of the present study agree with results of other investigators (Fredrickson *et al.*,2007; Basuny *et al.*, 2009; Khayat *et al.*,2011; Sanadi *et al.*, 2012), who reported significantly reduced serum level of TC, TRIG and LDL cholesterol in experimentally induced hypercholesterolemia in rats and rabbits supplemented with lycopene and tomato paste. Sevim *et al.* (2013) also showed that administration of lycopene for twenty eight days produced a significantly decreased serum TC, TRIG and LDL cholesterol with a corresponding increased serum HDL cholesterol level in rats experimentally induced diabetes. These findings are consistent with those reported by Sevim *et al.*, (2013). The ability of lycopene to reduce plasma cholesterol and triglycerides in diabetic animals could be explained by the insulin releasing capacity of lycopene, since insulin is

required for the inhibition of hormone-sensitive lipase and hence increases the utilization of glucose and thereby decreasing the mobilization of free fatty acids from the fat depots. The decreased level of FFA is also associated with decreased actions of lipolytic hormones, which, in turn, decreased the activity of hormone sensitive lipases on fat deposits (Ramesh and Pugalendi, 2005). However, the results of present study showed that lycopene treatment to diabetic rats did not produce any significant increase on serum insulin level when compared with diabetic control group.

In addition, the hypocholesterolemic effect of lycopene may also be attributable to its antioxidant property, which is responsible for the decrease activity of 3-hydroxyl-3-methyl-glutaryl co-enzyme A (HMG CoA) reductase, which is the key regulatory enzyme in cholesterol biosynthesis (Olooto *et al.,* 2014), reduction in cholesterol absorption by the intestinal wall and/or induction of LDL-receptors within the peripheral tissue (Danesh and Kanwar, 2004; Olooto *et al.,* 2014). This observed improvement in the lipid profile status of diabetic treated rats revealed the cardio-protective properties of lycopene; and may be attributed to antioxidant effects of lycopene. This beneficial effect lycopene on the lipid profile status of diabetic animals may also be secondary to glycemic control observed in the present study. In addition, the significantly lowered cholesterol level recorded in the current investigation may have contributed to the observed significant high serum high-density lipoprotein cholesterol in the animals. Kwiterovich (2000) and James *et al*. (2010) had reported that about 30% of blood cholesterol is carried in the form of HDL-C. HDL-C function to remove cholesterol atheroma within arteries and transport it back to the liver for its excretion or reutilization, thus high level of HDL-C protect against cardiovascular disease. Therefore, the observed increase in the serum HDL-C level on administration of various doses of lycopene diabetic rats, indicates that the lycopene

have HDL-C boosting effect. More so, the stabilization of serum triglyceride and cholesterol levels in rats by lycopene may be attributed to glucose utilization and hence depressed mobilization of fat (Momo *et al*., 2006; Iweala and Oludare, 2011). This finding suggest that lycopene may be useful in reducing the complications of hyperlipidemia and hypercholesterolemia which often coexist in diabetics (Sharma *et al*., 2003).

Biomarkers of cardiovascular risk are atherogenic index (AI) and cardiac risk ratio or index (CRR), because both have been found to be the best related predictor of future cardiovascular events (*Grover et al.,* 1999; Radahmadi *et al.,* 2006; Wafa *et al.,* 2012). Atherogenic indice (AI) are the most useful index for predicting and quantifying coronary artery disease risk (Ekpenyong *et al.,* 2014). It is a single, independent predictor of morbidity and mortality in diabetic patients and is strongly associated with increased arteriosclerotic cardiovascular risk in diabetics as well as the general population (Steinberg *et al.,* 2013; Ekpenyong *et al.,* 2014). The association of TRIG, TC, LDL cholesterol and HDL cholesterol in this simple ratio reflects the balance between risk and protective lipoprotein forces; and AI reflect the delicate metabolic interactions within the whole lipotein complex (Angoorbala *et al.,* 2012). At the end of study period, results obtained showed that the atherogenic indices (AI) (LDL-cholesterol/HDL-cholesterol), log (TRIG/HDL-cholesterol) and cardiac risk ratio (CRR) were significantly increased, while HDL-cholesterol/TC was significantly reduced in diabetic untreated animals when compared with animals in the normal control group. This observation is consistent with the reports of previous researchers (Andulla *et al.,* 2009; Ahmed *et al.,* 2010; Udenze *et al.,* 2012; Ekpenyong *et al.,* 2014; Olooto *et al.,* 2014). Atherogenic indices are powerful indicators of the risk of heart disease: the higher the value, the higher the risk of developing cardiovascular disease and vice versa (Frohlich and Dobiásová, 2003; Dobiásová, 2004; Martirosyan *et al.,* 2007; Brehm *et al.,*

2004; Usoro *et al.*, 2006). AI provides information about the atherogenicity of plasma and quantifies the response to therapeutic intervention (Ojiako and Nwanjo, 2005). In this study, we observed that treatment of diabetic with graded doses of lycopene significantly reduced atherogenic indices (LDL-cholesterol/HDL-cholesterol), log (TRIG/HDL-cholesterol) and increased, HDL-cholesterol/TC in a dose dependent manner. Therefore, low atherogenic indices are protective against coronary heart disease (Usoro et al., 2006). This finding is in agreement with the reports of (Chang and Cheong, 2007; Ogbonnia *et al.*, 2008; Pourkabir *et al.*, 2010; Zheng *et al.*, 2013) who showed that supplementation of dietary tomato peel ultrafine powder has atheroprotective effects by significantly reducing levels of liver cholesterol and serum cholesterol STZ-induced diabetic and hyperlipidemic rats.

The significant reduction in the level of log (TRIG/HDL-cholesterol) portends a decrease risk of vascular disease, since high anterogenic index of log (TRIG/HDL-cholesterol) has been positively correlated with cardiovascular disease (Igwe *et al*., 2007). Low total cholesterol, triglyceride and high HDL cholesterol lower the ratio and the decrease in the ratio is desirable (Sembulingam and Sembulingam, 2013). From the present study, the values of LDL-cholesterol/HDL-cholesterol ratio is less than 2.3 and TC/ HDL cholesterol ratio is less than 3 in all diabetic lycopene treated groups while the values of HDL-cholesterol/Total cholesterol is greater than 0.3 in diabetic animals administered with lycopene, with highest increase observed with the highest dose. These values are desirable and therefore non atherogenic (Ojiako and Nwanjo, 2005). A total cholesterol /HDL ratio of ≤ 3 connotes a low risk, a ratio of around 4.5 an average risk and ratio of ≤ 8 a high risk of developing coronary artery disease (Saikia and Lama, 2011). In the present study an increase in serum HDL with a concomitant increase in percentage of protection from atherogenesis was observed in all diabetic rats that received

various doses of lycopene. This observed effect of lycopene suggests a possible protective role of lycopene against the development of atherosclerosis and coronary heart disease, as well as the dyslipidemic conditions that characterize diabetes mellitus. Low atherogenic indices are protective against coronary heart disease (Usoro *et al.,* 2006). High HDL exerts a protective effect by decreasing the rate of entry of cholesterol into the cell via LDL thus; increase the rate of cholesterol release from the cell (Ikewuchi and Ikewuchi, 2009a). This enhance the reverse cholesterol transport by scavenging excess cholesterol from peripheral tissues and deliver it to the liver and steroidogenic organs for subsequent synthesis of bile acids and lipoproteins and eventual elimination from the body (Assmann and Gotto, 2004; Ademuyiwa *et al.,* 2005; Brunzell *et al.,* 2008).

The secretion of stress hormones (glucagon, catecholamins, cortisol and growth hormone) and especially, cortisol increases during stressful conditions (Barglow *et al.,* 1985; Goldstein *et al.,* 1994; Radahmadi *et al.,* 2006). Hyperglycaemia leads to generation of free radicals due to auto-oxidation of glucose and glycosylation of proteins (Al-Faris *et al.,* 2010) and induces oxidative stress which becomes the chief factor that leads to diabetic complications (Kumawat *et al.,* 2009). The present study showed that serum cortisol level was significantly elevated in diabetic animals when compared with those obtained from normal control animals. This observed effect is consistent with reports of (Roy *et al.*, 1990; Roy *et al.* 1993; Bitar, 1998; Radahmadi *et al.,* 2006) who showed that diabetes is associated with elevated plasma levels of glucocorticoids and increased adrenal function. In the same vein, Chan *et al*. (2002) have reported an increase in the HPA axis activity in diabetic patients. Hypothalamo–pituitary–adrenal (HPA) axis has been shown to responds to stressful situations (Radahmadi *et al.,* 2006). The weight and the activity of the adrenal glands have also been reported to increase during the chronic illnesses such as

diabetes (Parker *et al.,* 1985) and elevate the cortisol metabolite concentration (Parker *et al.,* 1985).

During stressful conditions, the hypothalamus secretes corticotropin-releasing hormone (CRH), which travels to the anterior pituitary gland and stimulates the secretion of adrenocorticotrophic hormone (ACTH). ACTH, in turn, is released into the blood stream and eventually reaches the adrenal cortex, where it stimulates the release of cortisol. This release of cortisol in response to stressor is believed to be involved in promoting survival functions, such as increasing blood pressure and blood sugar levels (Sapolsky *et al.,* 2000; King and Hegadoren, 2002; Matousek *et al.,* 2010). Hence, increased serum cortisol level observed in our current study may be related to increased activity of adrenal gland. The ability of lycopene to significantly reduce the serum cortisol level in diabetic rats suggests that lycopene may be exerting ameliorative effects in diabetes induced hypercortisolism that was observed in the present study. The present investigation showed a significantly elevated serum MDA of diabetic animals in comparison with the normal control group. This finding is in agreement with the study of other researchers (Bonnefont-Rousselot *et al.,* 2000; West, 2000; Moussa, 2008; Varashree *et al.,* 2010). Studies have shown that high levels of glucose can produce permanent chemical alteration in proteins and increase lipid peroxidation in a variety of experimental models of hyperglycemia (Varashree *et al.,* 2010; Singh *et al.,* 2012). The rise in serum MDA indicated that any oxidative stress incurred sufficiently could cause free radical mediated peroxidation of lipid component in cell membrane, thus MDA is a good indicator for evaluating oxidative stress in degenerative disease such as diabetes mellitus (Padalkar *et al.,* 2012). The present investigation showed that administration of lycopene significantly reduces serum MDA concentration in all diabetic treated

groups. This finding corroborates the report of Singh *et al.* (2012) who demonstrated that oral administration of lycopene once daily for three months to diabetic patients attenuated the oxidative stress by significantly decreasing the levels of MDA. This observed effect of lycopene may probably be due to its various antioxidant defense strategies, most especially the scavenging of two of the ROS: singlet molecular oxygen and peroxyl radicals (Atessahin *et al.,* 2005).

Lycopene has been reported to act as a chain breaking antioxidant (Singh *et al.,* 2012). Lycopene reacts with peroxyl radicals formed in the propagation phage of lipid peroxidation to form carbon centred radicals. These carbon centred radicals then react readily and reversibly with oxygen to form new chain carrying peroxyl radicals which are stable than reactive oxygen species (Singh *et al.,* 2012). Lycopene, a potent antioxidant and having very good free-radical- scavenging capacity because of its unique structure (high number of conjugated double bonds), might have quenched the superoxide and other free radical anions, which are highly released in chronic diseases like diabetes mellitus (Rao and Agrawal, 1999; Upritchard *et al.,* 2000; Khachik *et al.,* 2002). Hence, the observed effects of lycopene in this present study may be attributed to this property. In the present study, results obtained indicated that the serum antioxidant enzymes (SOD, CAT and GPx) activities were significantly decreased in diabetic rats when compared with normal control animals. This finding is consistent with the reports of various researchers (Balasubashini *et al.,* 2004; Al-Azzawie and Alhamdani, 2006; Cheng and Kong, 2011; Singh *et al.,* 2012; Cheng *et al.,* 2013). Hyperglycemia is a main cause for elevated free radical levels, followed by production of ROS, which can lead to increased lipid peroxidation and altered antioxidant defense and further impair glucose metabolism in biological system (Balasubashini *et al.,* 2004). An imbalance between oxidation and antioxidant status has been shown to play an

important role in mediating oxidative stress (Rameshand Saralakumari, 2012). Overwhelming free radicals generated due to oxidative stress may develop several adverse effects commonly seen in diabetes such as neuropathy, nephropathy, retinopathy, and vascular disorders (Al-Azzawie and Alhamdani, 2006). The major antioxidant enzymes, including SOD, CAT, and GPx, are regarded as the first line of the antioxidant defense system against ROS generated *in vivo* during oxidative stress and act cooperatively at different sites in the metabolic pathway of free radicals (Cheng and Kong 2011). In our study, reduced activities of serum SOD, CAT, and GSH-Px were elevated following oral administration of lycopene for 4 weeks in diabetic animals. Antioxidant enzymes such as SOD, CAT, GPx and GR are primary enzymes that are involved in the direct elimination of free radicals (Oguntibeju *et al.,* 2010). This observation is in consonance with the reports of Singh *et al.* (2012), who showed oral ingestion of lycopene to diabetic patients recorded a significant increase in the activity of serum SOD, CAT and, GPx. The ability of lycopene treatment to diabetic animals to restore the depleted serum antioxidants enzymes in the current investigation may be attributed antioxidant property of lycopene.

The biochemical indices monitored in the kidney are useful 'markers' for assessment of tissue damage. The measurement of activities of various enzymes in the tissues and body fluids plays a significant role in disease investigation and diagnosis (Malomo, 2000) as well as assault on the organs/tissues and to a reasonable extent the toxicity of the drug (Yakubu *et al*., 2003b). Tissue enzymes can also indicate tissue cellular damage caused by chemical compounds long before structural damage that can be picked by conventional histological techniques (Akanji, 1986). The present investigation indicated that the concentration of MDA, a maker of lipid peroxidation was significantly higher in the kidney tissue of diabetic untreated rats when compared with the

animals in the normal control group. This result is in agreement with other investigators (Kinalski *et al.,* 2000; Bukan *et al.,* 2003; Sevim *et al.,* 2013), who reported a significantly increased kidney MDA in experimentally induced diabetes in animals. Increased TBARS and MDA production have been implicated in the progression of diabetes (Siddiqui *et al.,* 2011). This increase in the kidney MDA indicated enhanced lipid peroxidation, which could cause injury to the cells. Increased levels of lipid peroxides in the plasma is usually considered to be the consequence of high production and liberation of tissue lipid peroxides into circulation due to pathological changes (Selvam and Anuradha, 1990; Ravi *et al.,* 2004). Oxidative stress causes a biomolecular damage as a result of the attack of reactive species on components of living organisms and is known as oxidative damage (Halliwell and Gutteridge, 2007; Erejuwa, 2012). This is caused by increased production and / or reduction in the removal of reactive species by the antioxidant defences (Erejuwa, 2012).

Hyperglycaemia leads to generation of free radicals due to auto-oxidation of glucose and glycosylation of proteins (Al-Faris *et al.,* 2010) and induces oxidative stress which becomes the chief factor that leads to diabetic complications (Kumawat *et al.,* 2009). Abnormal elevated levels of free radicals and the simultaneous reduction of antioxidant defence can result in damage of cellular organelles and enzymes, increased lipid peroxidation and development of insulin resistance (Tirgar *et al.,* 2010). The elevated level of lipid peroxidation causes oxidative damage by increasing peroxy radicals and hydroxyl radicals (Shukla *et al.,* 2012), and is usually measured through the catabolite, MDA, in terms of TBARS as a maker of oxidative stress (Kumar *et al.,* 2012). The decrease in MDA of kidney tissues upon administration of lycopene to diabetic animals in the present study, clearly demonstrated the antioxidant property of lycopene. These findings suggest that the lycopene may exert antioxidant activity and protect the tissue

from lipid peroxidation. In the same manner, the kidney antioxidant enzymes (SOD, CAT and GPx) of diabetic control animals were significantly decreased in comparison with the animals in the normal control group. These observations are in consonance with the findings of Kinalski *et al.* (2000) and Bukan *et al.* (2003), who demonstrated a significant decrease in kidney antioxidant enzymes in diabetes.

The SOD, a superoxide radical scavenging enzyme is considered the first line of defense against the deleterious effect of oxygen radicals in the cells and it scavenges reactive oxygen radical species by catalyzing the dismutation of O_2^- radical to H_2O_2 and O_2^- (Singh *et al.,* 2012). SOD has been reported to be involved in the conversion of superoxide anion radicals produced in the body to hydrogen peroxide, thereby reducing the likelihood of superoxide anion interacting with nitric oxide to form reactive peroxynitrite. As a result, reduction in SOD activity in diabetic animals observed in the present study may be as a results of an increased influx of O_2^- radical and hence may reflects the cause of tissue injury (Singh *et al.,* 2012). This finding is consistent with the report of Kedziora-Kornatowska *et al.* (2000) who reported decreased renal activity of SOD three to six weeks after STZ administration. In contrast, treatment diabetic rats with lycopene and glibenclamide enhanced the activity of SOD in comparison with the diabetic control group. On the other hand, catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues. CAT decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals (Chance and Greenstein, 1992; Onyeka *et al.,* 2012). The present study showed that there was a significant decrease in the activity of kidney catalase level in diabetic control animals compared with the corresponding normal control group. This agrees with the finding of Rauscher *et al.,* (2001) who have reported similar observation. In the current study, the decreased CAT activity in kidney of diabetic animals was significantly improved following oral

administration of lycopene and glibenclamide. This observation is in consonance with the reports of Kuhad *et al.* 2008) and Ali and Agha (2009) who showed that administration of lycopene (90 mg/kg body weight) to streptozotocin-induced hyperglycemic rats caused increased antioxidant enzyme activities (ie, superoxide dismutase and catalase).

In addition, glutathione peroxidase (GPx) is a potent endogenous antioxidant that helps to protect cells from a number of noxious stimuli including oxygen derived free radicals (Kim.*et al.,* 2006; Onyema *et al.,* 2006). GPx is an antioxidant enzyme involved in the detoxification of hydrogen and lipid peroxides and also acts as a peroxynitrite reductase (Erejuwa *et al.,* 2011). A significant decrease in GPx activity might be accompanied by a significant increase in lipid peroxidation as evidenced by increased kidney MDA concentration observed in the present study. The result of the current finding agrees with the reports of other researchers (Aragno *et al.,* 1999; Moussa, 2008). However, administration of graded doses of lycopene and glibenclamide to diabetic animals to significantly increased level of the activity of GPx. These findings have been substantiated by other investigators (Kuhad *et al.,* 2008; Ali and Agha, 2009). Therefore, the increased activity of GPx might be a protective mechanism in response to increased concentrations of H_2O_2 and other lipid peroxides in kidney of diabetic animals treated with lycopene. These observations also indicate that lycopene has nephroprotective effects or the ability to protect against diabetes-induced kidney damage that may arise. Lycopene's configuration has been reported to be responsible for its ability to inactivate free radicals and to interfere with free-radical-initiated reactions, particularly lipid peroxidation, thereby preventing tissue injury (Seren *et al.,* 2008). The antioxidant enzymes are very good biochemical markers of stress and their elevated activity may confirm a potential for remediation (Kopyra and Gwozdz, 2003; Dauqan *et al.,* 2011).

In addition, it has been reported that lycopene has high efficient antioxidant and free radical scavenging capacity (Atessahin *et al.,* 2006). Lycopene has been shown to be one of the best biological suppressants of free radicals, especially those derived from oxygen. It has the highest singlet oxygen-quenching rate of all carotenoids in biological systems (Bhoomika and Vaghela, 2013). The findings in the present study denoted the ability of lycopene to protect the kidney tissue from oxidative damage through elevation of endogenous antioxidant enzymes (SOD, CAT and GPx). The attenuation of kidney tissue in diabetic animals treated with lycopene, which also positively correlated with the significantly reduced kidney MDA level a marker of oxidative stress, further strengthens the notion and also suggests that lycopene may have the ability to protect the kidneys from oxidative injury.

Pathologic alterations in the protein synthetic capacity have been reported in disease, which are reflected in the protein content of serum (Al-Joudi and Wahab, 2004). Abbas *et al.* (2012), has reported that the determination of total protein, albumin and globulin levels is aimed at evaluating the toxicological nature of various chemicals. In the present study, the significantly decreased levels of total serum proteins, albumin and globulin in the diabetic control rats when compared to normal control animals indicates increased free-radical induced liver damage caused by hyperglycaemia observed in the present study. A reduction in serum total protein content in diabetes has been reported by other researchers (Mansour *et al.,* 2002; Mariam *et al.,* 2011), and this is indicated by an increase in the lipid peroxidation and a decreased antioxidant defense system (Chandramohan *et al.,* 2009).

In diabetes, increased blood nitrogenous substances may be accounted for by the enhanced breakdown of both liver and plasma proteins (Prakasam *et al.,* 2004). In addition, The reason for the reduction in the level of albumin could be as a result of the regulation of albumin catabolism by neonatal Fc receptor (FcRn) which binds albumin in acidic environments and increases albumin catabolism and hence, leads to a decreased level of serum albumin (Chaudhury *et al.,* 2003). Proteins have long been regarded as a principal target for oxidants due to their abundance in biological systems (Medina-Navarro *et al.,* 2010). Serum concentrations of proteins and albumin can help to show the condition of the liver and also ascertain the different types of liver damage (Yakubu *et al.,* 2003). Albumin, the most abundant plasma protein consists of more than 60% of free serum proteins is synthesized and secreted by the liver and it has many vital functions such as maintaining plasma colloid osmotic pressure, anti-oxidation and transportation of metals, fatty acids, cholesterol, bile pigments, and drugs (Roche *et al.,* 2008; Shi *et al.,* 2010; Nayyar *et al.,* 2012).

Serum albumin is a key antioxidant agent and its structural modification as a result of induction by glucose or free radicals can impair its antioxidant potentials (Faure *et al.,* 2008). It also represents the key and predominant antioxidant in plasma, a body compartment known to be exposed to continuous oxidative stress (Roche *et al.,* 2008). A great proportion of total serum antioxidant properties can be attributed to albumin (Roche *et al.,* 2008). Treatment with various doses of lycopene appreciably increased the serum total protein, albumin and globulin levels in diabetic rats in comparison to the diabetic control rats. The ability of lycopene to normalize the levels of albumin in the hyperglycaemic state may be attributed to their free radical scavenging property of lycopene. This improvement could be attributed to increased protein synthesis,

increasing incorporation of certain amino acids as a result of increasing insulin secretion, increase of hepatic uptake of glucogenic amino acids, stimulation of amino acid incorporation into protein and decreased proteolysis by activating the enzyme that catalyzing amino acids transamination. Also, good correlation between protein synthesis and insulin level has been reported by Nahla and Ismail (2006) and Sayed *et al.,* (2011). Although administration of lycopene did not produce a significant serum insulin secretion in the diabetic animals in the present study. Conversely, the results of the present study indicated a significantly increased serum albumin/globulin ratio in diabetic control, when compared with the animals in the normal control group. In contrast, Komolafe *et al.* (2014) and El-Shenawy and Abdel- Nabi (2006) reported significantly reduced albumin/globulin ratio in experimentally-induced induced diabetic animals. The albumin/globulin (A/G) ratio is a biochemical parameter utilized in the interpretation of changes in serum proteins that accompany disease (Al-Joudi and Wahab, 2004). Diabetes mellitus is grossly reflected by intense changes in the protein metabolism and by a negative nitrogen balance and loss of nitrogen from most organs (Prakasam *et al.,* 2004; Pasupathi *et al.,* 2009). Treatment of diabetic rats with lycopene reduced the serum albumin/globulin ratio appreciably in comparison with the diabetic control animals.

In the current investigation, there was a significant decrease in the serum sodium ion concentration when compared with the normal control animals. Kidney function has been reported to be compromised in uncontrolled diabetes mellitus. Glycosuria, which is a pertinent diagnostic feature of diabetes, imposes dehydration via glucose osmotic diuresis (Eteng *et al,* 2008; Item *et al.,* 2009; Tanko *et al.,* 2013). Hence, the depleted serum sodium ion may be attributed to dehydration which is accompanied with severe loss of electrolytes including

sodium, potassium, calcium, chloride and phosphates. However, oral administration of lycopene to diabetic rats restored the serum sodium ion level almost close to normal. On other hand, the study also showed a significant increased serum urea level in diabetic control rats in comparison with the normal control animals. Plasma urea are recognized markers of glomerular filtration rate (GFR) and in nephropathy (Kumar and Clark, 2005; Madsen *et al.,* 2005). This result is an agreement with the report of (Sapna *et al.,* 2009), that showed increased serum urea level in diabetic patients. An elevation of serum urea usually signifies decreased renal function (Sapna *et al.,* 2009). More so, in diabetes there is increased catabolism of amino acids resulting in high urea formation from the urea cycle (Sapna *et al.,* 2009). Hence in this study, the elevation of serum urea with hyperglycemia can be suggested as indicator for renal dysfunction (Trujillo *et al.,* 2013), and reduced filtering capacity of kidneys which leads to accumulation of waste products within the system of diabetic animals.

Treatment diabetic rats with lycopene and glibenclamide produced a significantly reduced serum urea level, suggesting its ability to protect against diabetes-induced kidney damage, by preventing altered protein metabolism and/or impaired renal function that often exist in diabetes mellitus. Diabetes is characterized by increased volume and metabolites excretions via the kidneys, usually in excess of normal thresholds. These usually give rise to derangements in homeostatic balance with respect to electrolytes (Item *et al.,* 2009; Tanko *et al.,* 2013). Although no significant difference was recorded in the serum potassium, chloride and bicarbonate ions concentrations in diabetic control rats when compared with normal control animals. Following treatment with lycopene the serum level of potassium, chloride and bicarbonate ions did not differ significantly with those of diabetic control animals when compared.

Erythrocyte is one of the main cells used as an oxidative stress marker in living animals, including humans, because their cell membranes, as well as their antioxidant enzymes, are sensitive to the presence of free radicals in general (Gutiérrez-Salinas *et al.*, 1993; José *et al.*, 2010). Erythrocytes and the erythrocyte membranes are critical target in the lipid peroxidation process due to constant exposure to high oxygen tension and elevated polyunsaturated fatty acids (PUFA) in their membrane (Kollanjiappan *et al*., 2002), coupled with their inability to possess nucleus and other organelles (Đorđeviæ *et al*., 2008). Hyperglycaemia leads to generation of free radicals due to auto-oxidation of glucose and glycosylation of proteins (Al-Faris *et al.,* 2010) and induces oxidative stress which becomes the chief factor that leads to diabetic complications (Kumawat *et al.,* 2009). The result obtained showed a significantly increased erythrocyte MDA concentrations in the erythrocyte membrane of diabetic control animals, when compared to those obtained in the normal control group. This significant increase in erythrocyte MDA concentration in STZ-induced diabetic control rats indicates that hyperglycaemic increased free-radical induced lipid peroxidation in the erythrocytes and their membranes. These findings have been supported by data obtained from other researchers (Qujeq *et al.,* 2005; Ahmed *et al.,* 2006; Gauri and Vijaya, 2008; Hussein *et al.,* 2011; Yang *et al.,* 2012; Mohamed *et al.,* 2013) who have demonstrated increased lipid peroxidation and protein oxidative damage as evidenced by higher MDA concentration in the erythrocytes of STZ-induced diabetic rats. Similarly, Sushi (1989) has also reported hyperglycemia-induced membrane lipid peroxidation in human erythrocytes.

The exact mechanism that leads to the hyperglycaemia-induced membrane lipid peroxidation in RBC of diabetics is not known. However, previous studies in a cell-free system have suggested

that in hyperglycaemia, glucose can enolize and thereby reduce molecular oxygen yielding a-keto aldehydes and free radical intermediates (Sushi, 1989). In diabetes abnormal elevated levels of free radicals and the simultaneous reduction of antioxidant defence result in increased lipid peroxidation and development of insulin resistance (Tirgar *et al.,* 2010). Red blood cells (RBCs) are unique, highly specialized and the most abundant cells in the human organism. RBCs are highly susceptible to oxidative damage due to the high cell concentration of oxygen and hemoglobin, a powerful promoter of the oxidative process (Pandey *et al.,* 2010). They are one of the first cells to be affected by adverse conditions (Bryszewska *et al.,* 1995). Circulating red blood cells act as a sink for free radicals since both superoxide radicals (O_2^-) and hydrogen peroxide (H_2O_2) have the ability to penetrate membranes of the cells (Andallu and Varadacharyulu, 2003). They are also subject to a continuous flux of O_2 and H_2O_2 , which results from auto-oxidation of haemoglobin (Andallu and Varadacharyulu, 2003). Conversely, lycopene treatment to diabetic rats attenuated the increased erythrocytes MDA concentration observed in the present study. This suggests that lycopene may be beneficial in ameliorating hyperglycaemia-induced oxidative damage to erythrocytes. This effect of lycopene may be attributed to its high oxygen-quenching capacity and singlet molecular oxygen (O_2) and peroxyl radical-scavenging ability (Stahl and Sies, 2003), which consequently contribute to the defence against lipid peroxidation as reflected by significantly decreased erythrocytes MDA concentration observed in the present investigation.

Red blood cells are distinctive, highly specialized and the most abundant cells in humans and contain high levels of both enzymatic and non-enzymatic cytoplasmic antioxidants (Pandey and Rizvi, 2010). They are the first cells in the body to be exposed to stressful stimuli and hence, prone to oxidative stress (Pandey and Rizvi, 2010). And because of its role as O_2 and CO_2

transporter, the erythrocytes are under constant exposure to reactive oxygen species (ROS) and oxidative stress (Matough *et al.,* 2012). Indeed, the erythrocytes have been used for the evaluation of the impact of free-radical induced oxidative stress in humans and animal models because of several reasons; for example (i) these cells are continually exposed to high oxygen tensions, (ii) unable to replace damaged components, (iii) their membrane lipid bilayers are rich in polyunsaturated fatty acids side chains which make them vulnerable to peroxidation and (iv) they have enzymatic and non-enzymatic antioxidant systems (Konyalioglu and Karamenderes, 2005).

Results of the present investigation showed that a significantly increased erythrocyte osmotic fragility were recorded at 0.3, 0.5, 0.7 and 0.9 % of NaCl concentrations in streptozotocin-induced diabetic untreated animals when compared with those obtained in the normal control group. This finding corroborates the reports of other investigators who showed that erythrocyte osmotic fragility was significantly increased in streptozotocin-induced diabetic animals (Shin *et al.,* 2007; Megha and Sehyun, 2009; Matough *et al.,* 2012; Arun, 2013). This current finding indicates the ability of streptozotocin-induced hyperglycaemia to compromise the integrity of the red blood cell membrane. The normal function of the RBC is largely hinged on the maintanance of the integrity of its membrane. The vulnerability of the red blood cell membrane integrity which resulted to increased erythrocyte fragility in the diabetic untreated group may have arisen from the increased lipoperoxidative (MDA) changes in the erythrocyte membranes as evidenced by increased MDA concentration in the erythrocytes of diabetic untreated animals observed in the present study. These results agree with findings of other researchers (Jain, 1989; Qujeq *et al.,* 2005; Ahmed *et al.,* 2006; Gauri and Vijaya, 2008; Hussein *et al.,* 2011; Matough *et al.,* 2012;

Yang *et al.,* 2012; Mohamed *et al.,* 2013) that reported a significant positive correlation between the hyperglycaemia-induced membrane lipid peroxidation and the increased osmotic fragility of the erythrocyte membrane, which can cause changes in the properties of the RBC membrane and erythrocytes of STZ-induced diabetic rats. Erythrocyte is a convenient model to study oxidative damage of cell membranes by various pro-oxidants as well as the chemicals (**Sharma et al., 2012)**. ROS-catalyzed oxidative damage to membrane lipids may impair the stability of erythrocytes and cause oxidative hemolysis or osmotic fragility (Tavazzi *et al.,* 2000; **Sharma et al., 2012**).

The erythrocyte membrane has been reported to be highly sensitive to oxidative stress as it contains high amount of polyunsaturated fatty acids (PUFA) as well as higher concentration of oxygen and heme (Mansour *et al.,* 2009; Nikolaidis and Jamurtas, 2009). Erythrocyte osmotic fragility test gives an in-vitro measure of the tensile strength of erythrocyte membrane and it is an indirect method of evaluating lipid peroxidation in animals (Uzum *et al.,* 2006; Ogundeji, *et al.,* 2013; Akande *et al.,* 2014). It is a measure of erythrocyte strength and its ability to withstand varying osmotic gradients and it has being reported to be increased during oxidative stress (Aldrich *et al*, 2006; Adenkola *et al.,* 2010). The greater the erythrocyte osmotic fragility, the weaker the tensile strength of erythrocyte membrane (Ogundeji, *et al.,*2013). Similarly, hyperglycaemia associated with diabetes has been shown to increase the erythrocyte osmotic fragility and membrane lipid peroxidation in human erythrocytes (Jain, 1989; Saba *et al.,* 2010). Increased MDA level has been known to be associated with increased fragility of erythrocytes during oxidative stress (Steinberg *et al.,* 2007; Saba *et al.,* 2010). The mechanisms for increased fragility of erythrocytes have been reported to be due to increased glycosylation of the erythrocyte membrane protein or/and alteration of the Na^+/K^+ ATPase on the erythrocyte

membrane (Arun, 2013). Administration of graded doses of lycopene and glibenclamide to diabetic animals recorded a significantly dose dependent decrease in percentage erythrocyte osmotic fragility, suggesting that lycopene and glibenclamide conferred some degree of stability to the erythrocytes of the diabetic rats when compared with the diabetic control group. The results are consistent with the observation of other researchers (Bhuvaneswaria *et al* 2001, Velmurugan *et al*., 2001; Velmurugan *et al*., 2002, El-Demerdash *et al*., 2004, El-Komy and Hassan, 2005, Moreira *et al*., 2005, Atessahin *et al.*, 2006; Yilmaz *et al*., 2006) who reported that lycopene improved oxidative stress-induced lipid peroxidation in rats. Moreso, the increasing erythrocyte osmotic resistance (or less osmotically fragile) observed in the diabetic animals treated with lycopene shows the protective ability of lycopene against increased osmotic stress of the erythrocytes; that is, increasing hypotonicity. Hence, the observed effect in the current study may also be due to the ameliorative effects or/and anti-oxidative activities of lycopene on the erythrocyte oxidative damage associated with diabetes. This finding may be attributed to strong antioxidant property of lycopene (Atessahin *et al.*, 2005; Kumar and Kumar, 2009). Furthermore, another mechanism by which lycopene reduces the erythrocyte osmotic fragility in diabetic animals may probably be by mopping up free radicals that are usually involved in the destruction of membrane protein and lipid peroxidation. The finding in the present investigation which positively corroborate with same in erythrocyte MDA concentration, which showed a significantly reduced lipid peroxidative (MDA) level and concurrent elevation of serum antioxidant enzymes (CAT, SOD and GPx) in diabetic rats treated with lycopene in the present study.

Blood cell morphology, distribution and indices such as red blood cells, hemoglobin, package cell volume, platelets, white blood cells count, mean corpuscular volume, mean corpuscular

hemoglobin and mean corpuscular hemoglobin concentration are good indicators of systemic response to external stress (Srivastava and Choudhay, 2010; Usama *et al.,* 2013). In addition, the knowledge of haematological characteristics is an important tool used as an effective and sensitive index of assessing the physiological and pathological changes in animal models (Zhou *et al.,* 2009). The analysis of blood indices has proven to be a valuable approach for analyzing the health status of animal models as these indices provide reliable information on metabolic disorders, deficiencies, chronic stress status and blood relating functions before they are present in a clinical setting (Bahmani *et al*., 2001). Alterations in blood parameters may be due to changes in cellular integrity, membrane permeability of cells or even due to exposure to toxic chemicals (Hoffbrand and Pettit, 1997).

The lower values of Hb, RBC and PCV in the STZ-induced diabetic untreated group in comparison with those obtained in the normal control rats observed in the present study agreed with those reported by other researchers (Arun and Ramesh, 2002; Oyedemi *et al.,* 2011; Matough *et al.,* 2012). However, the significantly reduced levels of Hb, RBC and PCV in the diabetic animals may be attributed to the infections on the normal body systems (Oyedemi *et al.,* 2011). Moreso, reactive oxygen species have also been implicated in the mechanism of red cells damage (Rao *et al.,* 2003; Mohammed *et al.,* 2009). Diabetes mellitus has been reported to be accompanied by increased production of free radicals (Baynes, 1999; Mohammed *et al.,* 2013), which causes damage to cellular proteins, membrane lipids and nucleic acids, and eventually cell death (Arun and Ramesh, 2002; Oyedemi *et al.,*2011). Therefore, the anemia recorded in the present investigation may be sequel to the haemolysis caused by increased erythrocyte osmotic fragility by STZ-induce hyperglycaemia observed in the present study, and as well as increased non-enzymatic glycosylation of RBC membrane proteins (Oyedemi *et al.,* 2011), which was not

assessed in the current investigation. Oxidation of these proteins and hyperglycaemia in diabetes mellitus causes an increase in the production of lipid peroxides that lead to haemolysis of RBC (Arun and Ramesh, 2002; Olszewska *et al.,* 2012). This finding was positively correlated with significantly higher RBC membrane lipid peroxide (MDA) levels in diabetic rats that were recorded in the current study. However, treatment of diabetic animals with all doses of lycopene and glibenclamide respectively significantly improved the levels of haemoglobin concentration, red cell count and packed cell volume, with a better effect recorded with 40 mg/kg of lycopene when compared with the diabetic control group.

The probable mechanism by which lycopene exerts this effect may be link to increased protein synthesis or mobilization, which are largely produced in the liver as well as secretion of erythropoietin in the stem cells of the animals. Erythropoietin is a glycoprotein hormone which stimulates stem cells in the bone marrow to produce red blood cells (Ohlsson and Aher, 2006; Oyedemi *et al*, 2011). The stimulation of this hormone enhances rapid synthesis of red blood cell which is reflected by increased levels of Hb, red blood cell and packed cell volume. Erythropoietin is a glycoprotein hormone which stimulates stem cells in the bone marrow to produce red blood cells (Ohlsson and Aher, 2006). The stimulation of this hormone enhances rapid synthesis of RBC and this often leads to improved level of MCH and MCHC (Abu-Zaiton, 2010). Hence, the haemopoietic effect of lycopene may probably be on stimulation of erythropoietin, thrombopoietin and granulopoietin production in response to anaemia, thrombocytopenia and leucopenia associated with STZ- induced diabetes observed in the current investigation. In like manner, the MCV, MCH and MCHC were significantly decreased in the diabetic control rats when compared with rats of the normal control group. The streptozotocin-induced alterations in MCV, MCH and MCHC have been reported to be due to direct or

feedback responses of structural damage to RBC membranes resulting in haemolysis and impairment in haemoglobin synthesis, stress-related release of RBCs from the spleen and hypoxia (Marei *et al*., 1998; Shah, 2006). Also the significant decrease in MCV suggests that the diabetic rats had tendency towards microcytosis, which is a type of anaemia caused by iron deficiency resulting in low RBC volume (Sembulingam and Sembulingam, 2013). Furthermore, MCH and MCHC have been used as a guide in the classification of anaemia (Baker and Silverton, 1985). The significant decrease in these in red cell parameters observed in the present study may be due to iron deficiency; and the RBC in this condition is said to be hypochromic (Sembulingam and Sembulingam, 2013). Hence, treatment of diabetic animals with lycopene and glibenclamide significantly restored changes in MCV, MCH and MCHC values when compared the normal control group. The MCH and MCHC are used to define the concentration of haemoglobin present in a single RBC (Sembulingam and Sembulingam, 2013) and to suggest the restoration of oxygen-carrying capacity of the blood. However, the mechanism of action by which lycopene exert this effect was not investigated in the present study.

Streptozotocin is a well known chemical that suppresses the immune system by damaging WBC and certain organs in the body (Oyedemi *et al.*, 2011). The present study showed that intraperitoneal injection of streptozotocin into rats significantly reduced the total WBC count and its differentials such as lymphocytes and neutrophils. The reduction of these parameters could be linked to suppression of leucocytosis from the bone marrow which may account for poor defensive mechanisms against infection (Oyedemi *et al.*, 2010). Consequentially, they might have effects on the immune system and phagocytic activity of the animals (Torell *et al.*, 1986). Treatment of diabetic animals with lycopene produced an ameliorative effect as reflected by significant increase in WBC, neutrophlis and lymphocyte counts respectively when compared

with the diabetic control group rats. This is an indication of increased cell mediated immunity in the diabetic rats that were administered with lycopene, which was corrected in those rats treated with lycopene in a manner that is similar to the effect of a standard antidiabetic drug.

The results obtained in the present study recorded a significantly elevated neutrophil/lymphocyte ratio and platelet count in the diabetic untreated animals when compared with those obtained in the normal control animals. Neutrophil/lymphocyte ratios have been reported to increase in response to stressors. The neutrophil-lymphocyte ratio (NLR) provides an inexpensive index for systemic inflammation (Duffy *et al.,* 2006; Mustafa and Adülkerim 2013). An increased ratio, indicate high stress (Davis *et al.,* 2008). In addition, high ratios of neutrophils/lymphocytes in blood samples reliably indicate high glucocorticoid levels (Davis *et al.,* 2008). This positively correlated with the significantly elevated serum cortisol level recorded in the present research work. Furthermore, this close relationship between stress hormones (cortisol) and neutrophil/lymphocyte ratio has been reported in haematological assessments of stress, as it aids the interpretation of results (Davis *et al.,* 2008).

Platelets play a role in blood clotting, which is a meshwork of fibrin fibres. The fibres usually adhere to any vascular opening and hence prevent further blood clot. It plays a crucial role in reducing blood loss and repairing of vascular injury (Oyedemi *et al.,* 2010). It is likely the STZ administered to diabetic untreated animals induced inflammation. The possibility of such inflammatory reaction in this study is confirmed by significantly high neutrophil-lymphocyte ratio recorded. However, in the present study, markers of inflammation were not estimated. Platelet aggregation ability has been reported in diabetes mellitus in chronic hyperglycaemia as a

result of lack or deficiency of insulin (Jarald *et al.,* 2008). Kayode *et al* (2009) and Vaghasiya and Chanda (2010) have shown that inflammation caused by high neutrophil-lymphocyte ratio could result in atherosclerotic lesion. In addition, specific leukocyte fractions have been reported to have higher predictive value in assessing cardiovascular risk and such predictive value is even higher when the neutrophil-lymphocyte ratio is used (Horne *et al*, 2005).

It is worthy to note that the development of atherosclerotic lesions, regardless of other risk factors is characterized by the disruption of the normal function of the endothelial cells, which may be due to high level of LDL-c with corresponding reduction in HDL level recorded in the present investigation. This could probably result in high yield of free radicals that cause damages to the endothelium (Kayode *et al* 2009; Ita *et al.,* 2013). The development of diabetes-related atherosclerosis has been reported to includes endothelial injury, smooth muscle cell proliferation, foam cell development and infiltration, platelet activation, and increased inflammation (Lusis, 2000). Chronic inflammation is directly associated with atherosclerosis, alongside other markers of inflammation (which were not assessed) (Ita *et al.,* 2013) in the present study; and coagulation which might have been up-regulated by high level of circulating platelet, which may possibly explain the significantly high values of neutrophil/lymphocyte ratio as well as and platelets count recorded in this study.

The results of the present investigation showed a significantly depleted hepatic glycogen content in STZ-induced diabetic untreated rats compared with the corresponding normal control animals. This observation agree with the reports of other investigators (Akatsuka *et al.,*1983; Lintus *et al.,* 1995; Grover *et al.,* 2002; Mallick *et al.,* 2006; Kumarappan *et al.,* 2012), who reported that

impaired or decreased hepatic glycogen synthesis in diabetic state. Glycogen is the major intracellular storable form of glucose and its level in various tissues such as liver is a direct reflection of insulin activity (Gandhi *et al.,* 2011). This finding positively corroborated with same in the present study in which the serum insulin level was significantly reduced following STZ administration in rats.

The regulation of glycogen metabolism has been reported to occur by the multifunctional enzymes glycogen synthase and glycogen phosphorylase that play a major role in the glycogen metabolism (Janero, 1990; Agius, 2008; Kumarappan *et al.,* 2012). The reduced glycogen store in rats with experimentally induced diabetes has been attributed to reduced activity of glycogen synthase and increased activity of glycogen phosphorylase (Kumarappan *et al.,* 2012). The reduction in glycogen synthetase and a concomitant elevation in the glycogen phosphorylase activities during diabetic condition have also been reported (Daisy *et al.,* 2012). The findings of the present study suggest that this fall in activities of enzyme, which were not estimated in the current investigation, may be due to decreased insulin level in the diabetic animals and, hence, result in inactivation of glycogen synthetase system (Narendhirakannan *et al.,* 2006). Daisy *et al.* (2012) reported increased glycogenolysis in diabetes as a result of insulin lack. This observation may have resulted to the reduced liver glycogen content in diabetic rats recorded in the present work. Following lycopene and glibenclamide treatment to diabetic rat, there was a significant increase in hepatic glycogen level in comparison to the normal control animals. However, the marked increased liver glycogen levels were not in proportion to insulin secretion as treatments with lycopene only at all doses did not produce any significant increase on the serum level, unlike the standard drug (glibenclamide) which recorded a significantly increased serum insulin

level when compared with the diabetic control group. These findings positively correlated with same in the current study in which the histological study on the liver glycogen tissue showed a dose dependent restoration of glycogen level in hepatocytes of diabetic animals treated with lycopene when compared with the diabetic control group.

The results suggest that the possible mechanism(s) of anti-diabetic action of lycopene may be in modulating of glycogen metabolism by enhancing the activity of hepatic glucokinase both in lycopene and glibenclamide treated diabetic animals. Since hepatic glucokinase is the most sensitive indicator of the glycolytic pathway in diabetes and its increased activity observed in the present study may be responsible for the increased the utilization of blood glucose for glycogen storage in the liver (Iynedjian *et al*., 1988). Another mechanism may be due to the reactivation/increased activity of glycogen synthase system (which was not estimated) by lycopene as a result of increased insulin secretion from the regenerated pancreatic β-cells. However, the present study showed that the serum insulin level of STZ-diabetic animals treated with lycopene increased, but was not statistically significant when compared to diabetic control group.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

The findings in the present study can be summarized as follows:

1. Administration of lycopene to diabetic animals produced a significantly decreased blood glucose level. The effect was comparable to glibenclamide (standard antidiabetic drug).
2. The activity of hepatic glucokinase was significantly enhanced in diabetic rats treated with various doses of lycopene. The observed effect was in a dose dependent fashion
3. Administration of various doses of lycopene to diabetic rats did not produce any significant increase on serum insulin level. Conversely, glibenclamide produced a significantly elevated serum insulin level
4. The possible anti-hyperglycaemic mechanism of actions of lycopene in the present investigation may be suggested to be due to increased activity of hepatic glucokinase and may not be due to stimulation of release of insulin from the pancreatic beta cells since serum insulin level not significantly increased
5. There was an improved hepatic glycogen store in diabetic animals that received graded doses lycopene, as reflected by a dose-dependent increase in the percentage liver glycogen level, with highest increase observed with the highest dose.
6. The histological findings of liver section stained with Period acid Schiff (PAS) also substantiate this observation as the he histological results indicated significantly restored cytoplasmic glycogen in hepatocytes of diabetic animals.
7. The significantly elevated liver enzyme activities (AST, ALT and ALP) were ameliorated following lycopene treatment to rats. The effects were in a dose-dependent manner for

AST and ALT, while 20 mg/kg of lycopene recorded better effect compared with other doses.

8. Lycopene significantly reduced the total cholesterol, triglyceride levels, LDL-c with a corresponding increase in the level of HDL-C. These reductions could be beneficial in preventing diabetic complications as well as improving lipid metabolism in diabetics.
9. Biomarkers of cardiovascular risk such as atherogenic index (AI) and cardiac risk ratio (CRR) were significantly improved in diabetic rats treated with lycopene, with an increased dose dependent percentage protection, indicating possible protective effects of lycopene against the development of dyslipidaemia and atherosclerosis.
10. Administration of lycopene to diabetic rats ameliorated hyperglycaemia-induced hypercortisolism, serum lipidperoxidative (MDA) changes as well as upregulated serum antioxidant enzymes (SOD, CAT and GPx) reserves. The ability of lycopene treatment to diabetic animals to restore the depleted serum antioxidants enzymes (SOD, CAT and GPx) in the current investigation may be attributed to antioxidant property of lycopene.
11. Lycopene treatment to diabetic rats attenuated kidney oxidative damage by significant reduction in kidney MDA concentration and increased kidney SOD, CAT amd GPx activities, suggesting the ability of lycopene to protect the kidneys from oxidative injury.
12. Lycopene administration to diabetic rats produced a significantly elevated serum total protein, albumin, globulin as well as albumn/globulin ratio levels
13. Administration of lycopene to diabetic animals improved renal dysfunction by restoring depleted serum sodium ion and produced a significantly reduced serum urea level, suggesting its ability to protect against diabetes-induced kidney damage. However, there were not significant changes in serum levels of potassium, chloride and bicarbonate ions.

14. Lycopene treatement to diabetic rats significantly reduced STZ-induced hyperglycaemic erythrocyte lipid peroxidative (MDA) changes and increased erythrocyte osmotic fragility. And this is probably through its potent antioxidative activities.
15. The increased RBC count, Hb concentration, PCV diabetic animals treated with lycopene suggests the haemopoietic effect of lycopene which may probably be due stimulation of erythropoietin. In addition, the significantly restored changes in MCV, MCH and MCHC values showed improved anaemic condition observed in diabetic animals and also suggest the restoration of oxygen carrying capacity of the blood. However, the mechanism of action by which lycopene exert this effect was not investigated in the present study.
16. The reduced WBC count, lymphocytes and neutrophils in diabetic animals were significantly elevated following lycopene treatment. While the nuetrophil/lymphocyte ratio and platelets count were lowered significantly.

6.2 Conclusion

The study confirmed the involvement of oxidative stress in the progression of diabetes; and that activities of antioxidant enzymes critically influence the susceptibility of various tissues to oxidative stress in diabetes. The study further demonstrated the potentials of lycopene in ameliorating the physiological and biochemical alteration in streptozocin-induced induced diabetes mellitus in Wistar rats. The effects may be attributed to potent antioxidant property of lycopene.

6.3 Recommendations

i. Investigation on the effets of lycopene should be done on inflammatory markers of diabetic animals such as TNF-α, interleukin-1 (IL-1), interleukin-6 (IL-6); as elevations in inflammation maker activity has been reported to plays a critical role in several stages of microvascular and cardiovascular diseases that occurs in diabetes.

ii. Further studies should also be done to assess the effects of lycopene on HbA1c (glycated haemoglobin) of diabetic rats, because HbA1c is a marker of cumulative glycaemic exposure. It is also used to assess/monitor the levels glycaemic control in diabetes during treatment.

iii. Further studies should be carried out investigate the precise mechanism(s) involved in the protective effect of lycopene against atherogenic heart disease associated with diabetes mellitus.

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LIST OF APPENDICES

Appendix 1: Effects of Lycopene Administration on Blood Glucose Levels in Streptozotocin-induced Diabetic Wistar Rats

	Fasting blood glucose level (mg/dL)				
Treatment Groups (n=5)	Week 0	Week 1	Week 2	Week 3	Week 4
NC + OL	91.0 ± 5.74[a]	90.8 ± 7.50[a]	73.4 ± 5.87[a]	76.0 ± 3.86[a]	79.8 ± 2.94[a]
DC + OL	364.4 ± 44.50[b]	392.6 ± 33.52[b]	465.2 ± 39.81[b]	487.0 ± 25.64[b]	431.4 ± 48.84[b]
D+LYC10 mg/kg	373.8 ±36.45[b]	278.2 ± 26.40[c]	216.4 ±19.55[c]	186.2 ±9.20[c]	171.1 ± 7.65[c]
D+LYC 20 mg/kg	371.2 ± 40.82[b]	277.43 ±24.33[c]	240.2 ±21.60[c]	183.0 ± 10.57[c]	118.4 ± 1.97[d]
D+LYC 40 mg/kg	370.6 ± 26.54[b]	279.8 ±38.47[c]	216.0 ±28.51[c]	164.4 ± 21.19[c]	100.8 ± 6.89[c]
D+GLB 2 mg/kg	374.2 ± 58.65[b]	260.3 ± 29.744[d]	188.0 ± 10.06[d]	150.2 ± 20.28[d]	108.8 ± 16.74[c]

[a,b,c,d] = Means on the same column with different superscript letters differ significantly ($P < 0.05$) compared with the control groups

DC+OL = Diabetic rats treated with olive oil (0.5 ml), NC+ OL = Normal (Non-diabetic) rats treated with olive oil (0.5 mL) D+ LYC10 mg/kg = Diabetic rats treated with 10 mg/kg of lycopene, D+ LYC 20 mg/kg = Diabetic rats treated with 20 mg/kg of lycopene, D+ LYC 40 mg/kg = Daibetic rats treated with 40 mg/kg of lycopene and D+ GLB 2 mg/kg = Diabetic rats treated with Gilbenclamide 2 mg/kg

Appendix 2:Effects of Lycopene Administration on Hepatic Glucokinase Activitiy,Serum Insulin Level and Erythrocyte MDA Concentration in Streptozotocin-induced Diabetic Wistar rats

Treatment Groups	Hepatic Glucokinase (U/g of Liver)	Serum Insulin Level (µIU/mL	Erythrocyte MDA (µmol/g Hb)
NC+OL	16.62 ± 0.63^{a}	12.04 ± 0.93^{a}	1.10 ± 0.09
DC+OL	8.78 ± 1.11^{b}	3.02 ± 0.24^{b}	2.10 ± 0.14
D+LYC10 mg/kg	11.96 ± 0.54^{c}	4.02 ± 0.70^{b}	1.48 ± 0.07
D+LYC 20 mg/kg	14.23 ± 0.88^{d}	3.96 ± 1.41^{b}	1.26 ± 0.08
D+LYC 40 mg/kg	15.78 ± 0.27^{e}	5.06 ± 0.96^{c}	1.02 ± 0.09
D+GLB 2 mg/kg	17.68 ± 0.75^{f}	7.76 ± 0.42^{d}	1.44 ± 0.11

^{a,b,c,d,e,f} = Means on the same column with different superscript letters differ significantly ($P < 0.05$) compared with the control groups

DC+OL = Diabetic rats treated with olive oil (0.5 mL), NC+ OL = Normal (Non-diabetic) rats treated with olive oil (0.5 mL) D+ LYC10 mg/kg b w = Diabetic rats treated with 10 mg/kg b w of lycopene, D+ LYC 20 mg/kg b w = Diabetic rats treated with 20 mg/kg b w of lycopene, D+ LYC 40 mg/kg b w = Daibetic rats treated with 40 mg/kg of lycopene and D + GLB 2 mg/kg b w = Diabetic rats treated with Gilbenclamide 2 mg/kg b w.

Appendix 3: Effects of Lycopene Administration on Serum Lipid Profile in Streptozotocin induced Diabetic Wistar Rats

Treatment Groups (n=5)	Serum Total Cholesterol (mmol/L)	Serum Triglyceride (mmol/L	Serum High Density Lipoprotein (mmol/L)	Serum Low Density Lipoprotein (mmol/L)
NC + OL	2.30 ± 0.06^{a}	0.58 ± 0.08^{a}	1.26 ± 0.05^{a}	0.92 ± 0.07^{a}
DC+OL	2.70 ± 0.09^{b}	1.36 ± 0.09^{b}	0.86 ± 0.06^{b}	1.57 ± 0.10^{b}
D+LYC10mg/kg	2.54 ± 0.05^{b}	1.00 ± 0.07^{c}	1.18 ± 0.05^{c}	1.16 ± 0.06^{c}
D+LYC 20 mg/kg	2.30 ± 0.09^{c}	0.72 ± 0.06^{c}	1.26 ± 0.16^{c}	0.89 ± 0.20^{d}
D+LYC 40 mgkg	2.30 ± 0.08^{c}	0.52 ± 0.04^{c}	1.44 ± 0.17^{c}	0.53 ± 0.22^{d}
D+ GLB 2 mg/kg	2.20 ± 0.13	0.54 ± 0.07^{c}	0.98 ± 0.08^{b}	1.11 ± 0.05^{c}

^{a,b,c,d} = Means on the same column with different superscript letters differ significantly

($P < 0.05$) compared with the control groups

DC+OL = Diabetic rats treated with olive oil, NC+ OL = Normal (Non-diabetic) rats treated with olive oil, D+LYC10 mg/kg b w = Diabetic rats treated with 10 mg/kg b w of lycopene, D+LYC 20 mg/kg b w = Diabetic rats treated with 20 mg/kg b w of lycopene, D+LYC 40 mg/kg b w = Daibetic rats treated with 40 mg/kg b w of lycopene and D+GLB 2 mg/kg bw = Diabetic rats treated with Gilbenclamide 2 mg/kg b w.

Appendix 4: Effects of Lycopene Administration on Serum Cortisol, Malondialdehyde Concentration and Some Endogenous Antioxidant Enzymes activities in Streptozotocin-induced Diabetic Wistar Rats

Treatment Groups	Serum Cortisol (µg/dL)	Seerum Malondialdehyde Concentration (nmol/ml)	Serum Superoxide Dismutase (U/ L)	Serum Catalase (U/ L)	Serum Gluthathione Peroxidase (U/L)
NC + OL (0.5 ml)	8.58 ± 1.05^{a}	1.84 ± 0.08^{a}	2.14 ± 0.07^{a}	51.60 ± 0.81^{a}	45.20 ± 1.16^{a}
DC + OL (0.5 ml)	12.48 ± 0.33^{b}	2.80 ± 0.07^{b}	1.64 ± 0.08^{b}	43.20 ± 1.16^{b}	37.20 ± 1.11^{b}
D+ LYC10 mg/kg	5.36 ± 0.45^{b}	2.22 ± 0.07^{b}	1.90 ± 0.05^{b}	46.60 ± 1.503^{b}	40.00 ± 2.09^{b}
D+ LYC 20 mg/kg	6.42 ± 0.48^{b}	1.76 ± 0.20^{c}	2.14 ± 0.09^{c}	50.40 ± 1.75^{b}	43.80 ± 2.04
D+ LYC 40 mg/kg	8.86 ± 0.44^{b}	1.52 ± 0.19^{c}	2.40 ± 0.14^{c}	55.20 ± 1.43^{c}	47.60 ± 0.68^{c}
D+ GLB 2 mg/kg	7.12 ± 0.96^{b}	1.80 ± 0.07^{c}	2.28 ± 0.12^{c}	52.40 ± 0.87^{c}	46.20 ± 0.58^{c}

^{a,b,c,d} = Means on the same column with different superscript letters differ significantly ($P < 0.05$) compared with the control groups

DC+OL = Diabetic rats treated with olive oil (0.5 ml), NC+ OL = Normal (Non-diabetic) rats treated with olive oil (0.5 mL) D+ LYC10 mg/kg b w = Diabetic rats treated with 10 mg/kg b w of lycopene, D+ LYC 20 mg/kg b w = Diabetic rats treated with 20 mg/kg b w of lycopene, D+ LYC 40 mg/kg b w = Daibetic rats treated with 40 mg/kg of lycopene and D + GLB 2 mg/kg b w = Diabetic rats treated with Gilbenclamide 2 mg/kg.

Appendix 5: Effects of Lycopene Administartion on Malondialdehyde Concentration and Some Endogenous Antioxidant Enzymes Activities in Kidney of Streptozotocin-induced Diabetic Wistar Rats

Treatment Groups	Malondialdehyde Concentration (nmol/mg protein)	Superoxide Dismutase (U/mg protien)	Catalase (U/mg protein)	Gluthathione Peroxidase (U/mg protein)
NC+OL (0.5 ml)	1.60 ± 0.15^{a}	2.42 ± 0.10^{a}	52.20 ± 1.39^{a}	46.62 ± 0.66^{a}
DC + OL (0.5 ml)	2.45 ± 0.10^{b}	1.66 ± 0.05^{b}	43.20 ± 1.16^{b}	36.00 ± 0.84^{b}
D+ LYC10 mg/kg	1.80 ± 0.19^{b}	1.80 ± 0.08^{c}	44.80 ± 0.90^{b}	41.20 ± 0.50^{a}
D+ LYC 20 mg/kg	1.36 ± 0.18^{c}	2.08 ± 0.21^{c}	47.80 ± 0.90^{b}	46.00 ± 1.79^{c}
D+ LYC 40 mg/kg	1.42 ± 0.12^{c}	2.32 ± 0.12^{c}	52.60 ± 1.86^{b}	49.20 ± 1.80^{ca}
D+ GLB 2 mg/kg	1.44 ± 0.11^{c}	2.22 ± 0.06^{c}	52.00 ± 0.95^{c}	46.80 ± 0.97^{c}

^{a,b,c,d} = Means on the same column with different superscript letters differ significantly ($P < 0.05$) compared with the control groups

DC+OL = Diabetic rats treated with olive oil (0.5 ml), NC+ OL = Normal (Non-diabetic) rats treated with olive oil (0.5 ml) D+ LYC10 mg/kg b w = Diabetic rats treated with 10 mg/kg b w of lycopene, D+ LYC 20 mg/kg b w = Diabetic rats treated with 20 mg/kg b w of lycopene, D+ LYC 40 mg/kg b w = Daibetic rats treated with 40 mg/kg of lycopene and D + GLB 2 mg/kg b w = Diabetic rats treated with Gilbenclamide 2 mg/kg.

Appendix 6: Effects of Lycopene Administration on Erythrocyte Osmotic Fragility in Streptozotocin-induced Diabetic Wistar Rats

Treatment Groups	Percentage Nacl Concentration					
	0.0	0.1	0.3	0.5	0.7	0.9
NC+ OL (0.5 ml)	100.00 ±0.0[a]	100.00 ± 0.0[a]	89.60 ±1.17[a]	8.20 ± 0.74[a]	2.00 ± 0.32[a]	1.20 ± 0.20[a]
DC+ OL (0.5ml)	100.00 ± 0.0[a]	100.00 ± 0.0[a]	98.40 ±0.51[a]	32.00 ±1.00[b]	11.80 ± 0.66[b]	7.60 ± 0.51[b]
LYC + 10 mg/kg	100.00 ± 0.0[a]	100.00 ± 0.0[a]	97.60 ±1.17[a]	12.20 ± 0.66[c]	3.00 ± 0.45[c]	1.60 ± 0.25[b]
LYC + 20 mg/kg	100.00 ± 0.0[a]	100.00 ± 0.0[a]	94.00 ±1.82[a]	8.80 ± 0.37[c]	2.00 ± 0.32[c]	1.80 ± 0.49[b]
LYC + 40 mg/kg	100.00 ± 0.0[a]	100.00 ± 0.0[a]	86.20 ±1.49[b]	8.40 ± 0.75[c]	1.60 ± 0.25[c]	1.40 ± 0.25[b]
GLB 2mg/kg	100.00 ± 0.0[a]	100.00 ± 0.0[a]	80.40 ± 6.52[b]	10.6 ± 1.25[c]	3.40 ± 0.75[c]	1.40 ± 0.25[b]

[a,b,c,d] = Means on the same column with different superscript letters differ significantly ($P < 0.05$) compared with the control groups

DC+OL = Diabetic rats treated with olive oil (0.5 ml), NC+ OL = Normal (Non-diabetic) rats treated with olive oil (0.5 mL) D+ LYC10 mg/kg = Diabetic rats treated with 10 mg/kg of lycopene, D+ LYC 20 mg/kg = Diabetic rats treated with 20 mg/kg of lycopene, D+ LYC 40 mg/kg = Daibetic rats treated with 40 mg/kg of lycopene and D+ GLB 2 mg/kg = Diabetic rats treated with Gilbenclamide 2 mg/kg.