EFFECTS OF ETHANOL EXTRACT AND FRACTIONS OF *Chrysophyllum albidum* STEM BARK ON BIOCHEMICAL STATUS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

BY

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UNIVERSITY OF BENIN

BENIN CITY

MARCH, 2026

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A THESIS SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF PHILOSOPHY IN BIOCHEMISTRY OF THE UNIVERSITY OF BENIN, BENIN CITY

MARCH, 2026

CERTIFICATION

We certify that this study was carried out by **Steve Osagie ASUELIMEN**

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(Head of Department)

CERTIFICATION OF THESIS

We, the undersigned attest and declare that the thesis of **Steve Osagie ASUELIMEN** titled, "Effects of Ethanol Extract and Fractions of *Chrysophyllum albidum* Stem Bark on Biochemical Status in Streptozotocin-Induced Diabetic rats" has successfully passed the anti-plagiarism test and does not violate any copyright regulations

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Prof. E. Chukwu Onyeneke **Date**

(Head of Department)

DEDICATION

This research is dedicated to Almighty God, for His goodness and mercy towards me.

I also dedicate this work to my father, Late Mr. P. O. Asuelimen and my mother, Mrs. F. O. Asuelimen, for their unwavering support towards my education.

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ABSTRACT

Diabetes mellitus (DM), a metabolic disease characterized by chronic hyperglycemia causes damage to important tissues and organs (heart, liver, blood vessels, kidneys and nerves). The aim of this study was to evaluate the effect of ethanol extract of *Chrysophyllum albidum* stem bark and its fractions on some biochemical status in streptozotocin (STZ)-induced diabetic rats. Crude ethanol extract prepared from pulverised stem bark of the plant was fractionated using analytical grade solvents (n-hexane, ethylacetate, and methanol). Adult male rats (Wistar strain, n = 56) weighing between 150 and 200 g were randomly assigned to eight groups (7 rats/group): control, diabetic, metformin, extract, and hydroethanol, n-hexane, ethyl acetate and methanol fractions. With the exception of control group, DM was induced in the rats via intraperitoneal injection of STZ (50 mg/kg body weight). This was followed by treatment (daily) with either metformin, ethanol extract or fractions of *C. albidum* stem bark for 14 days. At the expiration of the treatment period, plasma/tissue samples obtained from the rats were used for biochemical analyses. Blood glucose concentration and body weight were monitored on weekly basis. Indices of liver and kidney function; oxidative status in selected tissues (plasma, liver, kidney, pancreas), lipid peroxidation index and haematological parameters were measured. The results obtained showed that induction of DM with STZ significantly increased fasting blood glucose (FBG) level, organ weights (liver, kidney), malondialdehyde (MDA), indices of liver and kidney function, lipid profile, and some haematological parameters (white blood cell, lymphocyte, granulocyte, mid-cell), but it decreased the activities/levels of hepatic/renal/pancreatic antioxidant enzymes [catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx)], body weight, pancreas weight, plasma total protein, albumin, and electrolytes (Na^+, K^+, HCO_3^-), high-density lipoprotein cholesterol (HDL-C), platelet count, and red blood cell indices when compared to control ($p < 0.05$). However, treatment with *C. albidum* stem bark extract/fractions markedly reduced FBG level, organ weights (liver, kidney), and MDA, but it enhanced the activities of the antioxidant enzymes and pancreas weight ($p < 0.05$). Similarly, extract/fractions treatment improved lipid profile and haematological parameters, while restoring indices of liver and kidney function. The results were comparable to those of metformin (standard drug). Histopathological examination of pancreatic and liver tissues of diabetic untreated rats showed cell reduction of the islet of Langerhans and pancreatic acini, congestion of the central vein, as well as loss of normal hepatocytic architecture, indicating severe pancreatic and liver damage. However, treatment with *C. albidum* extract/fractions revealed improvement in liver/pancreas histology. Histopathological examination of pancreatic and liver tissues further supported the biochemical findings. The results obtained in this study have shown that ethanol extract of *C. albidum* stem bark and its fractions can markedly reduce typical derangements associated with STZ-induced diabetes mellitus (that is, hyperglycaemia, hyperlipidemia, hepatotoxicity, nephrotoxicity, and oxidative stress.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Diabetes mellitus (DM) is a metabolic disease marked by chronic hyperglycemia as a result of the inability of pancreatic beta cells to produce insulin or due to insufficient insulin production, coupled with insulin resistance by tissues (Afroz *et al.,* 2019). Persistent hyperglycemia, when left untreated, can cause damage, dysfunction, and failure of various organs: pancreas, heart, liver (hepatopathy), kidney (nephropathy), eyes (retinopathy), nerves (neuropathy), and blood vessels (Akomolafe *et al*., 2019). Diabetes mellitus is the leading cause of death and disability worldwide, affecting people of all ages, sexes, and regions. Many patients with DM may present no symptoms, particularly in the onset of the condition. Typical symptoms of DM include polydipsia (excessive thirst), polyuria (excessive urination), polyphagia (excessive hunger/appetite), unexplained weight loss, blurred vision, and slow healing of sores (Riaz, 2009). Different types of DM exist; however, the major types are type 1, or insulin-dependent diabetes mellitus (IDDM); type 2, or noninsulin-dependent diabetes mellitus (NIDDM); and gestational diabetes, alternatively referred to as pregnancy-induced DM. Type 1 diabetes mellitus (T1DM) has been linked to autoimmune destruction of pancreatic β-cells (Ahmad *et al.,* 2022). This condition causes complete lack of insulin (or insulin deficiency) due to autoimmune destruction of pancreatic β-cells (American Diabetes Association, 2021). Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance, coupled with impaired insulin secretion by pancreatic β-cells (Kahn, 2008). Type 2 DM constitutes 85 – 95 % of all diabetic cases worldwide. Having a vital genetic component, it is strongly linked to obesity and sedentary lifestyle (Lancet, 2023). The pathogenesis of T2DM has been linked to hereditary and environmental factors (Lancet,

2023). Gestational diabetes is a metabolic disorder marked by hyperglycemia in pregnant women. Both mother and baby are impacted when it happens exclusively in some pregnant individuals. Gestational diabetes has been linked to risk factors, including maternal age, family history, and obesity. It is associated with T2DM and ischemic heart disease (Li *et al.,* 2020). Typically, individuals with no history of DM prior to pregnancy are diagnosed with gestational diabetes during the fifth to sixth week or seventh to ninth week of pregnancy. Often associated with increased risk of complications during pregnancy and delivery, gestational diabetes can affect both the mother and fetus (Sun *et al.,* 2022).

The prevalence of DM is greatly influenced by lifestyle modification (change from traditional to western-type form of living). In 2021, it was estimated that 240 million people were living with undiagnosed DM (Magliano and Boyko, 2021). The International Diabetes Federation (IDF) Diabetes Atlas report of 2017 documented that about 451 million individuals have DM worldwide, and it was projected that the cases could rise to about 693 million by the year 2045 (AlBadri *et al*., 2018). In Nigeria, the prevalence stands at 5.77 % of the population, with many being diagnosed at much younger ages (less than 45 years) (American Diabetes Association, 2009). Rising cases of DM are linked with changing dietary patterns, increased alcohol intake, and stressful lifestyles (Anwar and Meki, 2003).

As a pathological hallmark of DM, insulin resistance is a condition wherein the body fails to respond normally to insulin (Acharya and Ghaskadbi, 2010). Muscle and fat cells are early responders to insulin resistance as they promote the generation of reactive oxygen species (ROS) due to hyperglycemia (Asagba *et al.,* 2019). Although ROS play a crucial role in cell signaling and defense mechanisms, their excessive accumulation in tissues has been linked to oxidative stress, a condition associated with various diseases and ageing (Hong *et al.,* 2024). Oxidative

stress caused by free radicals has been linked to several diseases, including DM (Paul *et al.,* 2015). In most developing countries characterized by high level of poverty, the majority of the populace go through stressful lifestyles thus predisposing them to oxidative stress, which has been implicated in diabetic micro- and macro-vascular complications (Zelzer *et al*., 2018). Studies have demonstrated the involvement of oxidative stress in the pathogenesis of diabetic complications (Papachristoforou *et al.,* 2020; Pisochi *et al.,* 2021). Bobadoye *et al*. (2016) reported that insulin resistance in humans is associated with reduced intracellular antioxidant defense. Decreasing oxidative stress with antioxidant compounds has been shown to prevent the development of DM and its complications (Li *et al*., 2017).

Streptozotocin is a broad-spectrum antibiotic produced by *Streptomyces achromogens* bacteria (Akinlade *et al*., 2021). It is an analogue of glucose that is selectively accumulated in pancreatic β-cells via glucose transporter 2 (GLUT-2) in the pancreatic plasma membrane (Al Nahdi *et al.,* 2017). It is diabetogenic due to its targeted GLUT 2-dependent action in the pancreatic β-cells (Hosokawa *et al.,* 2001). After entering pancreatic β-cells via GLUT 2 transporter, it causes DNA damage due to DNA alkylating activity of its methyl nitrosourea moiety (Ghasemi *et al.,* 2014), which results to DNA fragmentation (Al Nahdi *et al.,* 2017). The broken DNA stimulates the enzyme (poly ADP-ribose synthetase) to fix the damage DNA. Poly ADP-ribosylation causes depletion of cellular NAD^+ and ATP (Andrabi *et al.,* 2014). Decreased ATP synthesis is demonstrated by dephosphorylation, providing more substrates for xanthine oxidase reaction, resulting in the build-up of hydrogen peroxide and hydroxyl radicals (Al Nahdi *et al.,* 2017), which induced-oxidative stress, resulting in mitochondrial dysfunction in pancreatic β-cells. This results in insulin deficiency and hyperglycemia, mimicking T1DM and, in modified protocols, T2DM (Furman, 2015). Streptozotocin-induced diabetic rat model is a commonly employed

method for investigating DM pathophysiology and potential therapeutic management (Mondal *et al.,* 2025).

Conventional anti-diabetic drugs (sulfonylureas, biguanides, alpha glucosidase-inhibiting agents, meglitinide derivatives, and thiazolidinediones) are currently used in addition to insulin therapy for the management of DM. However, their use is limited by side effects, reduced potency, high cost, and accessibility issues, particularly in developing countries (Hua, 2020; Mumtaz *et al.,* 2023). These drawbacks require an increasing search for improved and complementary therapies, particularly those derived from medicinal plants. As a result, medicinal plants with hypoglycemic potential, especially those rich in strong antioxidant compounds, could serve as useful adjuncts in the treatment of DM (Jebur *et al.,* 2016).

Chrysophyllum albidum, a fruit-bearing tree belonging to the Sapotaceae family, is primarily found in lowland rainforest areas of East and West Africa (Emudainohwo *et al.,* 2015). The fruit is known in English as African star apple; other native names in Nigeria include '*Otien*' (Edo), '*Agbalumo*' (Yoruba), and '*Udara*' (Ibo) (Houessou *et al.,* 2012). Various parts of *C. albidum* plant are used in traditional medicine. The stem bark of *C. albidum* is used in traditional medicine to treat malaria and yellow fever (Florence and Adiaha, 2015). In addition to its emollient properties, decoctions of the leaves are used for the management of diarrhoea and stomach aches. In Western Nigeria, ointments made from the seed's cotyledons and leaves are used to treat skin and vaginal infections (Emuainohwo *et al.*, 2015). Antimicrobial and wound-healing properties of *C. albidum* extracts (seeds and roots) have been reported (Okoli and Okere, 2010). The leaves, stem bark, and fruit are reportedly used for the management of DM, blood pressure, malaria, and ulcer (Ibrahim *et al*., 2017). *Chrysophyllum albidum* fruit is a popular nutritious snack that is relished by the young and old as it is believed to have health-promoting

properties (Ihekwereme *et al*., 2017). However, its anti-diabetic property has not been fully explored.

1.2 Statement of Problem

As a growing disorder with several associated complications, DM causes mortalities, especially among elderly people. It constitutes an alarming public health problem, worldwide. The most frequent complications are retinopathy, nephropathy, neuropathy, myocardial infarction, and premature atherosclerosis. There is a growing need for more research to understand the genetic, environmental, and socioeconomic factors that contribute to the disease's progression as well as the effectiveness of different treatment modalities.

1.3 Justification of the Study

Diabetes mellitus (DM) remains a marked world health challenge, with the disease prevalence continuing to rise. Management of DM with conventional anti-diabetic drugs such as sulfonylureas, biguanides, alpha glucosidase-inhibiting agents, meglitinide derivatives, and thiazolidinediones often has limitations such as side effects, reduced potency, high cost, and accessibility issues, particularly in developing countries. These drawbacks require an increasing search for improved and complementary therapies, especially those derived from medicinal plants. Despite the use of *C. albidum* in ethnomedicine, only a few studies are available on the antidiabetic potential of the plant's stem bark.

1.4 Aim and Objectives

The aim of the study was to evaluate the effects of ethanol extract of *Chrysophyllum albidum* stem bark and its fractions on biochemical status in STZ-induced diabetic rats.

The Specific Objectives were to:

1. extract *C. albidum* stem bark with ethanol and fractionate the crude extract using methanol, n-hexane and ethyl acetate.
2. induce DM in experimental rats using STZ (50 mg/kg bwt) and treat the rats with the extract and fractions of *C. albidum* stem bark
3. measure blood glucose levels, haematological parameters, liver and kidney function indices in the rats
4. measure oxidative stress, lipid peroxidation markers in tissue homogenates/plasma and subject portions of excised rat tissues to histopathological examination.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Chrysophyllum albidum*

Chrysophyllum albidum is a medicinal plant commonly called African white star apple. It belongs to the family of Sapotaceae (which has up to 800 species); it is a lowland rainforest tree that grows up to 25 to 37 m in height at maturity (Adebayo *et al.,* 2011). The Scottish botanist George Don described it as a forest fruit tree (Ehiagbonare *et al.,* 2008). It is common throughout the tropical Central, East and West African regions and other parts of the world (Amusa *et al.,* 2013). At maturity, the fruit is round in shape and has an acute tip. It can be 6 cm long and 5 cm wide. When ripe, it may be a pinkish or light-yellow within the peel, which contrasts with the orange to golden-yellow skin. Three or five seeds, which are typically not consumed, are contained within the pulp. When cracked open, the dark brown seed coats expose the white cotyledons beneath. In addition to being opaque, the seed coverings are hard, bony, and shiny. The fruit is only available from December to March. The plant has economic importance in Nigeria (Oboh *et al.,* 2009). The seeds can be saved for future use in neighbourhood tournaments or thrown away. The fruit pulp is useful for jams and is eaten as a snack by many locals (Amusa *et al.,* 2013). Known as African star apple in English, other native names of *C. albidum* in Nigeria include *Otien* (Edo), *Agbalumo* (Yoruba), and *Udara* (Ibo) (Houessou *et al*., 2012).

2.1.1 Scientific Classification of *C. albidum*

Kingdom: Plantae Family: Sapotaceae

Division: Angiosperm Genus: *Chrysophyllum*

Class: Eudicots Species: *C. albidum*

Order: Ericales

Figure 2.1: *Chrysophyllum albidum* showing stem, leaves and fruits

Source: (Ibrahim *et al*., 2017).

2.1.2 Ethno-Medicinal Uses of *C. albidum*

Traditional medicine uses the stem bark of *Chrysophyllum albidum* to cure malaria and yellow fever (Florence and Adiaha, 2015). In addition to its emollient properties, the leaf has medicinal uses in treating diarrhoea and stomachaches. According to Emuainohwo *et al.* (2015), ointments made from the seed's cotyledons and the leaf are used to treat skin and vaginal infections in Western Nigeria. Anti-bleeding, anti-contaminant, and wound-healing properties of *C. albidium* extract (seeds and roots) have been reported (Okoli and Okere, 2010).

2.1.3 Phytochemical Composition of *Chrysophyllum albidum*

Study on the phytochemical composition of *Chrysophyllum albidum* showed that the stem bark, seed cotyledon, leaves, and root contained tannins, phenols, flavonoids, alkaloids, and cardiac glycosides (Okoli and Okere, 2010). Various bioactive compounds (saponins, alkaloids, flavonoids, phenols, glycosides, terpenoids and steroids) were also identified in methanol leaf extracts from *C. albidum* (Ushie *et al.,* 2014). Previous research conducted by MacDonald *et al.* (2014) also revealed the presence of alkaloids, flavonoids, saponins and tannins in *C. albidum*. The methanolic extract of the cotyledon seeds of *C. albidum* contains skatole, tetrahydro-2-methylharman, and eleagnine (Amusa *et al.,* 2013). The fruit has a concentration of vitamin C per 100 g, which is 10 % higher than *Psidium guajava* (guava) and *Anacardium occidentale* (cashew) and 10 % higher than *Citrus sinensis* (oranges). In addition to adding flavour and vitamins to food, *C. albidum* is a great source of iron and other minerals (Adisa, 2000). The fruits are a beneficial source of resin and wood protector since they contain 90% anacardic acid. Previous study reported by Emudainohwo *et al.* (2015) revealed that *C. albidum* contains 74% of unsaturated fatty acids as the main oil components.

2.1.4 Nutritional Components of *Chrysophyllum albidum*

Research conducted by Imaga and Urua (2013) on the nutritional components of *C. albidum*

revealed that ethanol and aqueous extracts of *C. albidum* contain 48.38% and 47.02% moisture, 2.75 and 2.68% crude protein, 24.26% carbohydrates, 4.175 and 4.68% ash, 10.94% crude fat, and 208.53 and 206.50 kcal of energy value, respectively. In 100 g sample of *C. albidum* extract, the following minerals were found: potassium (2.05), iron (42.45), zinc (34.45), magnesium (34.05), calcium (24.55), manganese (4.1), and sodium (123.05). In mg/100 g, *C. albidum* contained vitamin K (35.36), vitamin B1 (18.68), folate (2.02), vitamin C (3.084), and vitamin B6 (3.26). Quantitative analysis of *C. albidum* revealed the presence of carbohydrate (11%), crude fibre (4%), lipids (3%), protein (7%), calcium (17.11 ppm), iron (<1 ppm), phosphorus (9.92 ppm), vitamin C (25.03 ppb), A (10.74 ppb), and B1 and B2 (<1 ppb) (Ureigho and Ekeke, 2010). *Chrysophyllum albidum* fruit samples (both untreated and treated with 2, 4-dichlorophenoxyacetic acid) showed the presence of K, P, S, Ca, Mg, Fe, Al and Zn (Chukwuemeka, 2006).

2.1.5 Pharmacological Activities of *C. albidum*

2.1.5.1 Antioxidant Potential

The antioxidant potential of ethanol extract/fractions of *Chrysophyllum albidum* leaves was investigated (DPPH free radical scavenging activity) by employing the *in vitro* and *in vivo* experimental models (Adebayo *et al.*, 2011). The study showed marked variations in catalase activity, malondialdehyde (MDA) and glutathione (GSH) levels. *Chrysophyllum albidum* has antioxidant potential by scavenging free radicals, decreasing lipid peroxidation and increasing the endogenous antioxidant enzymes levels (Adebayo *et al.*, 2011). *Chrysophyllum albidum* has natural antioxidant boosters and has been used for the treatment of some free radicals implicated oxidative stress disorders (Abiodun *et al.,* 2011). Idowu *et al.* (2006) also reported that eleagnine, an alkaloid isolated from *C. albidum* seed cotyledon has antioxidant activities.

2.1.5.2 Antidiabetic and Hypolipidemic Potential

The anti-hyperglycemic and hypolipidemic effect of ethanol extract of *Chrysophyllum albidum* seed cotyledon and leaf have been evaluated (Olorunnisola *et al.,* 2008; Adebayo *et al.,* 2010). The study on the antidiabetic and hypolipidemic effects of ethanol extract of *Chrysophyllum albidum* seed cotyledons in alloxan-induced diabetic rats showed that administering the extract (100 and 200 mg/kg orally) for seven days significantly decreased blood glucose levels and restored lipid profile parameters in the treated diabetic rats when compared with the diabetic control (Olorunnisola *et al.,* 2013). Additionally, the ethanol extract of *C. albidum* root bark demonstrated moderate antidiabetic and hypolipidemic effects in alloxan-induced diabetic rats (Onyeka *et al.,* 2013).

2.1.5.3 Hepatoprotective Effect

Hepatoprotective activity of *Chrysophyllum albidum* leaves extract against carbon tetrachloride (CCl_4)-induced liver damage in rats has been reported by Abiodun *et al.* (2011). *Chrysophyllum albidum* significantly decreased the elevated liver enzymes (AST, ALT, ALP) and total bilirubin level in rats treated with carbon tetrachloride (Abiodun *et al.*, 2011). Histopathological examinations of hepatic tissue revealed the protective effect of *C. albidum* extract against carbon tetrachloride-induced centrilobular fatty degeneration and necrosis in rats (Emudainohwo *et al.,* 2015). Previous study reported by Ibrahim *et al.* (2011) also showed restoration of elevated levels of liver enzymes (AST, ALT, ALP) in plasma of diabetic rats treated with *C. albidum* supplementation.

2.1.5.4 Antimicrobial Properties

The antimicrobial potential of *C. albidum* has been linked to its wide range of phytochemicals (Hochma *et al.,* 2021). *Chrysophyllum albidum* possesses numerous phytochemicals that elicit

antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa, Escherichia coli* and *C. tetani,* as well as an antifungal effect against *Candida albicans* (Okoli and Okere, 2010). The majority of the phytochemicals in *C. albidum* are terpenoids. Cox-Georgian *et al.* (2019) and Guimarães *et al.* (2019) reported their antibacterial and antiseptic effects. Previous studies have shown the antimicrobial properties of chloroform and ethanol extracts of *C. albidum* root bark against the test isolates (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, Aspergillus niger*, *Penicillium notatum, Mucormucedo and Candida albicans)* (MacDonald *et al.*, 2014). Antimicrobial effect of *C. albidum* leaf extract on gastrointestinal tract pathogenic bacteria and fungi in humans was also investigated by Ajetunmobi and Towolawi (2014). Findings revealed that the extract has broad-spectrum antibiotic and antimicrobial activities.

2.2 Diabetes Mellitus (DM)

Diabetes mellitus is a major chronic metabolic disorder marked by hyperglycemia, caused by the inability of pancreatic beta cells to produce insulin or insulin resistance in tissues (Afroz *et al.,* 2019). Insulin, as an anabolic hormone, plays a major role in protein, carbohydrate, and lipid metabolism (Poznyak *et al.,* 2020). Insulin facilitates the uptake of glucose from the bloodstream into cells, liver, skeletal muscle, and adipose tissue (Honka *et al.,* 2018). Living things derive a significant amount of energy in the form of ATP from glucose metabolism. Insulin secretion and its ability to effectively drive glucose absorption into peripheral tissue are important for insulin to maintain blood glucose homeostasis.

2.2.1 Epidemiology of Diabetes Mellitus

Diabetes mellitus (DM) is a progressive and metabolic disorder marked by elevated plasma glucose. Before the development of systemic population-based studies, there were difficulties in

estimating DM prevalence in the United States and globally (Mekala and Bertoni, 2020). As the global incidence of obesity and bad lifestyles continues, DM is on the rise in every country. The recent estimations show that the prevalence rate of DM was 11.1 % in 2019, and it is projected to rise to 13 % by the year 2045. Africa has the lowest prevalence rate (4.7 %), with an expected increase to 5.2 % in 2045 (Saeedi *et al.,* 2019). Globally, South-East Asia and South America have either high or intermediate incidences (Saeedi *et al.,* 2019). According to Saeedi *et al.* (2019), a global prevalence of 463 million individuals with DM was documented, which accounts for a 9.3 % prevalence rate globally. The prevalence rate by 2030 and 2045 is estimated to be 10.2 % and 10.9 %. The region-stratified prevalence of diabetes mellitus (DM) has been determined for many countries, highlighting those with the highest number of diabetic patients globally in 2019 (International Diabetes Federation, 2019). China has the highest percentage of diabetic patients among the top 10 countries, with 116 million. India and the United States of America follow with 77 million and 31 million, respectively, indicating that the United States is one of the countries with the highest risk of developing DM in the future. The countries that are projected to be the high-end diabetic groups with diabetic patients are Mexico, Brazil, and Pakistan (12, 16, and 19 million), respectively.

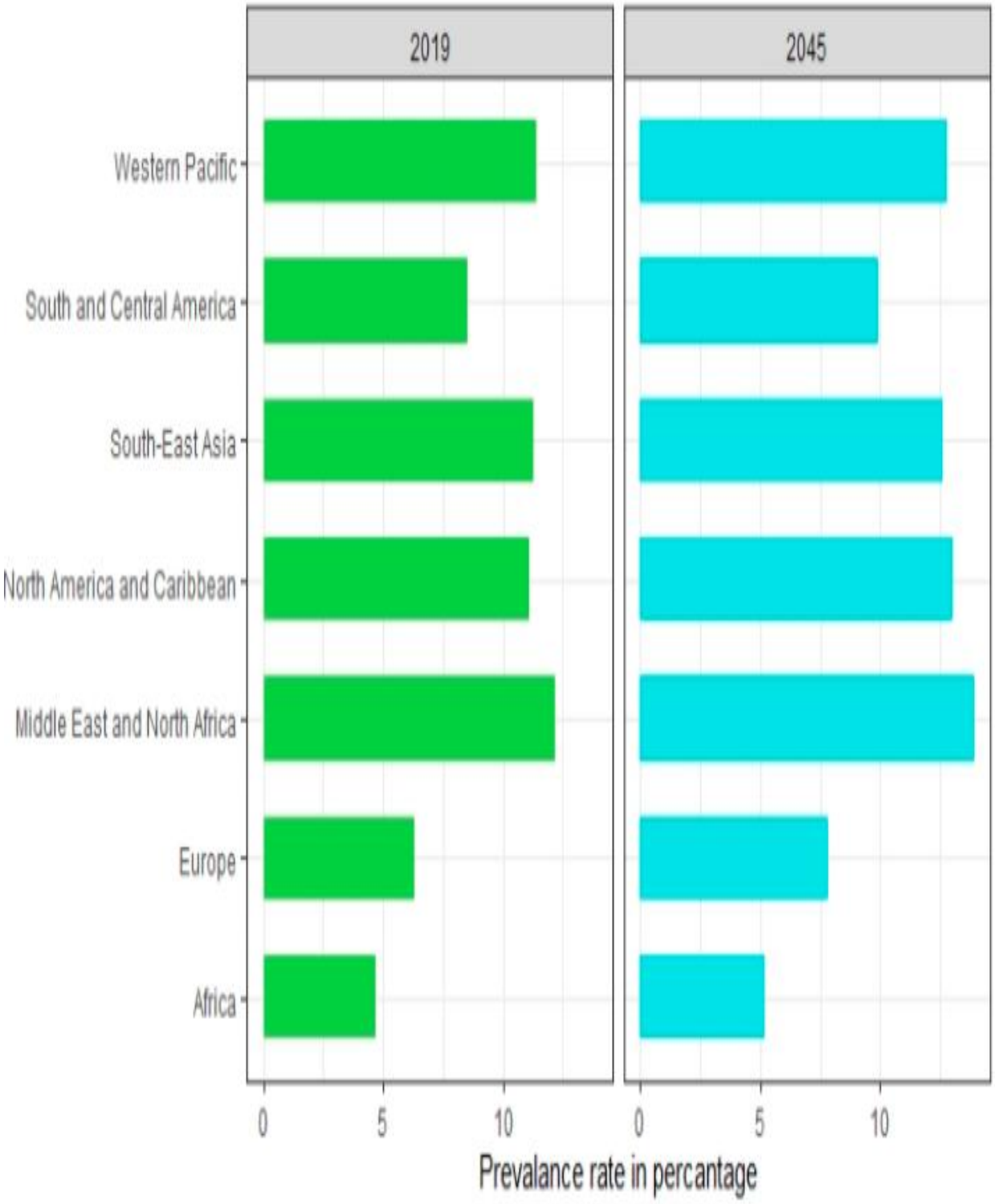

Figure 2.2: Prevalence of DM by global regions by 2019 and 2045 (estimated).

Source (Alam *et al.,* 2021)

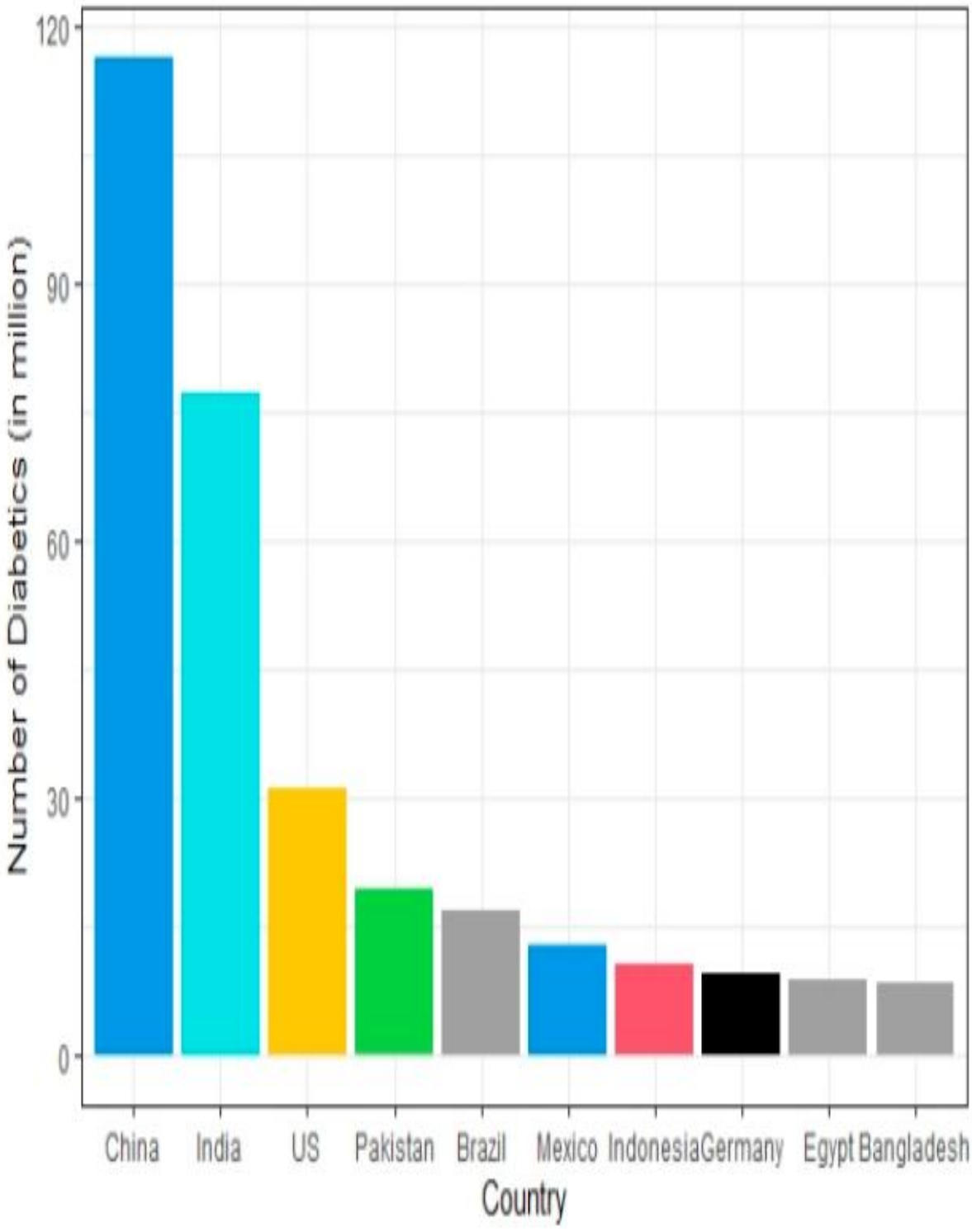

Figure 2.3: Countries with the highest number of diabetic patients worldwide in 2019

Source (Alam *et al.,* 2021)

2.2.2 Classification of Diabetes Mellitus (DM)

Among various types of DM, the most common ones are type 1 (T1DM), type 2 (T2DM), and gestational diabetes (also known as pregnancy-induced diabetes). Type 1 diabetes mellitus (T1DM) has been linked to autoimmune destruction of pancreatic β-cells (Ahmad *et al.,* 2022). This condition causes complete lack of insulin (or insulin deficiency) due to autoimmune destruction of pancreatic β-cells (American Diabetes Association, 2021). It usually occurs during childhood or puberty and symptoms develop rapidly. It occurs in patients with little or no endogenous insulin secretory potential and require insulin injection for survival while type 2 DM (T2DM) is marked by insulin resistance coupled with inability of pancreatic β-cells to produce appropriate quantities of insulin to overcome the insulin resistance (Gieroba *et al.,* 2025). The majority of people with DM have type 2 (T2DM), which is often linked to being overweight (obesity). It occurs after age 35 and symptoms develop gradually. Gestational diabetes is a metabolic disorder marked by hyperglycemia in pregnant women.

2.2.2.1 Type 1 Diabetes Mellitus (Insulin-Dependent Diabetes Mellitus)

Insulin dependent diabetes mellitus or Type 1 diabetes is a persistent autoimmune attack marked by insulin deficiency and persistence hyperglycemia (DiMeglio *et al.,* 2018). It is caused by autoimmune destruction of the pancreatic beta cells, which will in turn leads to absolute insulin deficiency (American Diabetes Association, 2021). Type 1 DM (T1DM) affects individuals globally, and urgent precaution is needed to prevent serious diabetic complications such as heart attack, nephropathy, retinopathy, neuropathy and stroke. Management of T1DM is strictly on insulin therapy (insulin injection). However, this method is unable to achieve optimal plasma glucose control in many patients (Akil *et al.,* 2021).

Insulin dependent diabetes mellitus constitutes 5% - 10% of patients diagnosed with diabetes (Maahs *et al.,* 2010) and is due to damage of pancreatic β-cells (Devendra *et al.,* 2004; Daneman, 2006). Insulin dependent diabetes mellitus accounts for 80% - 90% of DM in children (Craig *et al.,* 2009; Debelea *et al.,* 2014). The number of youth age (0 - 14) diagnosed with T1DM globally in 2013 was 497100 and the number of recent diagnosed cases was 78900 (International Diabetes Federation, 2013). The high prevalence in young people and adults (14 years of age) means that these data do not represent the total number of T1DM patients. Previous study estimated T1DM in the United States in 2010 to be 3 million (Chiang *et al.,* 2014). The estimated number of youth (less than 20 years) with T1DM in the United States was 166984 in the year 2009 (Pettitt *et al.,* 2014). The prevalence of T1DM globally is unknown, however, in the United States in youth (< 20 years) was 1.93 per 1000 in 2009 with 2.6% - 2.7% relative yearly increase (Debelea *et al,* 2014; Lawrence *et al.,* 2014). Autoimmune T1DM has potent human leukocyte antigen (HLA) associations, linking the *DR* to *DQ* genes. Human leukocyte antigen (HLA)-DR/DQ alleles have the potential to be either protective or predisposing (American Diabetes Association, 2014). In addition to the relevance of genetic predisposition in T1DM, various environmental factors have been linked to the origin of the disease (Couper and Donaghue, 2009; Canivell and Gomis, 2014).

Insulin dependent diabetes mellitus often progresses suddenly and can produce symptoms (polydipsia, polyuria, enuresis, lack of energy, extreme tiredness, polyphagia, sudden weight loss, slow-healing wounds, recurrent infections, blurred vision) (International Diabetes Federation, 2013) with diabetic ketoacidosis and serious dehydration in children and adults. Symptoms manifest more severely in adolescents than in adults. Idiopathic T1DM is an uncommon type of T1DM of no cause (idiopathic); it is mild when compared with T1DM. Most patients with this

type are of African or Asian origin and suffer from various degrees of insulin deficiency and episodic ketoacidosis (Merger *et al.,* 2013). Fulminant T1DM is a distinct form of TIDM, it was first described in the year 2000, and has some similar features with idiopathic T1DM such as not being immune-mediated (Imagawa and Hanafusa, 2006). It is marked by ketoacidosis short after the onset of hyperglycemia, increase plasma glucose levels (≥ 288 mg/dL) with trace levels of serum C-peptide, a marker of innate insulin secretion (Imagawa and Hanafusa, 2011). It has been demonstrated majorly in East Asian countries and accounted for probably 20% of acute-onset T1DM patients in Japan (5000 - 7000 cases) with an extremely increase and almost total pancreatic beta-cell destruction resulting in nearly no insulin secretion (Imagawa and Hanafusa, 2011; Shibasaki *et al.,* 2012). Environmental and genetic factors, majorly viral infection, have been linked to the disease. Antiviral immune response could initiate the destruction of pancreatic β-cells via the increased immune reaction with no trace autoantibodies against pancreatic β-cells (Imagawa and Hanafusa, 2006; Imagawa and Hanafusa, 2011). Linkage of fulminant T1DM with pregnancy has been reported (Imagawa and Hanafusa, 2011).

2.2.2.2 Type 2 Diabetes Mellitus (Noninsulin-Dependent Diabetes Mellitus)

Type 2 DM is a heterogeneous metabolic disease marked by insulin resistance coupled with decrease insulin secretion by pancreatic β-cells (Kahn, 2008). The majority of cases of DM (85 – 95 %) are T2DM. The autoimmune attack of the pancreatic β-cells does not lead to T2DM, in contrast to T1DM. The three major defects in the onset of hyperglycemia in T2DM are;

(1) Increased liver glucose production

(2) Decreased insulin secretion and

(3) Impaired insulin action/insulin resistance (Stumvoll *et al.* 2005).

Delay or inability of tissues to respond to insulin is called insulin resistance. It is mostly 'post receptor' (linked with problems with insulin-responsive cells and tissues rather than insulin secretion).

There is evidence that T2DM is hereditary. Furthermore, very high agreement rates of T2DM have been reported in monozygotic twins and about 25% of those with the disease have a family history of DM (Rother, 2007). Various lifestyles are known to be linked to the development of T2DM. These includes not exercising, smoking cigarettes, and alcohol intake. Obesity has been linked to approximately 55% of cases of T2DM (Olokoba *et al.,* 2012).

2.2.2.3 Gestational Diabetes Mellitus (GDM)

Gestational diabetes is a metabolic disorder marked by hyperglycemia in pregnant women. Both mother and baby are impacted when it happens exclusively in some pregnant individuals. Obesity, family history, and age have been linked to be the cause of GDM. It is associated with T2DM and ischemic heart disease (Li *et al.,* 2020). Individuals without history of DM before pregnancy are normally diagnosed during the fourth or seventh month of pregnancy. It is the major complication of pregnancy (Simjak *et al.,* 2018). Gestational diabetes can be treated via: insulin therapy and lifestyle modification, such as nutritional therapy (Oskovi-Kaplan and Ozgu-Erdinc, 2021).

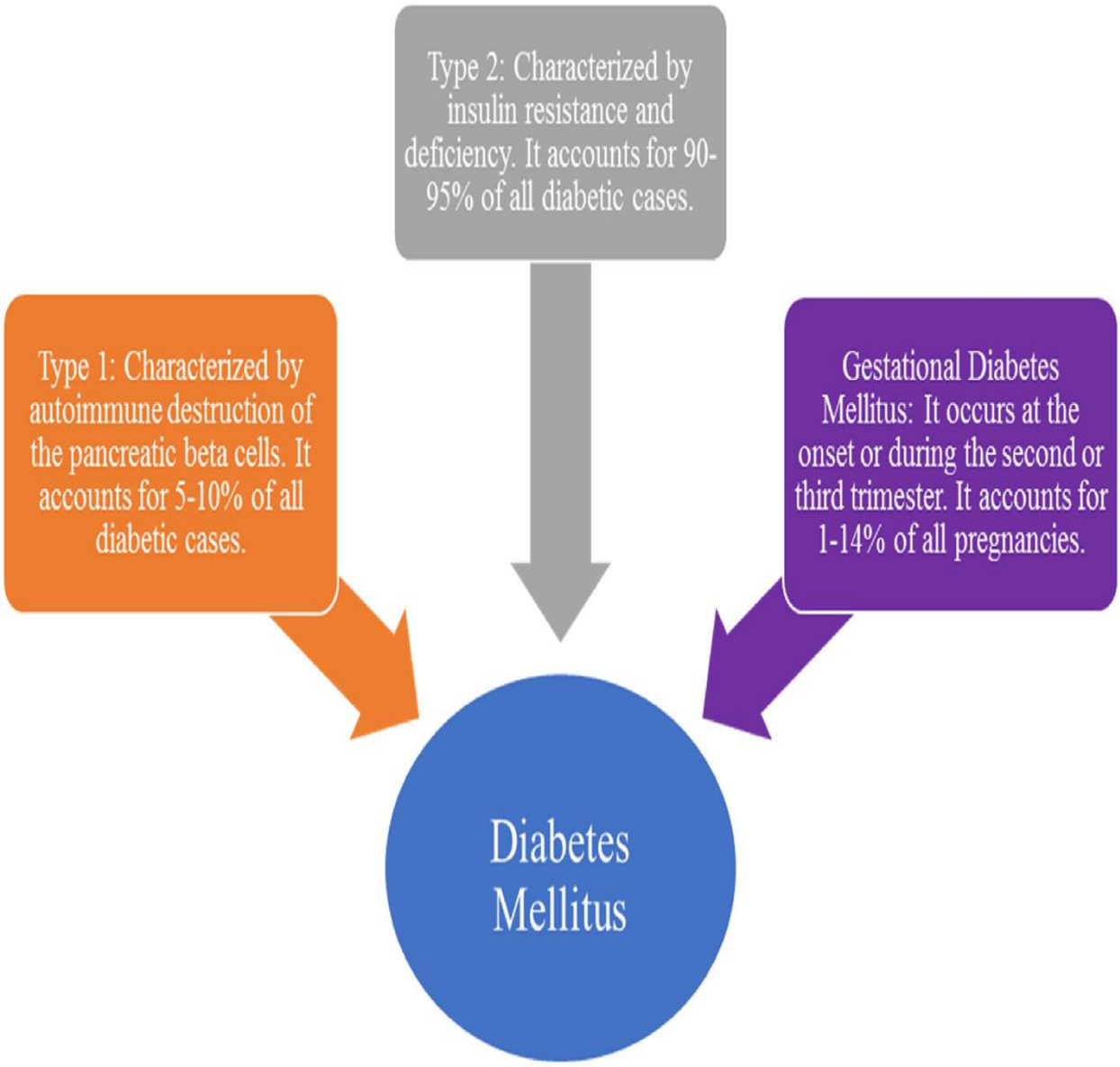

Figure 2.4: Types of Diabetes Mellitus

Source: (Ojo *et al.,* 2023)

2.2.3 Symptoms of Diabetes Mellitus

- Excessive thirst (polydipsia)
- Excessive urination (polyuria)
- Excessive hunger (Polyphagia)

- Constant weight loss
- Blurred vision
- Frequent infections (skin infections, thrush, gingivitis, and urinary tract infections)
- Slow healing of sores
- Skin problems (itchiness or acanthosis nigricans)
- Fatigue
- Dizziness/fainting
- Numbness/tingling (Riaz, 2009).

2.2.4 Diagnostic Criteria for Diabetes Mellitus

Plasma glucose estimation (FPG or OGTT) or glycated haemoglobin (HbA1c) are the two main methods used to diagnose diabetes mellitus. Determination of fasting plasma glucose (FPG), random blood glucose (RBG), oral glucose tolerance test (OGTT), and glycated haemoglobin (HbA1c) are used to establish blood glucose homeostasis. Diabetes mellitus can be diagnosed when the values of FBG, RBG, OGTT and HbA1c are greater than 126 mg/dL, 200 mg/dL, 200 mg/dL and 6.5%, respectively. In 2009, an expert in the field of diabetes studies suggested the use of HbA1c to diagnose DM in addition to monitoring DM treatment. This recommendation was also supported by the American Diabetes Association (2009), the Endocrine Society, the World Health Organisation (2011), and numerous other international scientific groups. The pros and cons of the various tests used to diagnose DM have been reviewed by Sacks *et al.* (2011). The pros of preferring HbA1c over FPG to diagnose DM include higher convenience and pre-analytical stability, lower CV (3.6%) when compared with FPG (5.7%) and 2h OGTT (16.6%), stronger association with microvascular complications majorly retinopathy, and a marker for glycemic regulation and glycation of proteins which has direct link between diagnosis of DM

and its complications (Cheng *et al.,* 2009; Davidson, 2011; Malkani and Mordes, 2011; Sacks, 2011; Shaw *et al.,* 2011; Days, 2012). It is preferable to repeat the HbA1c test in asymptomatic patients within two weeks to reaffirm a single apparently diagnostic result (McDonald and Warren, 2014). Numerous nations and ethnic groups have recommended HbA1c reference value of 6.5% (48 mmol/mol), however, the standard used to diagnose DM appear to be influenced by ethnicity (Dagogo-Jack, 2010; Ma *et al.,* 2013). Davidson (2011) reported 6.3% (45 mmol/mol) in an Egyptian study, 5.5% (37 mmol/mol) in a Japanese study, 6.0% (42 mmol/mol) in the National Health and Nutrition Examination Survey (NHANES III), 6.2% (44 mmol/mol) in a Pima Indian study, and three different reference values for Chinese (Ma *et al.,* 2013). Two reference values were suggested by the Australians (< 5.5% to "rule-out" diabetes, ≥ 7.0% to "rule-in" diabetes) (Lu *et al.,* 2010).

2.2.5 Complications of Diabetes Mellitus (DM)

Persistence hyperglycemia, when left untreated, can damage nearly every kind of cell in the body. Microangiopathic and macroangiopathic are the two major complications of DM. Diabetic retinopathy, neuropathy, hepatopathy, and nephropathy are microvascular complications of DM; atherosclerosis, stroke, and peripheral artery disease are macrovascular complications of DM (Fowler, 2008). Organs and tissues such as the eyes, liver, kidneys, nerves, heart, and blood vessels fail as a result of these vascular complications, which are chronic and persistence (Alam *et al.,* 2014). Diabetes complications provide a significant challenge to modern medicine, because they are linked to early morbidity, death, shorter life span, and high cost to diabetic patients.

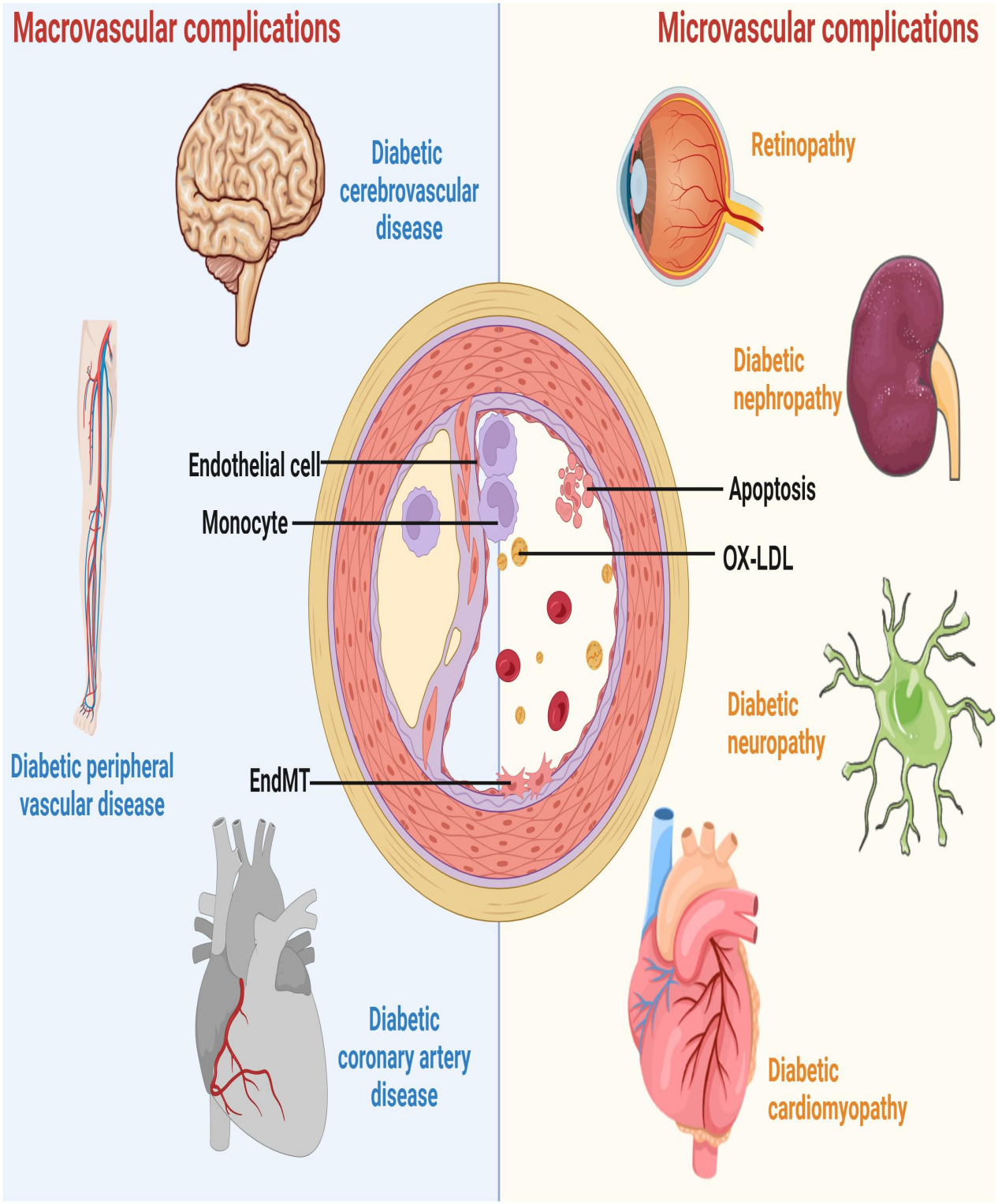

Figure 2.5: Microvascular/macrovascular complications of DM.

Source (Yang *et al.,* 2024)

2.2.5.1 Microvascular Complications of Diabetes Mellitus

2.2.5.1.1 Diabetic Retinopathy (DR)

Diabetic retinopathy, which damages the blood vessels in the retina (light-sensitive tissue), is the most prevalent microvascular complications of DM (Deshpande *et al.,* 2008). People with IDDM or NDDM are equally at risk of developing diabetic retinopathy; the likelihood of this condition worsens with time and elevated level of glycated haemoglobin, both of which point to poor glucose management (Marcovecchio *et al.,* 2011). Diabetic retinopathy is classified into two stages, non-proliferative DR and proliferative DR (Ahmed *et al.,* 2016). Chronic inflammation, stimulation of microglia, and loss of endothelial cells, nerve cells, and pericytes cause impaired vision in the early stage of DR. Furthermore, local ischaemia can progress to proliferative diabetic retinopathy, a condition that threatens eyesight (Gaddam *et al.,* 2019). Diabetic retinopathy is the major cause of adult-onset blindness globally, which is a burden because the disease can develop from mild and asymptomatic to severe and vision-threatening (Yau *et al.,* 2012). From an anticipated 127 million in 2010 to 191 million in 2030, the number of individuals with DR is expected to rise, according to Stitt *et al.* (2016). Treatment of early-stage retinopathy focuses on regulating blood sugar levels; treatment of advanced-stage retinopathy aims to slow the disease's progression and prevent vision loss (Zheng *et al.,* 2012; Gaddam *et al.,* 2019).

2.2.5.1.2 Diabetic Neuropathy (DN)

The leading cause of neuropathy globally is diabetes mellitus. About 8% of people with newly diagnosed DM will experience neuropathy, and over 50% of people with long-term diabetes will have this complication (Boulton *et al.,* 2005). Diabetes-induced neuropathy primarily affects sensory axons, although it may also affect autonomic and motor axons. However, the exact

mechanisms in which diabetes specifically targets sensory neurons are still up for debate (Feldman *et al.,* 2019). Among DN symptoms, distal symmetrical neuropathy (DSN) is the most prevalent (Callaghan *et al.,* 2012). Symptoms of DN include loss of feeling, tenderness, discomfort, and weakness that starts at the base of the foot and moves up the body in a length-dependent pattern. These symptoms are symmetrical; however, sensory symptoms are more prominent than motor involvement (Callaghan *et al.,* 2012). As DSN symptoms intensify and have adverse consequences, they can considerably diminish people's physical and emotional well-being. Distal symmetrical neuropathy-correlated numbness can induce balance problems, leading to falls (Callaghan *et al.,* 2012). There is a significant incidence of progressing lower-extremity amputations and an increased risk of ulcerations and amputations in people with severe DSN; 15% of patients experience ulcers while the disease progresses (Callaghan *et al.,* 2012). Research on the early diagnosis of DN and ways to inhibit its progression is currently restricted. Except for managing discomfort and strict control of blood glucose, no effective treatment is currently available. One possible explanation is that our understanding of the mechanisms that cause DN is still limited (Yagihashi *et al.,* 2011). Therefore, further investigation into the pathophysiology of DN could potentially facilitate the development of new therapeutic approaches.

2.2.5.1.3 Diabetic Nephropathy (DN)

Persistence chronic hyperglycemia damages and disrupts the microvasculature and renal cellular architecture, resulting in diabetic nephropathy or diabetic kidney disease (DKD), another morbid complication of DM (Sagoo and Gnudi, 2020). Metabolism, haemodynamics, intracellular signalling, and growth factors/cytokines are some of the many mechanisms that mediate these effects (Lim, 2014). The structural alterations in the nephron ultimately result in increased urine

albumin secretion, glomerular lesions, and a decline in glomerular filtration rate in diabetes (Lim, 2014). Diabetic nephropathy is a chronic and progressive condition that, due to the global diabetes epidemic, has emerged as the predominant cause of end-stage renal disease (ESRD) and the primary reason for renal replacement transplants globally (Center for Disease Control and Prevention, 2011). The economic and health burden of diabetic complications is already substantial, affecting 20–50% of diabetics; however, this number will rise as the incidence of diabetes continues to rise (Selby and Taal, 2020). The current approach to managing DN involves a multifactorial approach that focuses on lifestyle modifications, blood pressure and cholesterol control, and glycaemia control. Current treatments can slow down chronic renal disorder. These treatments include renoprotective, anti-proteinuric, and anti-hypertensive measures (Brenner *et al.,* 2001; Lewis *et al.,* 2001). The therapeutic impact of these therapies is still limited, and many diabetics will still acquire end-stage renal disease (ESRD) (Sagoo and Gnudi, 2020).

2.2.5.1.4 Diabetic Hepatopathy (DH)

People widely recognise the impact of DM on many tissues, but the hepatic tissue has received less attention. Liver impairment, or the requirement for a hepatic tissue transplant are hallmarks of diabetic hepatopathy, an advanced form of liver disease. Type 2 diabetics exhibit a broad spectrum of liver diseases, and diabetes is currently the leading cause of liver disease in the United States, according to most estimates (Tolman *et al.,* 2007). Cirrhosis, hepatocellular cancer, abnormal liver enzymes, NAFLD, cirrhosis, and acute liver failure are all part of this category (Natarajan *et al.,* 2020). Liver disease had a standardized death rate of 2.52, while CVD is 1.34. Abnormal glycogen buildup in hepatocytes characterises glycogenic hepatopathy (GH), a leading cause of fatty liver in T1DM (Saadi, 2012; Jardim *et al.,* 2013). According to Sweetser and

Kraicheyl (2010) and Saxena *et al.* (2010), hepatomegaly, nausea, vomiting, and increased liver enzymes are the symptoms of glycogen hepatopathy.

2.2.5.2 Macrovascular Complications of Diabetes Mellitus

Cardiovascular disease (CVD), cerebrovascular disease (CeVD), and peripheral arterial disease (PAD) are all examples of macrovascular disorders that impact the major blood vessels, such as arteries and veins (Dal-Canto *et al.,* 2019). About 20% to 30% of diabetic patients experience these complications, which greatly increase the morbidity and death rate associated with T1DM (Romon *et al.,* 2008; Tang *et al.,* 2016; Lee *et al.,* 2019).

As the harmful effects of DM increased globally, and new pharmacological medicines became available, the risk of CVD among diabetic people has decreased significantly in recent years. For example, in the Swedish population, the proportion of diabetic patients with modifiable CVD risks fell from 37.7% in 2003 to 19.1% in 2008, and this pattern was also seen in two other studies (Ford, 2011; Fharm *et al.,* 2012; Gregg *et al.,* 2012). The United Kingdom (UK) prospective diabetes research algorithms found that diabetic patients had an estimated 21.1% 10-year risk for CVD during 1999 – 2000, but the risk dropped to 16.4% in 2007 – 2008 (Ford, 2011). The incidence of diabetes combined with cerebrovascular disease (CeVD) has been declining in enrolled diabetes patients, similar to the trend in cardiovascular disease (CVD), however the prevalence of peripheral artery disease (PAD) in diabetes patients is on the rise (Lee *et al.,* 2019). It is believed that the primary pathogenic processes of macrovascular complications are injuries to the vascular endothelium. In addition, the development and advancement of diabetic macrovascular complications are associated with reduced platelet function, which in turn increases the risk of thrombosis and atherosclerosis progression (Grant, 2007). Oxidative stress has been linked to many pathogenetic pathways due to hyperglycemia, obesity, insulin resistance,

and other causes. Because of its central role in endothelial cell function, nitric oxide (NO) activity declines in insulin resistance and obesity, which in turn causes atherosclerotic alterations (Huang *et al.,* 2017). Another finding from this research is that NAFLD is strongly associated with an elevated risk of macrovascular complications, particularly coronary artery disease (CAD) (Viswanathan *et al.,* 2010; Lombardi *et al.,* 2020; Mantovani *et al.,* 2022). This relationship is explained by several mechanisms, such as the regulation of insulin resistance through hepatic lipid accumulation, the release of profibrotic mediators and factors into the bloodstream that promote inflammation, oxidative stress, and procoagulant and profibrotic effects, as well as myocardial remodelling and dysfunction, which can lead to various cardiac complications (Viswanathan *et al.,* 2010; Mantovani *et al.,* 2022; Stefan *et al.,* 2023).

2.2.5.2.1 Cardiovascular Disease (CVD)

Cardiovascular disease causes over 70% of deaths in people with type 2 diabetes (Beckman *et al.,* 2013). Diabetes mellitus (DM) has been linked to CVD. Diabetes quadruples the incidence of CVD episodes compared to non-diabetics, even after accounting for age, obesity, cigarette smoking, dyslipidaemia, and hypertension—the classic risk factors for CVD (Buyken *et al.,* 2007; Cade, 2008). Cardiovascular diseases, such as coronary heart disease (CHD), atherosclerotic heart disease (AHD), ischaemic heart disease (IHD), unstable angina pectoris (UAP), myocardial infarction (MI), and sudden cardiac death (SCD), can occur (Malakar *et al.,* 2019). One-third of those with T2DM worldwide have coronary heart disease (Einarson *et al.,* 2018). This means that people with DM have a much higher risk of myocardial infarction during the next seven years compared to those without diabetes, and this is true even after controlling for previous myocardial infarctions (Sarwar *et al.,* 2010).

2.2.5.2.2 Cerebrovascular Disease (CeVD)

Cerebrovascular disease is a disorder that affects the nervous system (neurons). Cerebrovascular accident (stroke) is the major symptom of cerebrovascular disease. About 20% to 40% of those with T2DM will develop cerebrovascular disease, which is a major contributor to mortality and severe morbidity globally (Tonyan *et al.,* 2021). Classification of CeVD is based on whether it is ischaemic or haemorrhagic. Half of the 6.6 million fatalities caused by stroke in 2019 were ischaemic strokes; another 44%, or 2.9 million, were caused by intracerebral haemorrhage; and 6%, or 0.4 million, were caused by subarachnoid haemorrhage (Tonomura *et al.,* 2020). The risk of stroke is higher in individuals with DM. The adjusted hazard ratio for ischaemic stroke among people with type 2 diabetes compared to those without was 2.27 (95% cardiac index, 1.95 - 2.65) in a meta-analysis of 102 prospective studies comprising 698,782 persons (Sarwar *et al.,* 2010). Furthermore, research has revealed that women with type 2 diabetes have a much higher risk of stroke when compared with men (Guo *et al.,* 2016). Several studies have indicated that CeVD is linked with age, smoking, obesity, hypertension, HDL-cholesterol, HbA1c, a history of vascular disease, heart failure, and atrial fibrillation (Proietti *et al.,* 2017; Oe *et al.,* 2021). Microalbuminuria may be a predictor of stroke, according to a previous study reported by Rocco *et al.* (2010) on the topic of CeVD. The significance of microbiota in the development of stroke has been reported by Tonomura *et al.* (2020).

The molecular pathways of the aforementioned predisposition factors that increase the chance of CeVD differ and are not well documented. However, the major point is that these risk factors are able to decrease the onset time of overt atherosclerosis and stimulate endothelial dysfunction (Ciccone *et al.,* 2011; Scicchitano *et al.,* 2019). The development of atherosclerosis is a characteristic of CeVD, and the pathophysiology of this disease is complex. Among the risk

factors, oxidative stress and inflammatory pathways have been linked as key players in the development of CeVD (Abramov *et al.,* 2007; Orellana-Urzua *et al.,* 2020).

2.2.5.2.3 Peripheral Artery Disease (PAD)

Diabetic individuals often experience peripheral, coronary artery disease and cerebrovascular disease. Atherosclerotic blockage of the lower limbs has been linked to PAD. Claudication is the major symptom of PAD. It manifests as cramping, soreness, or discomfort in the lower leg, thigh, or buttock when one exerts oneself, but it disappears when one rests (American Diabetes Association, 2003). Arterial occlusion marked by a reference value of 0.9 is used to assess PAD. There is a 2 – 4 fold increased risk of PAD in diabetic patients compared to the risk of coronary artery disease (CAD) or stroke (Lange *et al.,* 2003; Diehm *et al.,* 2004; Beckman and Creager, 2016). Studies have shown that smoking, obesity, high blood pressure, hypercholesterolemia, and dyslipidemia are some of the risk factors for PAD in diabetic patients (Weragoda *et al.,* 2016; Hiatt *et al.,* 2017). The duration of diabetes, the degree of hyperglycemia, age, gender, elevated serum fibrinogen levels, insulin resistance, microalbuminuria, increased levels of intercellular adhesion molecules, and elevated serum lipoprotein levels are additional factors that have been linked to an increased risk of PAD in diabetic patients (Weragoda *et al.,* 2016). Like other macrovascular complications, the molecular mechanism of PAD development and progression in DM is associated with disruption of the vessel wall via factors that affect haemostasis, abnormalities in blood cells, dysfunction of endothelial cells, and the promotion of vascular inflammation (Peneni *et al.,* 2013). Multiple processes interact with one another; for example, inflammation, endothelial function, and arteriogenesis can be affected by decreased nitric oxide production, while platelet and endothelial dysfunction can be induced by increased reactive oxygen species (Thiruvoipati *et al.,* 2015).

2.2.6 Role of Oxidative Stress in Diabetes Mellitus

Free radicals generated by reactive nitrogen species (RNS) and reactive oxygen species (ROS) are the major oxidants in biological systems. They are highly unstable and released as byproducts of cellular metabolism. Because of the lone pair electrons, they are highly reactive with protein, carbohydrate and DNA. Free radicals are well recognised for their dual role (beneficial, deleterious) in biological systems, since they can either be beneficial or deleterious to living systems (Valko *et al.,* 2007). Free radicals (ROS and RNS) have positive effects at low to moderate levels, including protecting cells from infections, increasing cell division, and facilitating cellular structure development (Droge, 2002; Pacher *et al.,* 2007; Sachdev and Davies, 2008). A high concentration of free radicals has been associated with induced oxidative stress and cellular damage (Bahorun *et al.,* 2006; Chaudhary *et al.,* 2023). Oxidative stress has been linked to various disease conditions. These encompass diabetes mellitus, neurodegenerative disorders (Parkinson's disease, Alzheimer's disease), cardiovascular diseases (atherosclerosis, hypertension), respiratory disease (asthma), cataract formation, rheumatoid arthritis, and various cancers (colorectal, prostate, breast, and lung cancers) (Reddy, 2023).

2.2.7 Molecular Mechanisms of Oxidatve Stress Development in Diabetes Mellitus

Persistent chronic hyperglycemia coupled with increased reactive oxygen species generation (oxidative stress) has been linked to the pathogenesis of vascular complications of diabetes mellitus (Bigagli and Lodovici, 2019). The molecular mechanisms correlated with oxidative stress in diabetes mellitus have been determined, and they are linked with lipid and glucose metabolism (Ighodaro, 2018). In hyperglycaemic conditions, oxidative stress development is stimulated by a number of metabolic pathways, including glycolytic pathway, hexosamine pathway, activation of protein kinase C (PKC), polyol pathway, and deactivation of insulin

signalling pathway (Chernikov *et al.,* 2017). When blood glucose levels are too high (hyperglycemia), the glycolysis reactions produce too many reactive oxygen species (ROS), which in turn damage DNA molecules as a result of oxidative stress and subsequent activation of DNA repair enzyme poly-ADP-ribose polymerase 1 (Robertson, 2004; Rolo and Palmeira, 2006). Glyceraldehyde-3-phosphate (GA3P) and other glycolysis intermediates (fructose-6-phosphate, glucose-6-phosphate) accumulate when poly-ADP-ribose polymerase 1 inhibits the activity of glyceraldehyde-3-phosphate dehydrogenase (Giacco and Brownlee, 2010). The build-up of glyceraldehyde-3-phosphate will in turn activate other prooxidant pathways (polyol and hexosamine pathways) (Rolo and Palmeira, 2006). In addition, an increase in glyceraldehyde-3-phosphate level can induce glucose autoxidation, which lead pro-oxidant (H_2O_2) that contributes to oxidative stress. High levels of glucose in the cell can induce glucose autoxidation; the process leads to the formation of glyoxal, a precursor of advanced glycation end products (AGEs), and definitely promotes cellular oxidative stress (Dariya and Nagaraju, 2020).

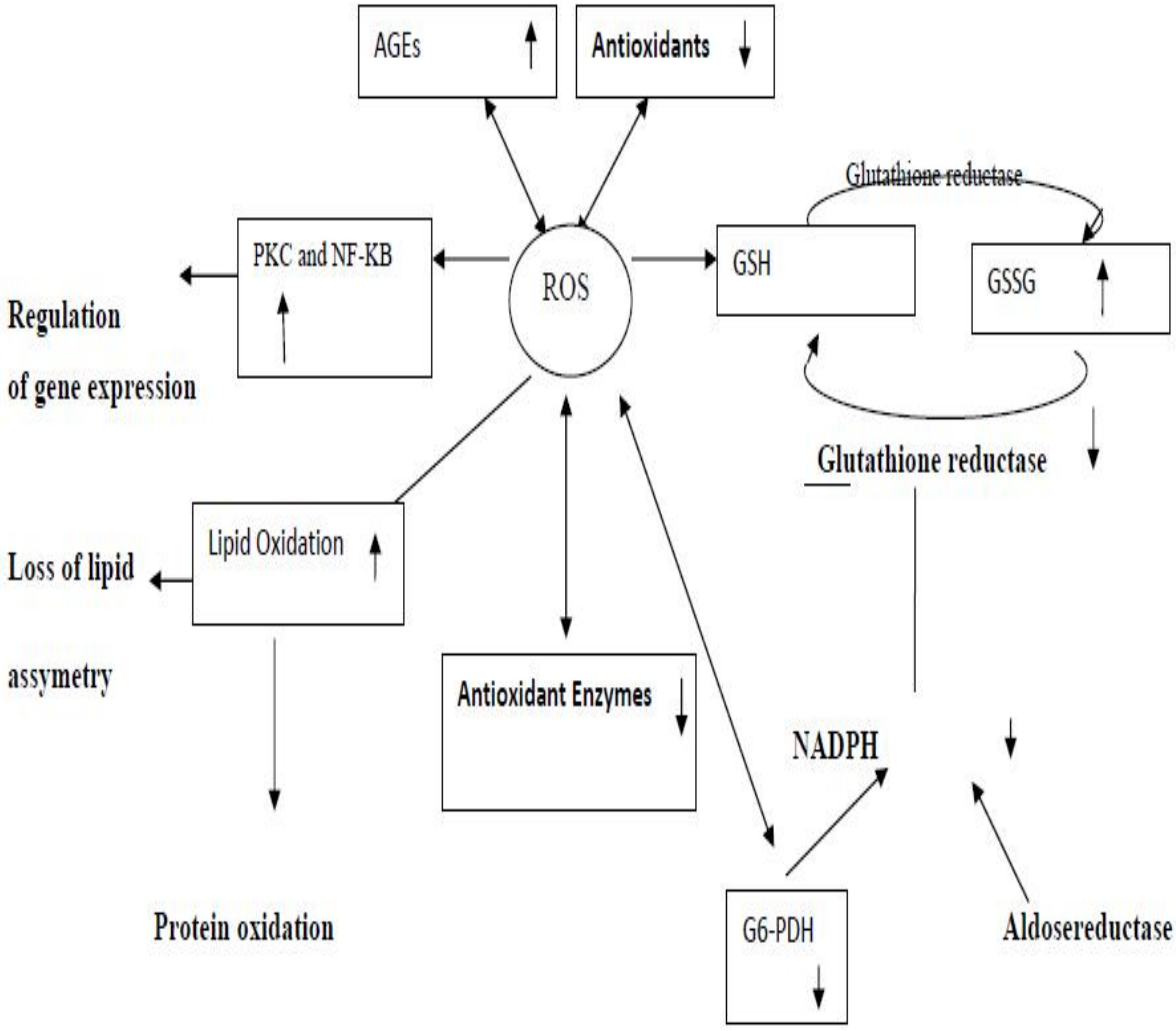

Figure 2.6: Oxidative stress-induced macromolecular damage and organ malfunction

Source (Niedowicz and Daleke, 2005)

2.3 Streptozotocin (STZ)

Streptozotocin (STZ)-induced diabetic rat model is a commonly employed method for investigating DM pathophysiology and potential therapeutic management (Mondal *et al.,* 2025).

Streptozotocin or 2-deoxy-2-[3-methyl-3-nitrosourea] 1-D-glucopyranose (Thurston and Pysz, 2021) is a broad spectrum antibiotic produced by *Streptomyces achromogens* bacteria (Akinlade *et al*., 2021). It has chemical formula ($C_8H_{15}N_3O_7$) and molar mass of 265 g/mol. According to Capdevila *et al.* (2022), STZ consists of two parts. The first part is the glucopyranosyl group, which helps the glucose transporter 2 (GLUT2) ingest it by pancreatic β-cells. The second portion is the nitrosourea group, which destroys pancreatic β-cells. In a study conducted by Al Nahdi *et al.* (2017), it was found that pancreatic β-cells selectively accumulates glucose analogue (STZ) through the GLUT2 of the cell membrane. Its methylnitrosourea moiety alkylates DNA, which damages DNA (Wszola *et al.,* 2021). Additionally, mitochondrial dysfunction can be induced by the methylnitrosourea side chain's ability to release nitric oxide. Streptozotocin has long been employed to induce diabetes in rats and mice models for experimental purposes. Streptozotocin has been employed in multiple research to induce diabetes since its diabetogenic action was initially documented in 1963 (Xiang *et al.*, 2010; Guo *et al.*, 2018; Vatandoust *et al.*, 2018). It has been reported that reactive oxygen species (ROS), reactive nitric oxide species (NO/RNS), and the development of inflammatory responses are the mechanisms by which STZ-induced cytotoxicity (Van Dyke *et al.,* 2008; Friederich *et al.,* 2009).

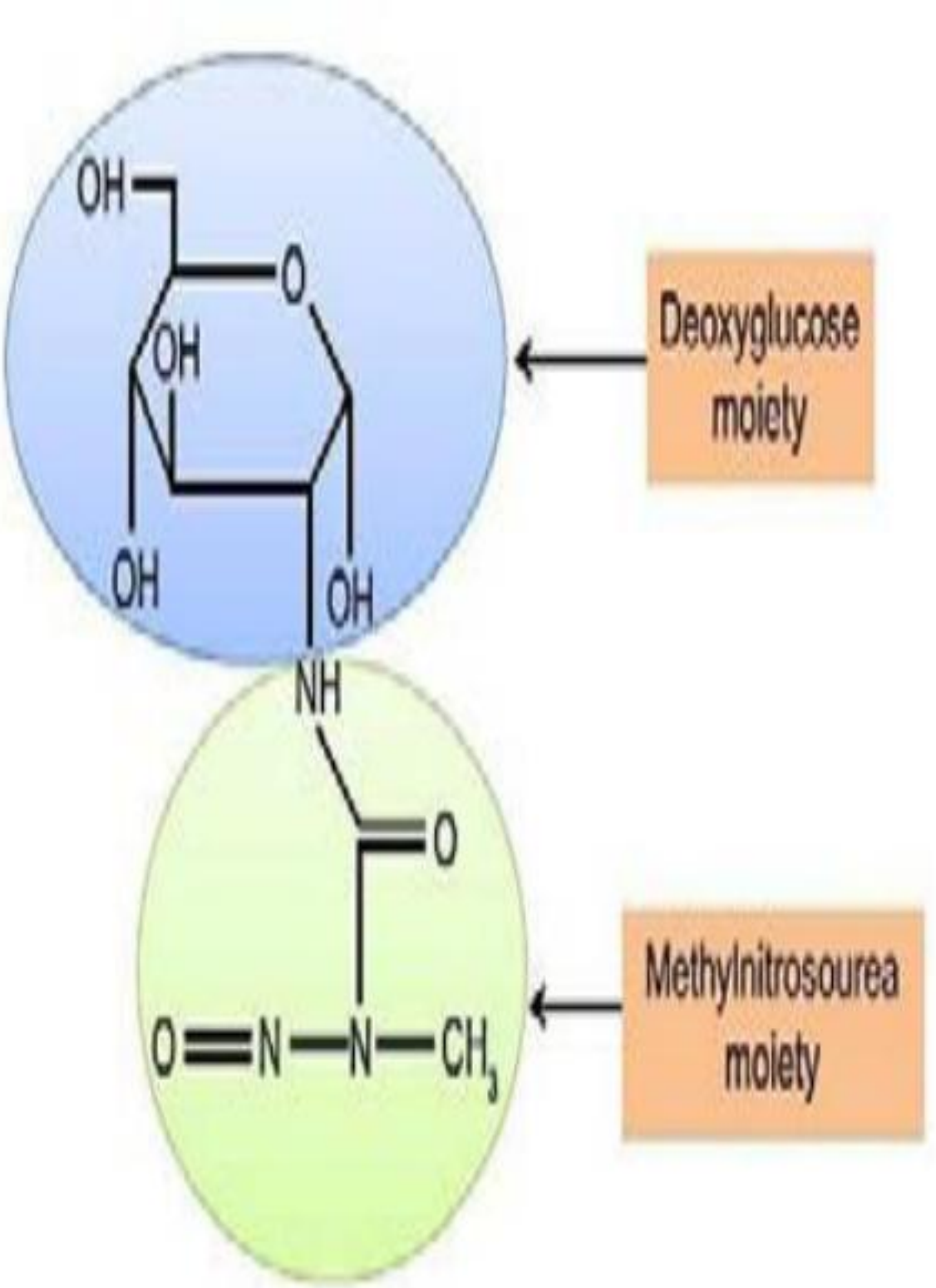

Figure 2.7: Structure of Streptozotocin

Source: (Wu and Yan, 2015)

2.3.1 Mechanisms of Action of STZ

Streptozotocin is an analogue of glucose that is selectively accumulated in pancreatic beta-cells via a GLUT-2 glucose transporter in the pancreatic plasma membrane (Al Nahdi *et al.,* 2017). Streptozotocin is diabetogenic due to its targeted GLUT 2-dependent action in the pancreatic β-cells (Hosokawa *et al.,* 2001). After entering the pancreatic β-cells via the GLUT 2 transporter, it causes DNA damage due to the DNA alkylating activity of its methyl nitrosourea moiety (Ghasemi *et al.,* 2014), which, in turn, results in DNA fragmentation (Arwa *et al.,* 2017). The broken DNA stimulates the enzyme (poly ADP-ribose synthetase) to fix the damage DNA. Poly ADP-ribosylation causes depletion of cellular NAD^+ and ATP (Andrabi *et al.,* 2014). The decreased ATP synthesis is demonstrated by dephosphorylation, providing more substrates for xanthine oxidase reaction, resulting in the build-up of hydrogen peroxide and hydroxyl radicals (Arwa *et al.,* 2017) which will in turn induced-oxidative stress. Additionally, N-methyl Nnitrosourea side chain has the potential to release nitric oxide (Van Dyke *et al.,* 2008; Friederich *et al.,* 2009), which inhibits the aconitase activity, resulting in mitochondrial dysfunction in pancreatic beta cells. This results in insulin deficiency and hyperglycemia, mimicking type 1 and, in modified protocols, type 2 diabetes (Furman, 2015) hence, justifying its selection as a diabetogenic agent in experimental studies.

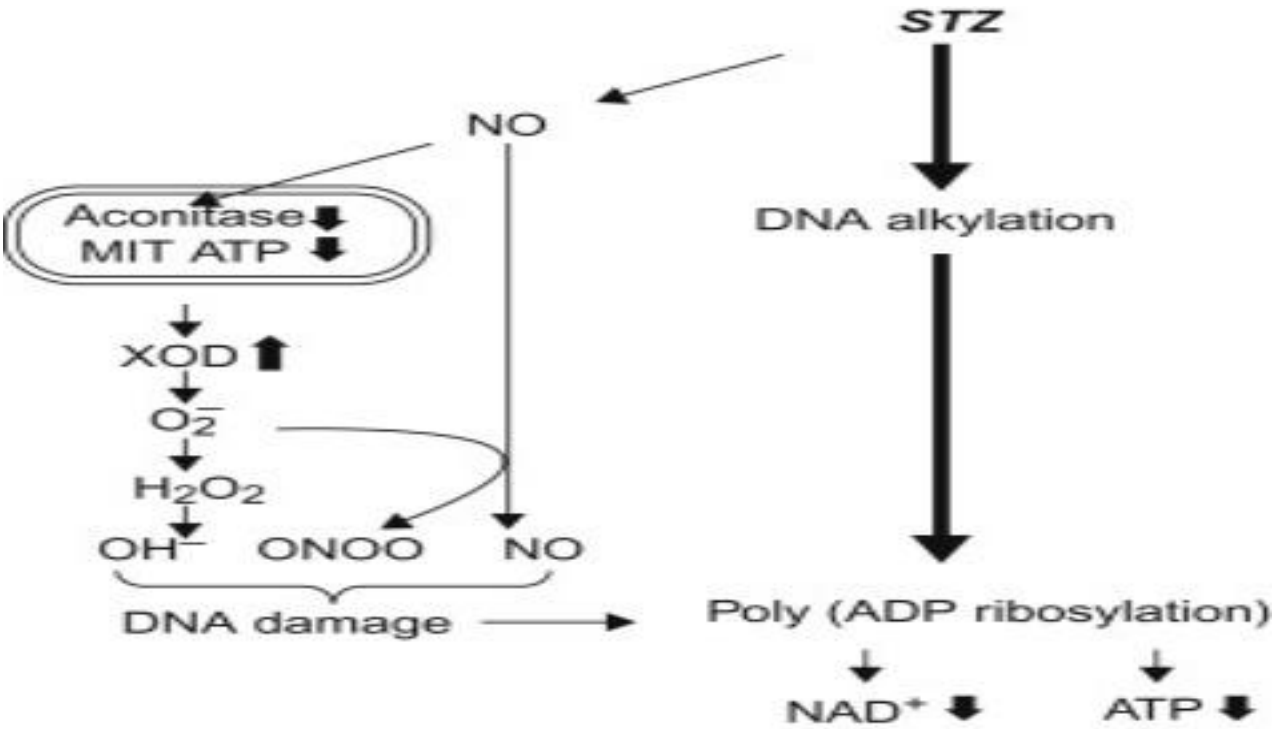

Figure 2.8: Mechanisms of streptozotocin-induced diabetes mellitus

Source: (Al Nahdi *et al.,* 2017)

2.4 Blood Glucose Homeostasis

Glucose homeostasis is a process of maintaining a constant concentration of glucose in the blood. Carbohydrate molecules, which include plant polysaccharides like starch and animal polysaccharides like glycogen, are the source of glucose for energy in the body. The liver can also synthesize glucose endogenously through gluconeogenesis and glycogenolysis pathways (Roder *et al.,* 2016; Lundqvist *et al.,* 2019). The process is vital for supplying energy to several tissues. The glucose-insulin regulating pathway maintains blood glucose homeostasis in healthy individuals. The normal range for blood glucose levels in individuals following fasting is 70 - 100 mg/dL. Hypoglycemia, a potentially fatal condition, is associated with levels below 70 mg/dL. Plasma glucose levels above 100 mg/dL are considered hyperglycemia, diagnosis of diabetes would typically be made in cases of persistent hyperglycemia. Failure of the blood

glucose homeostasis system to maintain the plasma glucose concentration within the normal range leads to diabetic complications (Li *et al.,* 2006; Ajmera *et al.,* 2013).

2.5 Insulin

Human pancreatic beta cells in the islets of Langerhans secrete the peptide hormone insulin, which regulates glucose metabolism (Mahgoub and Ali, 2019). Some research has shown that parts of neurons may also express a low level of insulin (Ruud *et al.,* 2017). Insulin is secreted in response to an increase in blood glucose level following a carbohydrate rich meal (Qaid and Abdelrahman, 2016). Insulin was first discovered in 1921 in extracts of the pancreas by Frederick Banting and Charles Best, while Nicolae Paulescu, independently worked on an insulin-containing substance (pancrein) (Lewis and Brubaker, 2021; Stansfield, 2012). Insulin was found to be a polypeptide hormone and its amino acid sequence was elucidated in 1952 (Vecchio *et al.,* 2018). Insulin is made up of two protein chains, with 21 and 30 amino acids in (A chain, B chain), a total of 51 amino acids, linked by disulfide bridges (Moroder and Musiol, 2017). Proinsulin, the precursor of insulin contains 74 amino acids, and a molecular weight of 5802 Da (Beischer, 2019). Proinsulin is secreted by the pancreatic β-cells in an inactive form, cleavage at A and B chain produces the active form (insulin), and C peptide (Weiss *et al.,* 2014). The (A and B) chains become linked together by disulfide bonds which is antiparallel on the C-terminal helix (Weiss *et al.,* 2014). Proinsulin, insulin, and C-peptide are stored in granules in the pancreatic β-cells, which will in turn release insulin into the circulation in response to a stimulus (Gan *et al.,* 2018). After 30 minutes of exposure to hyperglycemia, the insulin level in the blood increases (Ruzittu, 2020). Normal adult pancreatic β-cells contains approximately 200 units of insulin, and the amount of daily insulin secretion into the circulatory system ranges from 30 to 50 units (Matteucci *et al.,* 2015).

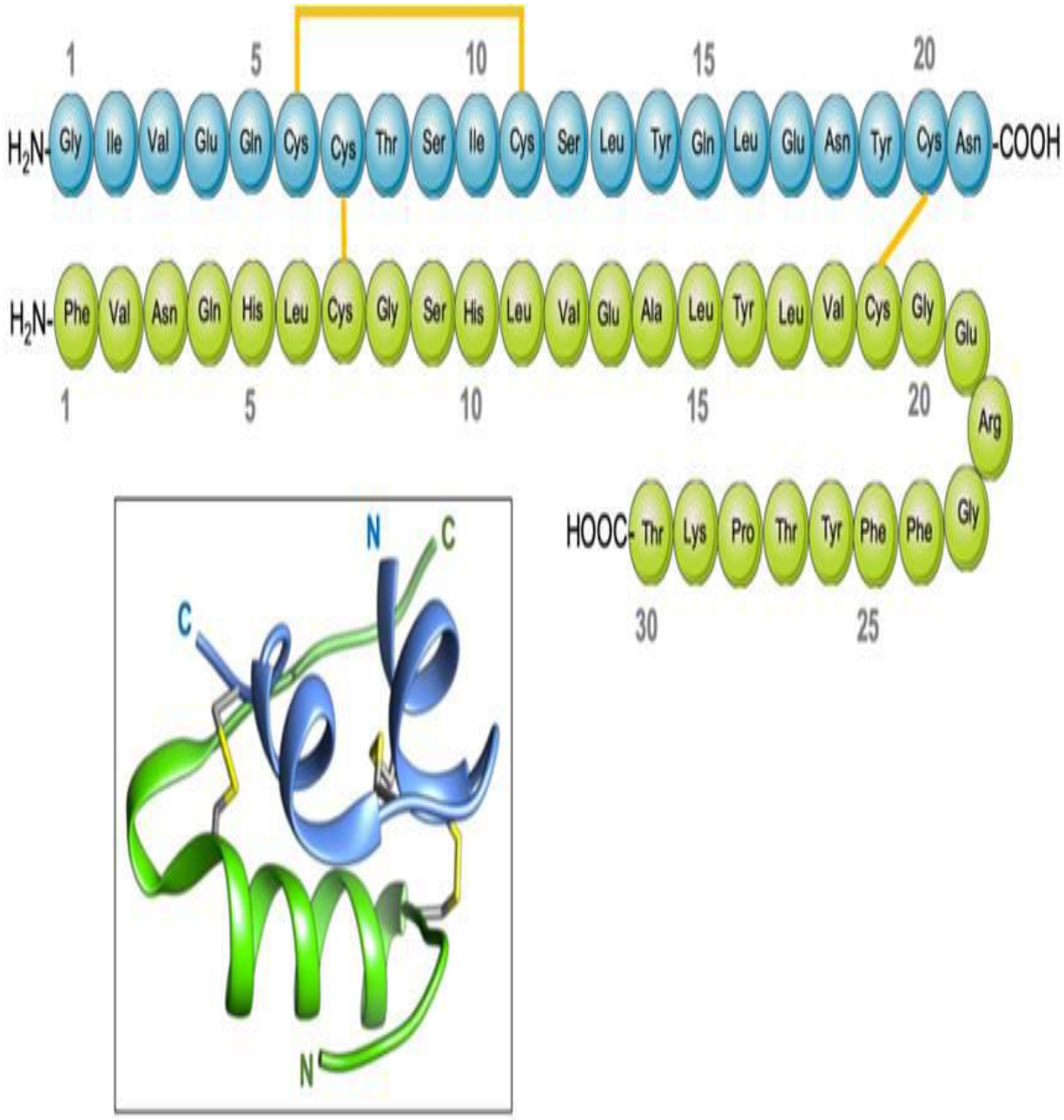

Figure 2.9: Structure of insulin

Source: (Alyas *et al.,* 2021)

2.5.1 Biosynthesis of Insulin

Insulin biosynthesis takes place in pancreatic β-cells found in the islets of Langerhan. Human insulin gene contains three exons separated by two introns (Fu *et al.,* 2013). The coding of

mature insulin is performed by the exons. Insulin expression is controlled by exon 1, which comprises the 50 untranslated 17 regions. The signal peptide, B-chain, and C-peptide are encoded by exon 2. Finally, exon 3 encodes the rest of the C peptide and the A-chain (Nishi and Nanjo, 2011; Støy *et al.,* 2021). The insulin precursor, preproinsulin, is a single-chain protein that includes the following: amino-terminal signal peptide, B-chain, C-peptide, and carboxy-terminal A-chain. During the production of insulin, the gene that encode insulin is transcribed to messenger ribonucleic acid using different transcription factors. The transcribed gene will in turn translated to preproinsulin at the cell surface of the endoplasmic recticulum which is then transported into the endoplasmic recticulum. Signal peptidase catalyzes the remover of signal peptide to produce proinsulin. Proinsulin, in turn, folds at the luminal side of the ER to form the three evolutionarily conserved disulfide bonds fundamental to insulin's structure (Chang *et al.,* 2003). Misfolding of the mutant proinsulin can initiate the unfolded protein response and lead to ER stress and pancreatic β-cell dysfunction (Oslowski and Urano, 2010). Proinsulin is transported from endoplasmic reticulum via golgi apparatus to secretory vesicles. Endopetidase catalyzes the cleavage of C-peptide in post-golgi vesicles at the insulin production stage. The C-peptide is cut off by endopetidase within the post-golgi vesicles to produce insulin. The hormone is then stored in the secretory vesicles of pancreatic β-cells as hexamers stabilized by zinc (Liu *et al.,* 2018).

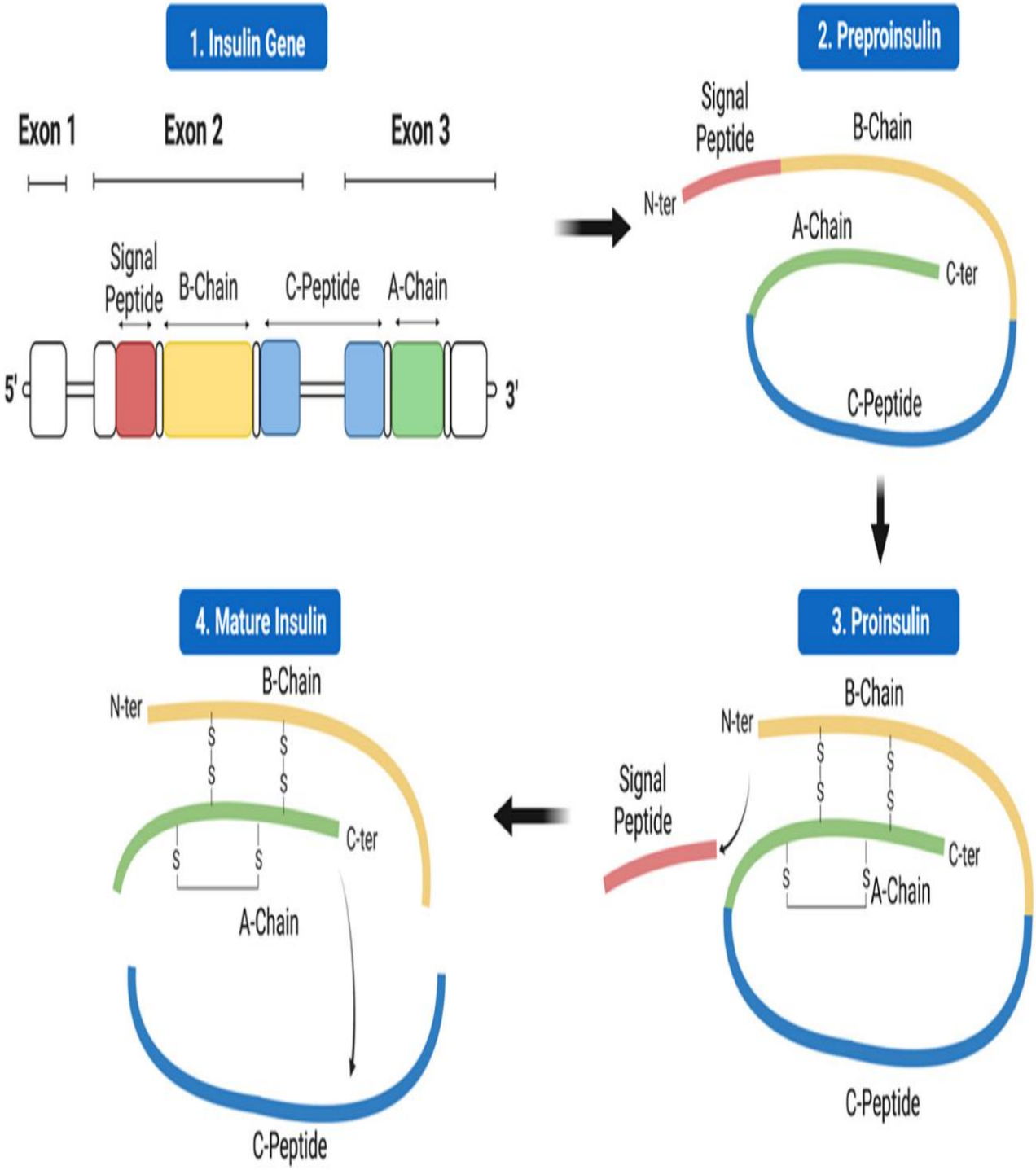

Figure 2.10: Biosynthesis of insulin and C-peptide

Source: (Ataie-Ashtiani and Forbes, 2023)

2.5.2 Biochemical Mechanisms of Insulin Secretion

Insulin exocytosis is a highly regulated process, and many factors are involved in promoting insulin release (Fu *et al.,* 2013). Carbohydrates are normally the primary source of fuel in food and glucose is the major insulin secretagogue (Fu *et al.,* 2013), traditional models of insulin exocytosis depends on the increase in the pancreatic beta-cells intracellular ATP: ADP ratio, following increased glucose metabolism. Following substrate-level phosphorylation and mitochondrial electron transport employing electron donors NADH and $FADH_2$ for ATP synthesis. Increasing glycolytic pathway and tricarboxylic acid cycle (TCA) directly increases mitochondrial ATP production. Increase in ATP: ADP ratio causes depolarization of the pancreatic β-cells plasma membrane by closure of beta-cells ATP-sensitive K^+ channels, which in turn stimulates the opening of voltage-gated Ca^{2+} channels (Jensen *et al.,* 2008; Newsholme and Krause, 2012). The resultant effect (influx of Ca^{2+}) leads to insulin exocytosis through fusion of a readily releasable pool of insulin-containing vesicles with the plasma membrane (Komatsu *et al.* 2013).

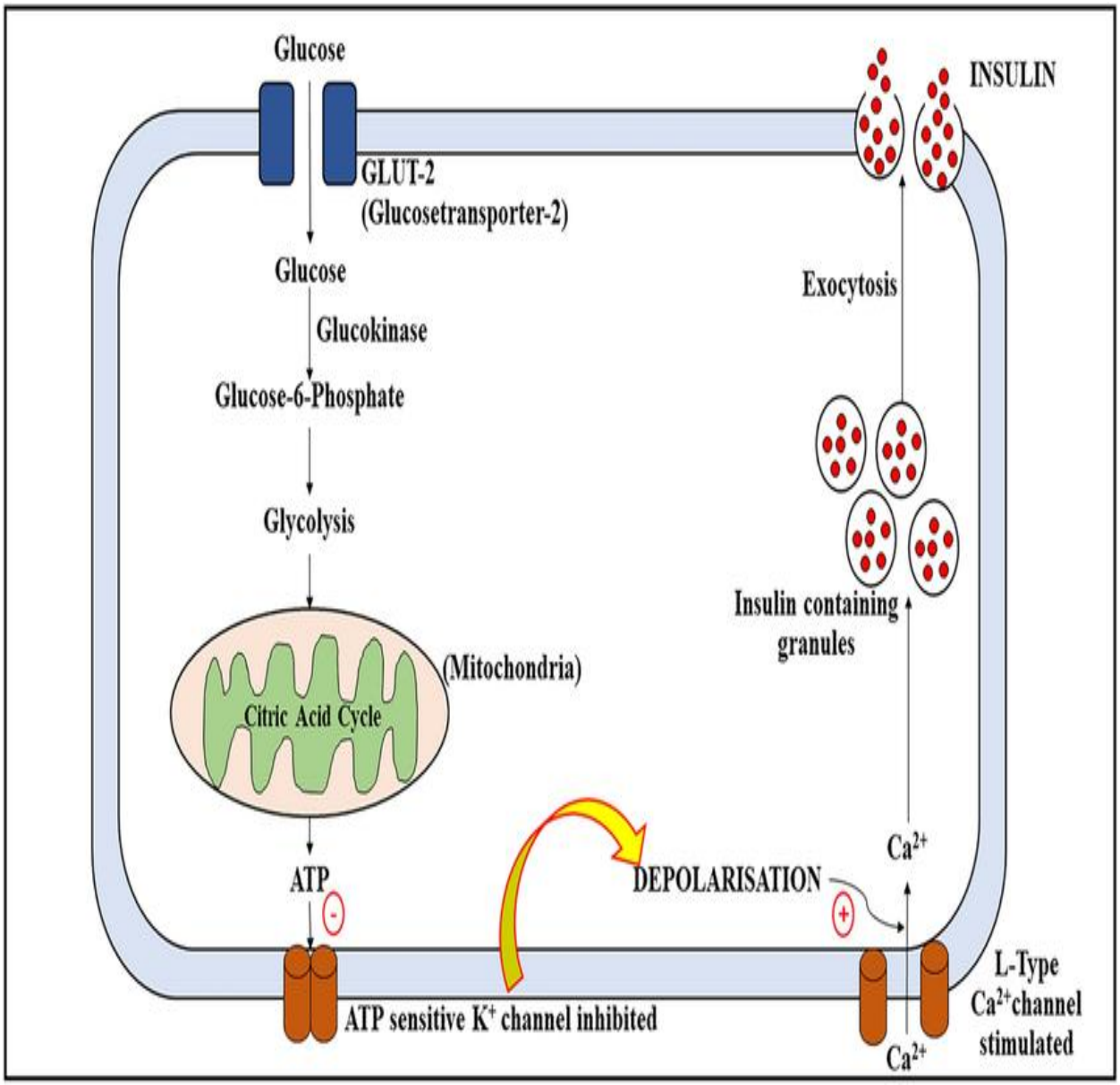

Figure 2.11: Mechanisms of insulin secretion

Source: (Marchetti *et al.,* 2017)

2.6 Liver

The liver is the largest gland in the human body; it is the second-largest organ overall, behind only the skin. It has a right-angled base and a left-angled peak, giving it a pyramidal or wedge form (Elobu *et al.,* 2021). The liver, a peritoneal organ, is dark pinkish-brown in colour; it

weighs 1.35 - 1.59 kg and accounts for 2 - 3% of the total body weight. The right mid-clavicular line provides an approximate measurement of 12 - 15 cm. It consists of two faces and four edges: visceral parietal faces, dorsal, ventral, right and left edges (Juza and Pauli, 2014). The hepatic tissue is located above the right kidney, intestines, and stomach in the upper right of abdominal cavity, under diaphragm. It progresses to hypochondrium on left side. Liver is held in place by peritoneal reflections, which are ligamentous attachments, and it is shielded from harm by lower right rib cage. The peritoneum connects the liver in four locations: the falciform ligament, the left and right triangular ligaments, and the coronary ligament (Krishna, 2013). A branch of the coronary artery system, the hepatic artery carries oxygenated blood to the liver. The hepatic veins, which are formed from the central veins, facilitate the direct flow of deoxygenated blood from the liver to the inferior vena cava, bypassing the diaphragm (Kalra *et al.,* 2023).

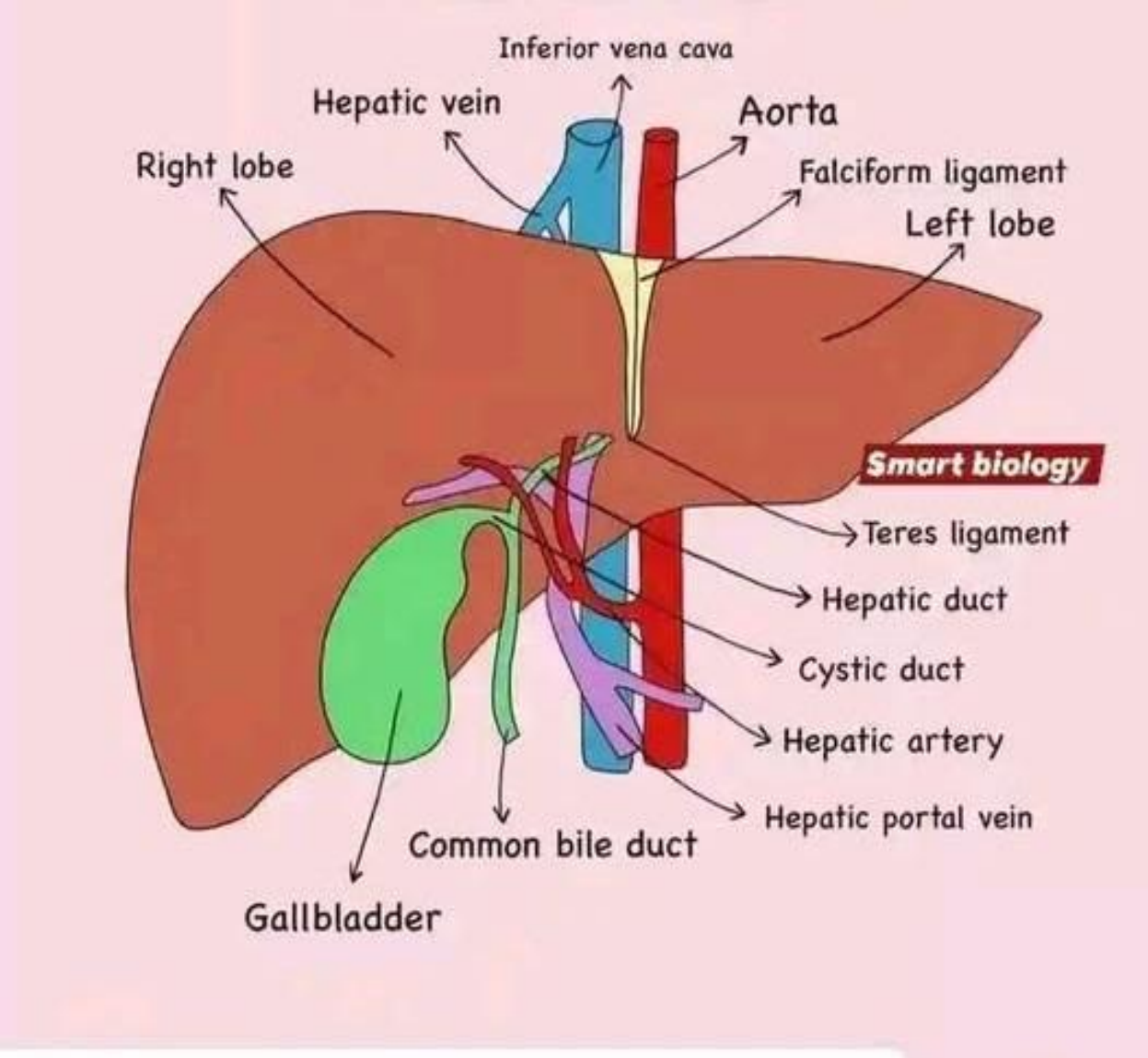

Figure 2.12: Structure of the liver

Source (Elobu *et al.,* 2021)

2.6.1 Functions of the Liver

Maintaining a steady metabolic rate is primarily the function of the hepatocytes. The following are the main functions of the hepatocyte:

1. Bile production, which aids digestion by removing waste and breaking down fats in the small intestine.
2. Plasma protein production.

3. Synthesis of cholesterol and other specialized proteins that aid in the transport of lipids throughout the body.
4. Regulation of blood glucose homeostasis
5. Controlling the concentration of amino acid, the building block of protein
6. Storage of vitamin A and iron
7. Production of urea (the end product of protein metabolism).
8. Metabolism of drugs and xenobiotics
9. Regulating blood clotting.
10. Metabolism of bilirubin

2.6.2 Role of Liver in Plasma Glucose Homeostasis

Plasma glucose homeostasis is essential for optimal health. The liver majorly regulates the glucose metabolism (glycolysis, gluconeogenesis, glycogenesis, and glycogenolysis) pathways that occur to maintain this homeostasis (Alamri, 2018). These pathways occur depending on blood glucose concentrations (hyperglycemia/hypoglycemia) (Han *et al.,* 2016). These interdependent processes are regulated by key enzymes (Fructose-1, 6-bisphosphatase and glucokinase). While the activities of these enzymes are regulated based on plasma glucose concentrations, the pancreas is responsible for the secretion of regulating hormones (insulin/glucagon), which will directly control these pathways in the liver (Sharabi *et al.,* 2015; Alamri, 2018).

2.7 Kidney

The kidneys are reddish-brown bean-shaped organs located retroperitoneally; the kidneys of healthy adult weigh (160 – 162 g) and (135 – 136 g) in men and women (Garza and Leslie, 2025). The left kidney is approximately 10 g heavier, slightly larger when compared with the right.

Standard measurements fall within the range of 5 – 7 and 10 – 12 cm cm in breadth and length. The right kidney is slightly lower than the left kidney because of the presence of the liver (El-Reshaid and Abdul-Fattah, 2014). As a result of the gradual degeneration of the renal parenchyma, kidney size declines with age and has a positive correlation with total body surface area, height, and weight (Garza and Leslie, 2025; Zhang *et al.,* 2025). Both pairs of renal arteries and veins carry blood to and from the kidneys. The ureter, which connects the kidneys to the bladder, is responsible for transporting urine from the kidneys to the bladder.

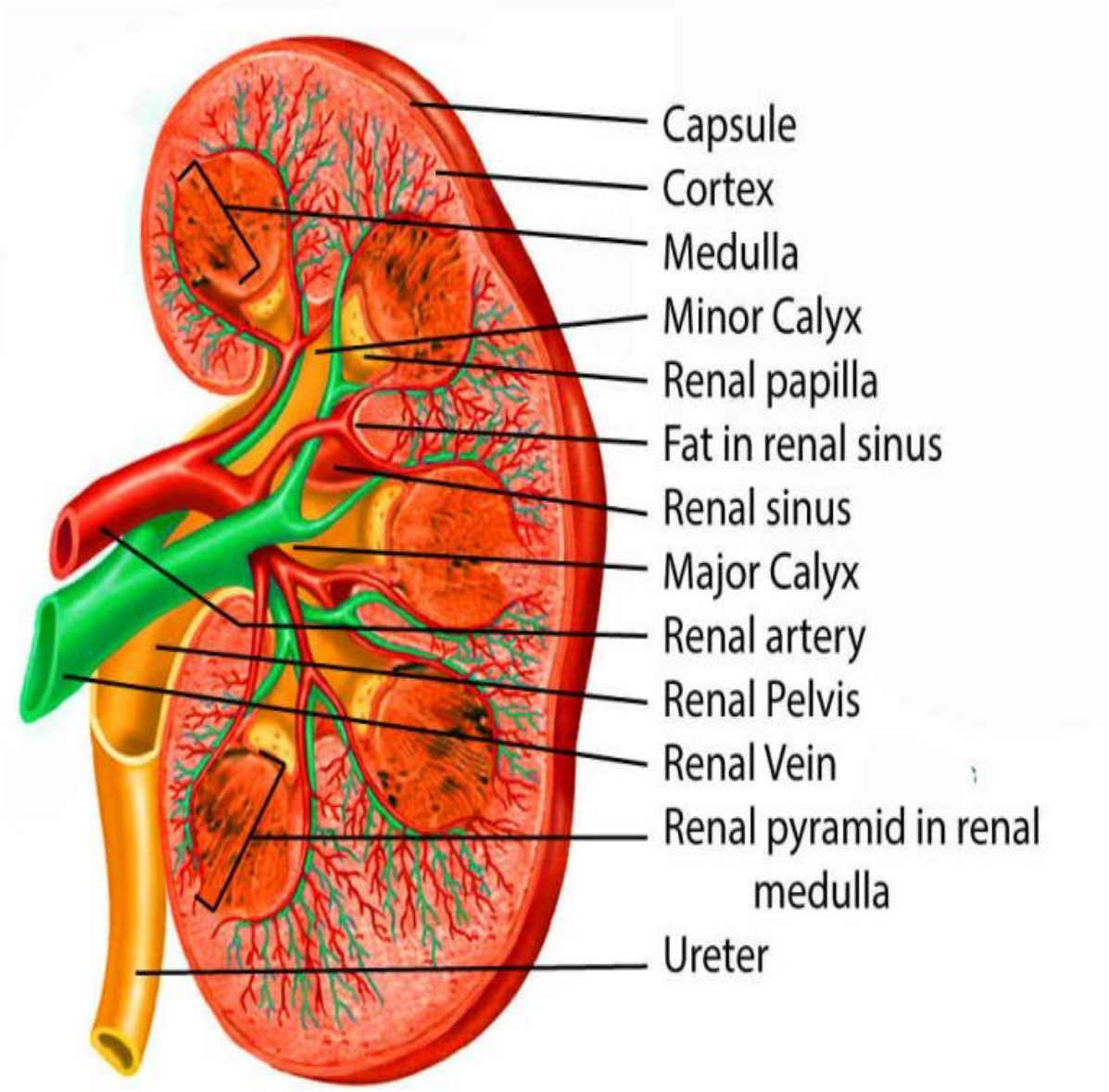

Figure 2.13: Structure of the kidney

Source (Garza and Leslie, 2025)

2.7.1 Functions of the Kidneys

The kidneys perform several important functions, including regulation of electrolytes (Na^+, K^+, Ca^{2+}, Cl^-, HCO_3^-), elimination of nitrogenous waste products (ammonia and urea), and the maintenance of acid-base balance through bicarbonate reabsorption and hydrogen ion excretion

(Hopkins *et al.,* 2022; Yu and Sharma, 2023). According to Fountain *et al.* (2023), the kidneys play a significant role in the renin-angiotensin-aldosterone system, which regulates blood pressure and intravascular volume. The kidneys play a pivotal role in reabsorption of essential substances, including glucose, amino acids, phosphate, calcium, and water (Ogobuiro and Tuma, 2023; Qadeer and Bashir, 2023). Additionally, the kidneys secrete important hormones such as calcitriol, the active form of vitamin D, which controls calcium and phosphate metabolism, and erythropoietin, which stimulates red blood cell production (Jelkmann, 2013; Lung and Komatsu, 2025).

2.7.2 Role of Kidneys in Blood Glucose Homeostasis

The kidneys play a pivotal role in regulating plasma glucose homeostasis (Ruan and Guan, 2009). In normal glomerular filtration, the kidneys significantly decreased chronic hyperglycemia (Girard, 2017; Vallon and Thomson, 2017). The kidneys play a significant role in maintaining plasma glucose homeostasis via gluconeogenesis, renal glucose uptake, and glucose reabsorption in the proximal tubules (Mather and Pollock, 2011; Segura and Ruilope, 2013; Moradi-Marjaneh *et al.,* 2019). Glucose reabsorption is one of the most crucial physiological functions of the kidneys, enabling complete recovery of filtered glucose, elimination of glucose from urine, and the inhibition of calorie loss (Segura and Ruilope, 2013).

2.8 Pancreas

The pancreas is a glandular organ that is essential in digestion/metabolic regulations. A normal adult human pancreas weighs approximately 100 g, 14 – 25 cm in length, and lobular and elongated in shape (Longnecker *et al.,* 2018). According to Saisho *et al.* (2007), the average human pancreas continues to grow until around the age of 30, and there is a significant variation in the weight of the adult pancreas. Located in the upper abdomen, it comprises of three sections:

the head, which is adjacent to the duodenum, and the body and tail, which stretch along the midline of the body to a spot close to the spleen. The exocrine function involves the production and secretion of digestive enzymes into duodenum through a complex ductal tree. According to Williams (2010), acinar cells are the cells in the pancreas responsible for producing these digestive enzymes. Approximately 85% of the pancreas is composed of acinar cells, which are arranged in acini. These cells produce and release enzymes that aid in the digestion of proteins, lipids, and carbohydrates (Matsuda, 2019). Separated from the acinar cell clusters are scattered patches (islets of Langerhans), which secrete endocrine hormones. Approximately one million islets of Langerhans exist in the pancreas; they weigh about 1 g and contribute approximately 1 – 2% of the pancreas' total mass (In't Veld and Marichal, 2010). The endocrine system secretes regulatory hormones from five distinct cell types of the islet cells: α-cells that produce glucagon (Brissova *et al.,* 2005), which make up 15 – 20% of the total islet cells; β-cells that secrete amylin, C peptide, and insulin (Brissova *et al.,* 2005), which represent 65 – 80% of the total cells; γ-cells that release pancreatic polypeptide (Katsuura *et al.,* 2002), which account for 3 – 5% of the total islet cells; δ-cells that secrete somatostatin (Brissova *et al.,* 2005), comprising 3 – 10% of the total cells; and ghrelin-secreting ε-cells (Wieup *et al.,* 2002), which represent less than 1% of the total islet cells. These hormones are important in blood glucose homeostasis.

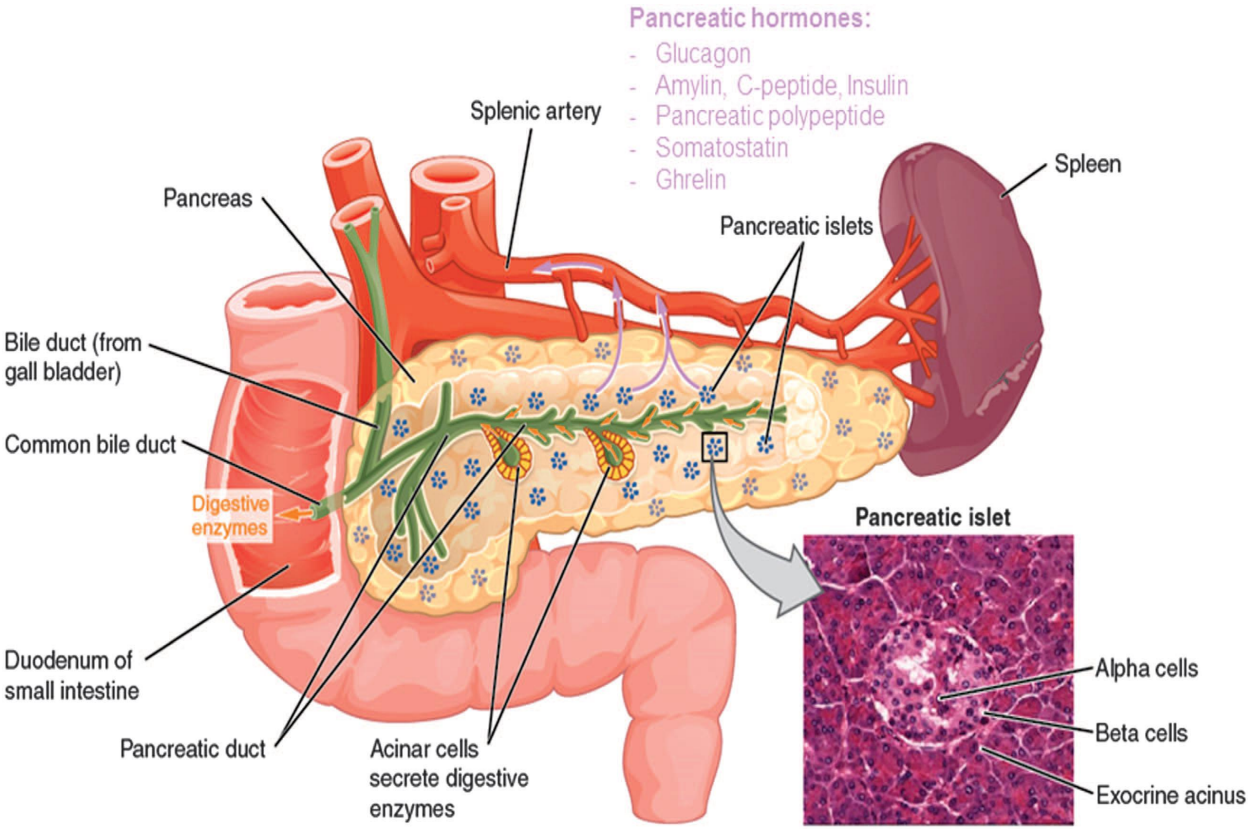

Figure 2.14: Anatomical organization of pancreas

Source (Longnecker *et al.,* 2018)

2.8.1 Pancreatic Islets and Blood Glucose Homeostasis

The pancreatic tissue consists of clusters of regulating hormone-secreting cells (islets of Langerhans), which comprise the endocrine part of the pancreas (Steiner *et al.,* 2010). They are mainly composed of β-cells, α-cells, and δ-cells. The β-cells secrete insulin, the hormone that stabilizes carbohydrate metabolism, facilitating glucose uptake by extrahepatic tissues/organs. The α-cells secrete glucagon, one of the hormones that stimulates gluconeogenesis and glycogenolysis in response to hypoglycemia. The δ-cells produce somatostatin, which acts in a paracrine manner to regulate insulin and glucagon secretion. Coordination between these cell types within the islet provides precise regulation of blood glucose homeostasis.

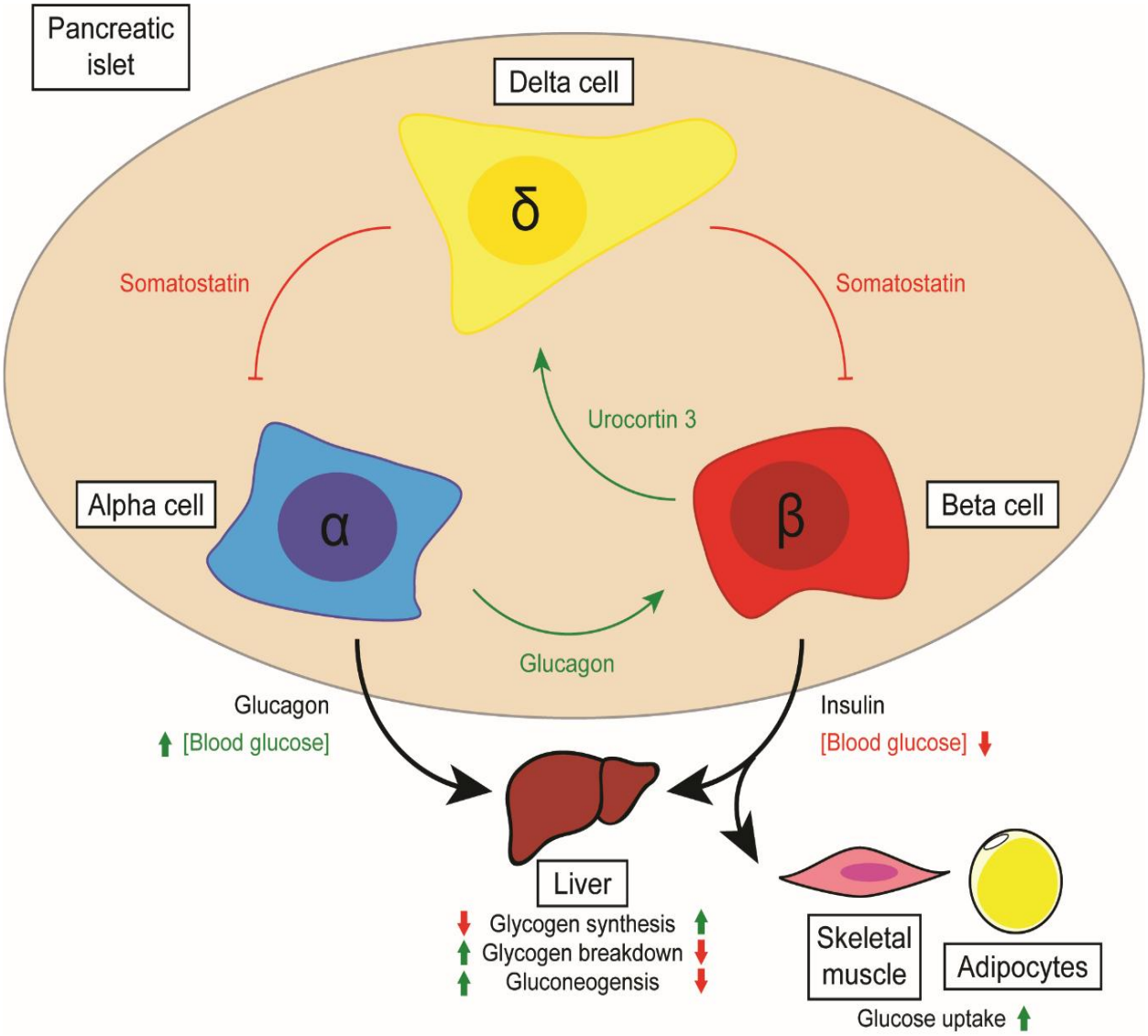

Figure 2.15: Paracrine and systemic actions of the pancreatic islet.

Source (Huang, 2022)

2.8.2 Role of Pancreas in Blood Glucose Homeostasis

The pancreas plays a crucial role in plasma glucose homeostasis. Glucose metabolism is highly dependent on hormones secreted by the pancreatic islet cells (Pendharkar *et al.,* 2016). The secretion of insulin and glucagon in response to hyperglycemia/hypoglycemia allows the body to regulate blood glucose concentrations. These hormones exert their effects in opposition to each other (Szablewski, 2011). In hyperglycemic conditions, β-cells secrete insulin to lower plasma

glucose levels. The liver, skeletal muscles, and fat cells are among the tissues/organs that receive insulin signals (Dimitriadis *et al.,* 2011). Insulin stimulates glycogenesis in the liver, while in skeletal muscle and adipose tissue, it promotes glucose uptake. Additionally, it acts as one of many nutritional signals to regulate brain metabolism (Duarte *et al.,* 2012). In hypoglycemic conditions, the pancreatic α-cells secrete glucagon to increase blood glucose levels. In the liver, glucagon stimulates gluconeogenesis and glycogenolysis to release glucose into the bloodstream (Briant *et al.,* 2016).

2.9 Fractionation

Fractionation is a process of separation of plant crude extracts into various fractions. It further concentrates the active compounds and improves their biological activities. The process continues until a pure compound is isolated (Rimando *et al.,* 2001; Doughari, 2012; Banu and Cathrine, 2015). When several solvents are selected for the fractionation, they are added based on the order of increasing polarity. Various physical methods, such as separation funnels, chromatographic techniques, fractional distillation, fractional crystallisation, fractional liberation, and sublimation, are used in the fractionation of bioactive compounds from crude extract (Doughari, 2012). In the separating funnel method, when selected solvents (n-hexane, ethyl acetate, and methanol) are used, fractionation begins by suspending the crude extract in ethanol and distilled water (ethanol:water at the ratio of 7:3). Subsequently, fractionated with n-hexane, ethyl acetate, and methanol to get n-hexane, ethyl acetate, and methanol fractions (Rimando *et al.,* 2001; Doughari, 2012; Ingle *et al.,* 2017). The remaining portion after the fractionation is known as aqueous or hydroethanol fraction (Rimando *et al.,* 2001; Doughari, 2012; Ingle *et al.,* 2017).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals and Reagents

The chemical/reagents used in this study included streptozotocin, sodium chloride, sodium hydroxide, n-hexane, ethyl acetate, chloroform, ethanol, methanol, thiobarbituric acid, Malondialdehyde (MDA), Tris-HCl buffer (pH 7.4), phosphate buffer (pH 7.4), as well as Randox kits. All the chemicals and reagents were of analytical grade, and they were products of Randox Ltd (UK) and Sigma-Aldrich, Ltd. (USA).

3.1.2 Plant Collection and Authentication

The plant sample (stem bark) was obtained from Ebelle, Igueben Local Government Area, Edo State. The leaves were identified by Professor Henry Akinnibosun from the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, where a herbarium specimen was deposited and voucher number UBH-G326 was obtained.

3.1.3 Experimental Animals

Fifty-six (56) adult male Wistar rats weighing 150 - 200 g were used for this study. The animals were bought from the animal house of the Department of Biochemistry, Federal University, Wukari, Taraba State. The animals were housed in normal/standard cages and given two weeks to adjust to the laboratory conditions. During the acclimatization period, the rats were fed *ad libitum* with standard pellet diet and kept at 25 ± 1°C on a 12-h light/12-h dark cycle.

3.2 Methods

3.2.1 Extract Preparation and Fractionation

The plant sample was washed and shade-dried for 3 weeks. The dried sample was pulverized using a mechanical blender. The weight of the pulverized sample was measured. The pulverized

plant sample (2500 g) was extracted with 12.5 litres of absolute ethanol. The mixture was macerated at room temperature with continuous stirring periodically for 72 h. The resulting extract was filtered through a muslin cloth and lyophilized (freeze-dried) using TOPT-10 freeze dryer (Xian TOPTION Instrument Co. Ltd., China) (Onoagbe *et al.,* 1999). The ethanol extract was subsequently fractionated with n-hexane, ethyl acetate and absolute methanol.

3.2.2 Acute Toxicity Test

The median lethal dose (LD_{50}) of the ethanol extract was carried out as described by Lorke (1983). In phase I, twelve (12) rats were assigned into four groups (3 rats per group). *Chrysophyllum albidum* crude extract was administered orally at a single dose of 0 mg/kg bwt, 10 mg/kg bwt, 100 mg/kg bwt and 1000 mg/kg bwt respectively. For 24 hours, they were monitored for any signs of toxicity, including mortality. Twelve (12) rats were randomly assigned into four groups (3 rats/group) in phase II. Each group received an oral single dose of *C. albidum* crude extract at a concentration of 0, 1600, 2900, and 5000 mg/kg bwt, respectively. In addition, they were monitored for any signs of toxicity/mortality. The product of the lowest lethal dose and the maximum non-lethal dose was used to compute the LD_{50}.

3.2.3 Induction of Diabetes Mellitus

Diabetes mellitus (DM) was induced using a single intraperitoneal injection of 50 mg/kg bwt streptozotocin (Prasath *et al.*, 2014). A test for persistent hyperglycemia was made after 72 h. Rats with blood glucose ≥ 200 mg/dL stable for the following 3 days were considered diabetic and used for the study.

3.2.4 Experimental Design

The rats were randomly assigned to eight groups (7 rats per group): control, diabetic, metformin,

ethanol, hydroethanol, n-hexane, ethyl acetate and methanol. Treatment with the drug/fractions lasted 14 days. Weight and blood glucose were measured on weekly basis.

Table 3.1: Experimental Design

Group	Treatment
Control	Distilled water
Diabetic	STZ only
Metformin	STZ + Metformin (50 mg/kg bwt)
Ethanol	STZ + Ethanol (200 mg/kg bwt)
Hydroethanol	STZ + Hydroethanol fraction (100 mg/kg bwt)
n-Hexane	STZ + n-Hexane fraction (100 mg/kg bwt)
Ethyl acetate	STZ + Ethyl acetate fraction (100 mg/kg bwt)
Methanol	STZ + Methanol fraction (100 mg/kg bwt)

3.2.5 Blood/Sample Collection

At the conclusion of the treatment period, blood samples were collected through cardiac puncture under mild ketamine anesthesia into heparin/EDTA containers. The blood was centrifuged at 3500 rpm for 15 min to obtain plasma which was used for biochemical analyses. Organs such as liver, kidney, and pancreas were excised and used for histological studies, as well as homogenates preparation.

3.2.6 Preparation of Tissue Homogenate

Tissues (liver, kidney, and pancreas) were promptly removed after euthanization, rinsed with cold 50 mM Tris-HCl buffer (pH 7.4) to remove blood stains, and weighed. The tissues were homogenized in a Teflon-glass homogenizer with 50 mM Tris-HCl buffer (1:5 w/v) at 1200

rev/min in cold water. The homogenates were centrifuged at 4000 rpm for 10 minutes, and the supernatants (S1) were collected for further analysis.

3.2.7 Biochemical Analyses

3.2.7.1 Determination of Plasma Alanine Aminotransferase (ALT) Activity

The ALT activity was determined as described by Reitman and Frankel (1957).

Principle:

α-Oxoglutarate + L-alanine $\xrightarrow{\text{ALT}}$ **L-glutamate + pyruvate**

The activity of ALT was determined by measuring the concentration of pyruvate hydrazone, which was produced by 2,4-dinitrophenylhydrazine, at 540 nm and 37^{o}C.

Procedure: Precisely, 0.5 mL of reagent 1 (phosphate buffer, α-oxoglutarate, and L-alanine) was added to test tubes containing 0.1 mL of sample and 0.1 mL of distilled water/reagent blank. The mixture was incubated at 37°C for 30 minutes. Exactly 0.5 mL of 2,4-dinitrophenylhydrazine (reagent 2) was added, mixed, and incubated at 20 - 25°C for 20 minutes. Precisely, 5 mL of reagent 3 (sodium hydroxide) was carefully added and mixed, and the sample's absorbance was measured against the reagent blank at 540 nm after 5 minutes. The concentration (U/L) of ALT was determined from the standard calibration table provided in the manual of randox kit.

3.2.7.2 Determination of Plasma Aspartate Aminotransferase (AST) Activity

The AST activity was determined as described by Reitman and Frankel (1957).

Principle:

α-Oxoglutarate + L-aspartate $\xrightarrow{\text{AST}}$ L - glutamate + Oxaloacetate

To determine AST activity, the concentration of oxaloacetate hydrazone, which was produced with 2,4-dinitrophenylhydrazine, was measured at 540 nm and 37°C.

Procedure: Precisely, 0.5 mL of reagent 1 (aspartate, phosphate buffer and α-oxoglutarate) was added to test tubes containing 0.1 mL of sample and 0.1 mL of distilled water/reagent blank. The mixture was incubated at 37°C for 30 minutes. Exactly 0.5 mL of 2,4-dinitrophenylhydrazine (reagent 2) was added, mixed, and incubated at 20 - 25°C for 20 minutes. Precisely, 5 mL of reagent 3 (sodium hydroxide) was carefully added and mixed, and the sample's absorbance was measured against the reagent blank at 540 nm after 5 minutes. The concentration (U/L) of AST was determined from the standard calibration table provided in the manual of randox kit.

3.2.7.3 Determination of Plasma Alkaline Phosphatase (ALP) Activity

The ALP activity was determined as described by Garen and Levinthal (1960).

Principle:

Para-nitrophenylphosphate + H_2O $\xrightarrow{ALP}$ phosphate + para-nitrophenol. Alkaline phosphatase will be measured by monitoring the concentration of phosphate hydrazone formed with 2, 4-dinitrophenylhydrazine at 405 nm and 37^{o}C.

Procedure: In a cuvette, 0.01 mL of plasma (test sample) was mixed with 0.5 mL of reagent (Diethanolamine buffer, $MgCl_2$, p-nitrophenylphosphate). The initial absorbance was measured at 405 nm, and subsequently after 1, 2 and 3 minutes. Alkaline phosphatase activity (U/L) was calculated using the formula provided as shown below:

ALP (U/L) = 2760 × ΔA at 405nm/min, Where ΔA = Changes in absorbance ($A_{1/2/3} - A_{initial}$).

3.2.7.4 Determination of Plasma Total Protein (TP) Concentration

The TP concentration was determined as described by Lowry *et al.* (1951) with mild modification.

Principle:

A coloured complex is formed when cupric ions in an alkaline medium interact with protein peptide bonds.

Procedure: Precisely, 1 mL of R1 (Biuret reagent) was pipetted into test tubes containing 0.02 mL each (sample, distilled water, and standard); the tubes were mixed and incubated for 30 minutes at 25°C. Using a colorimeter set to 546 nm, the absorbance of both the sample (A_{sample}) and the standard ($A_{standard}$) was measured against the reagent blank. Using the formula provided by the kit manufacturer, TP was estimated: Total protein (mg/dL) = $A_{sample}/A_{standard}$ x concentration of standard.

3.2.7.5 Determination of Plasma Albumin (ALB) Concentration

The ALB concentration was determined as described by Doumas *et al*. (1971).

Principle:

Serum albumin is quantified by measuring its binding to bromocresol green, an indicator known as 3,3',5,5'-tetrabromo-m-cresol sulphonephthalein. Absorbance at 578 nm is directly proportional to the concentration of albumin in the sample.

Procedure: Precisely, 3 mL of Bromocresol green (BCG) was pipetted into test tubes containing 0.01 mL each (distilled water, sample, and standard); the tubes were mixed and incubated for 5 minutes at 25°C. Using a colorimeter set to 578 nm, the absorbance of both the sample (A_{sample}) and the standard ($A_{standard}$) was measured against the reagent blank. Using the formula provided by the kit manufacturer, albumin was estimated: Albumin (mg/dL) = $A_{sample}/A_{standard}$ x concentration of standard.

3.2.7.6 Determination of Plasma Total Bilirubin (TB) Concentration

The TB concentration was determined as described by Jendrassik and Grof (1938).

Principle:

In an alkaline medium, bilirubin and diazotised sulphanilic acid react to produce an azobilirubin complex, which is blue in colour. At 578 nm, the azobilirubin complex absorbs light, and this absorption is proportional to the sample's bilirubin concentration. Caffeine releases albumin-bound bilirubin when it reacts with diazotised sulphanilic acid, allowing the determination of total bilirubin concentration.

Procedure: Precisely, 0.2 mL of R1 (hydrochloric acid, sulphanilic acid) and 0.05 mL of R2 (Sodium nitrite) were pipetted into test tubes (sample, and sample blank); the tubes were mixed, 1 mL of R3 (Caffeine, sodium benzoate) and 0.2 mL of plasma (test sample) were added to each of the test tubes, mixed and incubated for 10 minutes at 25°C. Finally, 1 mL of R4 (Tartrate, sodium hydroxide) was added to both test tubes, mixed and incubated for 5 minutes at 25°C. Using a colorimeter set to 578 nm, the absorbance of sample (A_{sample}) was measured against the sample blank (A_{TB}). Using the formula provided by the kit manufacturer, TB was estimated: Total bilirubin (mg/dL) = A_{TB} x 10.8.

3.2.7.7 Determination of Plasma Direct Bilirubin (DB) Concentration

The DB concentration was determined as described by Jendrassik and Grof (1938).

Principle:

Azobilirubin complex, which is blue in colour, is formed when direct bilirubin reacts with diazotised sulphanilic acid in an alkaline medium. Absorbance at 578 nm is directly proportional to the concentration of direct bilirubin in the sample.

Procedure: Precisely, 0.2 mL of R1 (hydrochloric acid, sulphanilic acid) and 0.05 mL of R2 (sodium nitrite) were pipetted into test tubes (sample and sample blank); the tubes were mixed, and 2 mL of 0.9 % NaCl and 0.2 mL of plasma (sample) were added to each of the test tubes, mixed and incubated for 10 minutes at 25°C. Using a colorimeter set to 546 nm, the absorbance

of the sample (A_{sample}) was measured against the sample blank (A_{TB}). Using the formula provided by the kit manufacturer, DB was estimated: Direct bilirubin (mg/dL) = A_{TB} x 14.4

3.2.7.8 Determination of Plasma Urea Concentration

The urea concentration was determined as described by Searcy *et al.* (1967).

Principle:

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_4^+ + \text{Salicylate} + NaClO \xrightarrow[OH^-]{\text{Nitoprusside}} \text{Indophenol} + NaCl$$

Urease breaks down urea into its component gases (ammonia and carbon dioxide). A green chromophore is produced when ammonia reacts with alkaline hypochlorite, sodium salicylate, and sodium nitroprusside, which serves as the coupling agent. The green chromophore absorbs at 600 nm; the absorbance is a direct correlation to the concentration of urea in the sample.

Procedure: Precisely, 10 µL of (plasma, distilled water, standard) were pipetted into test tubes. Exactly 1 mL of working reagent (urease, phosphate buffer pH 6.9, EDTA, sodium salicylate, and sodium nitroprusside) was pipetted into each of the tubes, mixed, and incubated at 37°C for 5 minutes. Lastly, 1 mL of R3 (sodium hypochlorite, sodium hydroxide) was added to each test tube, mixed and incubated for 5 minutes at 37°C. Using a colorimeter set to 600 nm, the absorbance of (sample/standard) was measured against a reagent blank. Using the manufacturer-supplied formula, urea concentration was determined: Concentration of urea (mg/dL) = A_{sample} / $A_{standard}$ x concentration of standard.

3.2.7.9 Blood Urea Nitrogen (BUN)

Blood urea nitrogen was calculated as follow:

Blood urea nitrogen (mg/dL) = Urea (mg/dL) x 0.467

3.2.7.10 Determination of Plasma Creatinine Concentration

The creatinine concentration was determined as described by Liu *et al.* (2012).

Principle:

A coloured complex is formed when picric acid reacts with creatine in an alkaline solution. The absorbance of the coloured complex at 492 nm is directly proportional to the concentration of creatinine in the sample.

Procedure: Precisely, 1 mL of working reagent (picric acid, sodium hydroxide) was pipetted into test tubes containing 0.1 mL each (sample and standard). After 30 seconds, at 492 nm, absorbance (A_1) of the mixtures was taken, and exactly 2 minutes later, absorbance (A_2) of the mixtures was taken. The change in absorbance (ΔA) of the sample and standard was calculated as $A_2 - A_1$, which was used to estimate the concentration of plasma creatinine in the sample. Concentration of creatinine (mg/dL) = ΔA sample/ΔA standard x concentration of standard.

3.2.7.11 Determination of Concentrations of Plasma Electrolytes

The Electrolytes (Na^+, K^+, Cl^-, HCO_3^-) concentration was determined as described by Tietz (1995).

3.2.7.11.1 Sodium ion (Na^+)

Principle:

A chromophore is produced when sodium reacts with a specific chromogen. The concentration of sodium in the sample is directly proportional to the absorbance of the coloured complex at 630 nm.

Procedure: Precisely, 1 mL of sodium reagent was pipetted into test tubes containing 10 µL each (sample/distilled water/standard); the test tubes were mixed, and incubated for 10 minutes, at room temperature. Using a colorimeter set to 630 nm, the absorbance of the sample (A_{sample})

and standard ($A_{standard}$) were measured against the reagent blank. Using the formula provided by the kit manufacturer, Na^+ was estimated:

Concentration of sodium ion (mmol/L) = A_{sample}/ $A_{standard}$ x concentration of standard.

3.2.7.11.2 Potassium ion (K^+)

Principle:

The formation of a turbid emulsion by potassium ions and tetraphenylborate (TPB) at an alkaline pH can be measured using a photometer at 578 nm. The increase in absorbance is a direct correlation with the concentration of potassium in the sample.

Procedure: Precisely, 1 mL of potassium reagent was pipetted into test tubes containing 20 µL each (sample/distilled water/standard); the test tubes were mixed, and incubated for 5 minutes, at room temperature. Using a colorimeter set to 578 nm, the absorbance of the sample (A_{sample}) and standard ($A_{standard}$) were measured against the reagent blank. Using the formula provided by the kit manufacturer, K^+ was estimated:

Concentration of potassium ion (mmol/L) = A_{sample}/ $A_{standard}$ x concentration of standard.

3.2.7.11.3Chlorideion (Cl^-)

Principle: A coloured complex with mercuric thiocyanate is formed when Cl^- reacts with ferric nitrate in an acidic environment. The concentration of Cl^- in the sample is directly proportional to the absorbance of the coloured complex at 505 nm.

Procedure: Precisely, 1 mL of chloride reagent was pipetted into test tubes containing 10 µL each (sample/distilled water/standard); the test tubes were mixed, and incubated for 5 minutes, at room temperature. Using a colorimeter set to 505 nm, the absorbance of the sample (A_{sample}) and standard ($A_{standard}$) were measured against the reagent blank. Using the formula provided by the kit manufacturer, Cl^- was estimated:

Concentration of chloride ion (mmol/L) = A_{sample}/ $A_{standard}$ x concentration of standard.

3.2.7.11.4 Bicarbonate ion (HCO_3^-)

Principle:

$PEP + HCO_3^- \xrightarrow{PEPC} Oxaloacetate + H_2PO_4^-$

$Oxaloacetate + Reduced\ cofactor + H^+ \xrightarrow{MDH} Malate + Cofactor$

Oxaloacetate is produced in a reaction catalyzed by phosphoenolpyruvate carboxylase (PEPC). In the presence of malate dehydrogenase (MDH), the reduced cofactor is oxidized by oxaloacetate. The concentration of the reduced cofactor is measured at 340 nm and is directly proportional to the total concentration of carbon dioxide in the sample.

Procedure: Precisely, 1 mL of reagent was pipetted into test tubes containing 10 µL each (sample/standard); the test tubes were mixed. Using a spectrophotometer set to 340 nm, the absorbance (A1) of the sample/standard was measured after 2 minutes. The absorbance (A2) of the sample/standard was measured after 10 minutes. Using the formula provided by the kit manufacturer, HCO_3^- was estimated:

Concentration of HCO_3^- (mmol/L) = $\Delta A_{sample}/\Delta A_{standard}$ x concentration of standard.

3.2.7.12 Determination of Plasma Total Cholesterol (TC) Concentration

The TC concentration was determined as described by Richmond (1973).

Principle:

Cholesterol esterase breaks down cholesteryl esters, which constitute a significant amount of blood cholesterol, into free fatty acids and cholesterol. Cholesterol oxidase produces hydrogen peroxide by oxidizing cholesterol. In the presence of phenols and peroxidases, a coloured complex (quinoneimine) is produced from hydrogen peroxide and 4-aminoantipyrine, which can be measured at 500 nm.

$$\text{Cholesterol ester} + H_2O \xrightarrow[\text{Esterase}]{\text{Cholesterol}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow[\text{Oxidase}]{\text{Cholesterol}} \text{Cholestene-3-one} + H_2O_2$$

$$2H_2O_2 + \text{phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{quinoneimine} + 4H_2O$$

Procedure: Precisely, 1 mL of cholesterol reagent was pipetted into test tubes containing 10 µL each (sample/distilled water/cholesterol standard); the test tubes were mixed and incubated for 5 minutes at 37°C. Using a colorimeter set to 500 nm, the absorbance of the sample (A_{sample}) and standard ($A_{standard}$) were measured against the reagent blank. Using the formula provided by the kit manufacturer, the concentration of TC was estimated:

Concentration of TC (mg/dL) = A_{sample} / $A_{standard}$ x concentration of standard.

3.2.7.13 Determination of Plasma Triacylglycerol (TG) Concentration

The TG concentration was determined as described by Tietz (1994).

Principle:

The presence of triacylglycerol is established by the use of enzymatic hydrolysis (lipase). The indicator (quinoneimine) is made by catalyzing the reaction of hydrogen peroxide, 4-aminophenazone, and 4-chlorophenol with peroxidase.

$$\text{Triacylglycerol} + H_2O \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{Fatty acids}$$

$$\text{Glycerol} + ATP \xrightarrow{\text{Glycerol kinase}} \text{Glycerol -3-phosphate} + ADP$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow[\text{Oxidase}]{\text{Glycerol-3-phosphate}} \text{Dihydroxyacetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-Aminophanazone} + \text{4-Chlorophenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + HCl + 4H_2O$$

Procedure: Precisely, 1 mL of triacylglycerol reagent was pipetted into test tubes containing 10 µL each (sample/distilled water/triacylglycerol standard); the test tubes were mixed and incubated for 5 minutes at 37°C. Using a colorimeter set to 500 nm, the absorbance of the sample (A_{sample}) and standard ($A_{standard}$) were measured against the reagent blank. Using the formula provided by the kit manufacturer, the concentration of TG was estimated:

Concentration of TG (mg/dL) = A_{sample} / $A_{standard}$ x concentration of standard.

3.2.7.14 Determination of Plasma High-Density Lipoprotein Cholesterol (HDL-C) Concentration

The HDL-C concentration was determined as described by Friedewald (1972).

Principle:

Quantitative precipitation of chylomicron fractions and low-density lipoproteins (LDL and VLDL) is achieved by adding phosphotungstic acid in the presence of magnesium ions. The supernatant fraction (HDL-C) after centrifugation is then measured to determine the HDL-C level in a given sample.

Procedure: Precisely, 500 µL of diluted precipitating agent (phosphotungstic acid, magnesium chloride) was pipetted into test tubes containing 200 µL each (sample/standard); the test tubes were mixed and incubated for 10 minutes at room temperature. The mixtures were spun for 10 minutes at 4000 rpm, and supernatants were collected. Precisely, 1 mL of cholesterol reagent was pipetted into test tubes containing 100 µL each (sample supernatant/standard supernatant/distilled water); the test tubes were mixed and incubated for 10 minutes at 25°C. Using a colorimeter set to 500 nm, the absorbance of the sample (A_{sample}) and standard ($A_{standard}$) were measured against the reagent blank. Using the formula provided by the kit manufacturer, the concentration of HDL-C was estimated:

Concentration of HDL-C (mg/dL) = A_{sample} / $A_{standard}$ x concentration of standard.

3.2.7.15 Determination of Plasma Low-Density Lipoprotein Cholesterol (LDL-C) Concentration

The LDL-C concentration was determined as described by Friedewald (1972) using the equation:

LDL (mg/dL) = TC – TG/5 – HDL-C

3.2.7.16 Determination of Malondialdehyde (MDA) Levels in Rat Plasma and Tissues

The MDA concentration was determined as described by Amstrong and Browne (1994).

Principle:

The formation of MDA-TBA adduct (1:2) as a result of the reaction between MDA and thiobarbituric acid (TBA) under acidic and high-temperature conditions is the basis of this test. When examined spectrophotometrically, this complex exhibits absorption maxima between 530 and 540 nm, with a maximum at 532 nm. The sample's absorbance is a direct correlation with the MDA concentration.

Procedure: Precisely, 100 µL of sodium dodecyl sulfate (SDS) was pipetted into test tubes containing 100 µL each (supernatant/standard); the test tubes were mixed, and 2.5 mL of thiobarbituric acid (TBA)/buffer reagent was added to each tube, mixed and incubated for 60 minutes at 95°C. Following the incubation period, the mixtures were cooled in an ice bath for 10 minutes at room temperature. For 15 minutes, the samples were spun in a centrifuge at 1200 x g. Using a spectrophotometer set to 532 nm, the absorbance of the clear supernatant was measured, and the concentration/level of MDA was estimated from the standard MDA.

3.2.7.17 Determination of Activities of Antioxidant Enzymes

3.2.7.17.1 Superoxide Dismutase

The activity of superoxide dismutase (SOD) was determined using Randox SOD detection kit according to the manufacturer’s instructions.

Principle:

A red formazan dye is produced by reacting 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (I.N.T.) with superoxide radicals generated by xanthine and xanthine oxidase (XOD). The level of inhibition of this process is then used to evaluate the activity of superoxide dismutase in a sample. Under these conditions, one unit of SOD inhibits the rate of decrease of INT by 50 %.

$$\text{Xanthine} \xrightarrow{\text{XOD}} \text{Uric acid} + O_2^{\bullet}$$

$$\text{INT} \xrightarrow{O_2^{\bullet}} \text{Formazan dye}$$

OR

$$O_2^{\bullet} + O_2^{\bullet} + 2H^{+} \xrightarrow{\text{SOD}} O_2 + H_2O_2$$

Procedure: Precisely, 1000 µL of R1 (mixed substrate) was pipetted into test tubes containing 30 µL each (supernatant/standard); the test tubes were mixed, and 150 µL of R2 (xanthine oxidase) was added to each tube, mixed, and the initial absorbance (A_1) was measured at 505 nm after 30 seconds. The final absorbance (A_2) was measured after 3 minutes. Superoxide dismutase (SOD) levels were estimated using a standard curve.

3.2.7.17.2 Catalase

The activity of catalase was determined as described by Claiborne (1995).

Principle:

The consumption of the H_2O_2 substrate at 240 nm is a measure of the catalase activity.

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

(Absorbance at 240 nm) ↓ (No Absorbance at 240 nm)

Although catalase can only be saturated with a substrate of up to 5 M H_2O_2, levels exceeding 0.1 M quickly render the enzyme inactive. Substrates need to be present at relatively low concentrations for catalase activity measurements to be accurate. This means that for pH 7.0 and

25°C, one standard definition of catalase activity is the quantity of enzyme required to degrade 1.0 μMole of H_2O_2 substrate (with an initial concentration of 10.3 mM) per minute. A thorough calibration of H_2O_2 must be carried out prior to the experiment to meet the exact beginning substrate requirement.

Procedure: To a clean cuvette, 1000 μL of sample dilution buffer was introduced and then set in the reference cuvette apparatus. After zeroing the instrument, the wavelength was set at 240 nm. Precisely, 950 μL of working assay buffer was pipetted into a clean semi-micro UV cuvette, and 50 μL of diluted standard or sample was added to the cuvette and mixed rapidly. The absorbance at 240 nm was measured immediately every 2 seconds or at the smallest time interval allowed for 0.25 minutes (15 seconds).

3.2.7.17.3 Glutathione Peroxidase

The activity of GPx was determined as described by Rotruck *et al.* (1973).

Principle:

Glutathione peroxidase (GPx) catalyses the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm is measured.

$$2GSH + ROOH \xrightarrow{GPx} ROH + GSSG + H_2O$$

$$GSSG + NADPH + H^+ \xrightarrow{GR} NADP^+ + 2GSH$$

Procedure: Precisely, 0.02 mL of diluting agent was pipetted into test tubes containing 0.02 mL each (diluted sample and distilled water); 1 mL of R1 (phosphate buffer, glutathione, glutathione reductase, NADPH, EDTA) and 0.04 mL of R2 (cumene hydroperoxide) were added to each test

tubes and mixed, and the initial absorbance of the sample and reagent blank was measured at 340 nm after 1 minute. The final absorbance was measured after 2 minutes.

3.2.7.18 Measurement of Haematological Parameters

Haematological indices were analyzed using Horiba ABX 80 haematology analyzer (manufacturer's instructions). Blood samples in EDTA bottles were injected into the chamber of automated haematology system analyzer and diluted with an isotonic solution of saline. Indices analyzed includes haemoglobin, red blood cell count, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin, platelet count, white blood cell count, and mid-cells.

3.2.8 Histological Examination of the Rat Organs

Rats were perfused and their organs (liver, kidney, and pancreas) were fixed with 10 % phosphosaline. The fixed tissues were processed to obtain paraffin wax embedded tissue blocks, which were sectioned (5 – 6 mm) thickness using microtome (Leica, Germany). The tissues were mounted on slides and stained with hematoxylin and eosin for histological examination under light microscope as described by Bancroft and Gamble (2008).

3.3 Data Analysis

Data analysis was carried out using one-way analysis of variance (ANOVA) followed by *post hoc* Tukey's HSD and Duncan multiple range tests (GraphPad Prism 10.4.2), and values are expressed as mean ± standard error mean (SEM). Values of $p < 0.05$ were considered statistically significant.

CHAPTER FOUR

RESULTS

4.1 Determination of Lethal Dose (LD_{50}) of *Chrysophyllum albidum* Stem Bark Extract

Table 4.1 showed the determination of LD_{50} of *C. albidum* extract. The results revealed that the oral LD_{50} of *C. albidum* stem bark extract was > 5000 mg/kg bwt.

Table 4.1: Oral LD_{50} of *C. albidum* Stem Bark Extract

Phase	Group	N	Dose (mg/kg bwt)	Remark
I	1	3	0	No Death
	2	3	10	No Death
	3	3	100	No Death
	4	3	1000	No Death
II	1	3	0	No Death
	2	3	1600	No Death
	3	3	2900	No Death
	4	3	5000	No Death

N = Number of rats, LD_{50} = Lethal dose = $\sqrt{}$ (highest non-lethal dose) × (lowest lethal dose)

4.2 Effects of *C. albidum* Stem Bark Extract/Fractions on Body Weights of Rats

Table 4.2 showed the effects of *C. albidum* stem bark extract/fractions on body weight in STZ-induced diabetic rats. There was a marked decrease in body weight of diabetic rats when

compared with the normal control ($p < 0.05$). However, administration of *C. albidum* extract and its fractions markedly restored the body weight loss. Similarly, the standard drug, metformin (50 mg/kg bwt), markedly elevated the rats' body weight when compared with diabetic untreated rats ($p < 0.05$). The hydroethanol fraction showed the highest body weight increase when compared with ethanol extract and other fractions.

Figure 4.1 showed the effects of *C. albidum* stem bark extract/fractions on body weight gain in STZ-induced diabetic rats. A negative body weight gain was observed in diabetic untreated rats when compared with the normal control. However, there was significant increase in body weight gain of diabetic rats treated with *C. albidum* extract/fractions ($p < 0.05$). The percentage body weight gain of rats treated with ethanol extract, and hydroethanol, n-hexane, ethyl acetate, and methanol fractions were 32.12, 37.83, 35.96, 34.43, and 28.49 %, respectively. Similarly, the standard drug, metformin, significantly increased the percentage body weight gain (23.38 %) when compared with diabetic untreated group ($p < 0.05$). Hydroethanol fraction showed the highest percentage body weight gain when compared with crude extract, metformin and other fractions.

Table 4.2: Effects of *C. albidum* Stem Bark Extract/Fractions on Rat Body Weight

Group	Day 0 (g)	Day 1 (g)	Day 7 (g)	Day 14 (g)
Control	154.00 ± 2.08	161.30 ± 4.10	176.00 ± 2.65	203.70 ± 3.48
Diabetic	176.30 ± 7.31[a]	163.00 ± 7.51[a]	151.70 ± 7.51[a]	130.70 ± 10.40[b]
Metformin	174.70 ± 6.08[ac]	155.00 ± 4.59[ac]	166.30 ± 4.41[ac]	191.3 ± 7.31[ad]
Ethanol	160.70 ± 2.96[ac]	143.00 ± 3.51[ac]	159.30 ± 6.44[ac]	189.00 ± 6.66[ad]
Hydroethanol	188.30 ± 13.37[bc]	164.00 ± 10.69[ac]	178.30 ± 12.03[ac]	224.00 ± 23.58[ad]
n-Hexane	163.70 ± 6.89[ac]	147.30 ± 7.22[ac]	173.30 ± 8.09[ac]	200.30 ± 10.59[ad]

Ethyl acetate	162.00 ± 3.22^{ac}	145.00 ± 3.22^{ac}	171.00 ± 2.52^{ac}	195.00 ± 7.10^{ad}
Methanol	168.30 ± 4.63^{ac}	153.00 ± 3.79^{ac}	169.70 ± 4.98^{ac}	196.70 ± 6.64^{ad}

Values are expressed as mean ± SEM (n=7). ^{b}p <0.05, ^{a}p> 0.05 in comparison with normal control. ^{d}p <0.05, ^{c}p>0.05 when compared with diabetic untreated rats.

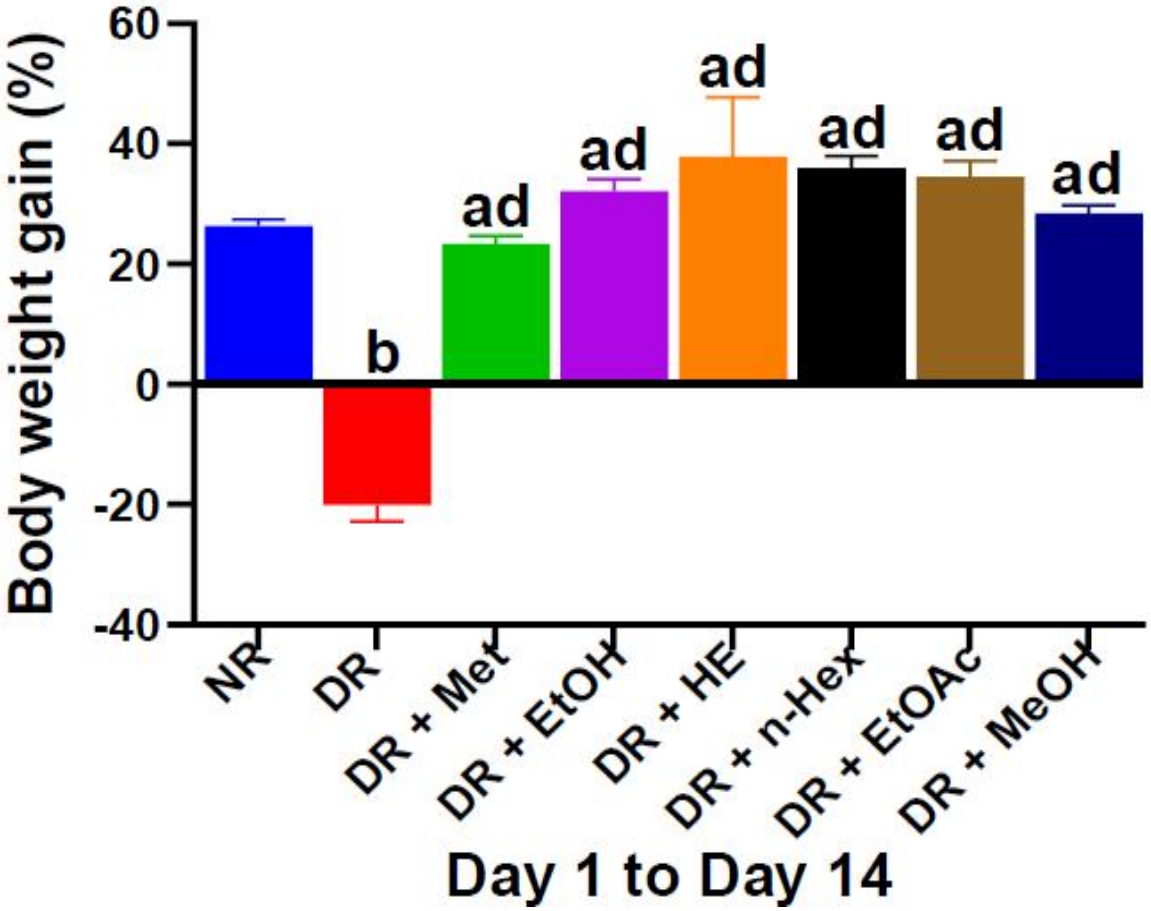

Figure 4.1: Effects of *C. albidum* stem bark extract/fractions on body weight gain in STZ-induced diabetic rats after 14 days treatment. Values are expressed as mean ± SEM (n=7). ^{b}p < 0.05, ^{a}p > 0.05 in comparison with the normal control. ^{d}p < 0.05, ^{c}p > 0.05 when compared with diabetic untreated rats. NR: Normal rat, DR: Diabetic rat, Met: Metformin, EtOH, HE, n-Hex, EtOAc and MeOH: Ethanol extract of *C. albidum* stem bark, Hydroethanol, n-Hexane, Ethyl acetate, and Methanol fractions of *C. albidum* stem bark, respectively.

4.3 Effects of *C. albidum* Stem Bark Extract/Fractions on Relative Organ Body Weight Ratio

Table 4.3 showed the effects of *C. albidum* stem bark extract/fractions on relative organ body weights ratio in STZ-induced diabetic rats. The results showed a marked increase in relative organ weight for liver/kidneys of diabetic untreated rats while a decrease was observed in pancreas when compared with the normal control rats ($p < 0.05$). Treatment of diabetic rats with *C. albidum* extract/fractions or standard drug (metformin) markedly restored the effect of STZ on relative weights of liver and kidney. Treatment with *C. albidum* extract/fractions restored the relative weight of pancreas at a non-significant level ($p > 0.05$). Similar trend was seen in the group administered standard drug, metformin, when compared with the diabetic untreated rats ($p > 0.05$).

Table 4.3: Effects of *Chrysophyllum albidum* Stem Bark Extract/Fractions on Relative Organ Body Weights Ratio of Rats

Group	Liver (%)	Kidney (%)	Pancreas (%)
Control	2.01 ± 0.10	0.38 ± 0.05	0.41 ± 0.01
Diabetic	4.51 ± 0.56^{b}	1.04 ± 0.51^{b}	0.30 ± 0.49^{a}
Metformin	2.27 ± 0.12^{ad}	0.48 ± 0.00^{ad}	0.38 ± 0.03^{ac}
Ethanol	2.40 ± 0.13^{ad}	0.50 ± 0.03^{ad}	0.37 ± 0.05^{ac}
Hydroethanol	2.18 ± 0.30^{ad}	0.47 ± 0.06^{ad}	0.42 ± 0.08^{ac}
n-Hexane	2.30 ± 0.13^{ad}	0.42 ± 0.03^{ad}	0.27 ± 0.00^{ac}
Ethyl acetate	2.48 ± 0.26^{ad}	0.46 ± 0.02^{ad}	0.33 ± 0.01^{ac}
Methanol	2.15 ± 0.11^{ad}	0.45 ± 0.01^{ad}	0.45 ± 0.03^{ac}

Values are expressed as mean ± SEM (n =7). $^{b}p < 0.05$, $^{a}p > 0.05$ in comparison with normal control. $^{d}p < 0.05$, $^{c}p > 0.05$ when compared with the diabetic untreated rats.

4.4 Effects of *Chrysophyllum albidum* Stem Bark Extract/Fractions on Blood Glucose

Figure 4.2 showed the effects of *C. albidum* stem bark extract/fractions on plasma glucose in STZ-induced diabetic rats. Intraperitoneal injection of STZ (50 mg/kg bwt) markedly elevated the blood glucose level of rats when compared with the normal control group ($p < 0.05$). However, administration of *C. albidum* extract/fractions considerably decreased the blood glucose levels to near normal ($p < 0.05$). Similarly, there was significant decrease in blood glucose level of diabetic rats treated with standard anti-diabetic drug, metformin ($p < 0.05$). The glycemic changes calculated for crude extract, and hydroethanol, n-hexane, ethyl acetate, and methanol fractions, and metformin were 51.27, 50.27, 53.98, 48.37, 75.28, and 66.15 %, respectively. The methanol fraction of *C. albidum* stem bark showed the highest glycemic change when compared with other fractions and metformin.

4.5 Effects of *Chrysophyllum albidum* Stem Bark Extract/Fractions on Liver Enzymes

Figure 4.3 showed the effects of *C. albidum* stem bark extract/fractions on AST, ALT and ALP in STZ-induced diabetic rats. Results obtained revealed a marked increase in plasma activities of AST, ALT, ALP, and AST:ALT in diabetic untreated rats when compared with the control ($p < 0.05$). However, treatment with *C. albidum* extract/fractions considerably decreased the activities of these enzymes relative to diabetic untreated rats ($p < 0.05$). Similar trend was observed with metformin ($p < 0.05$).

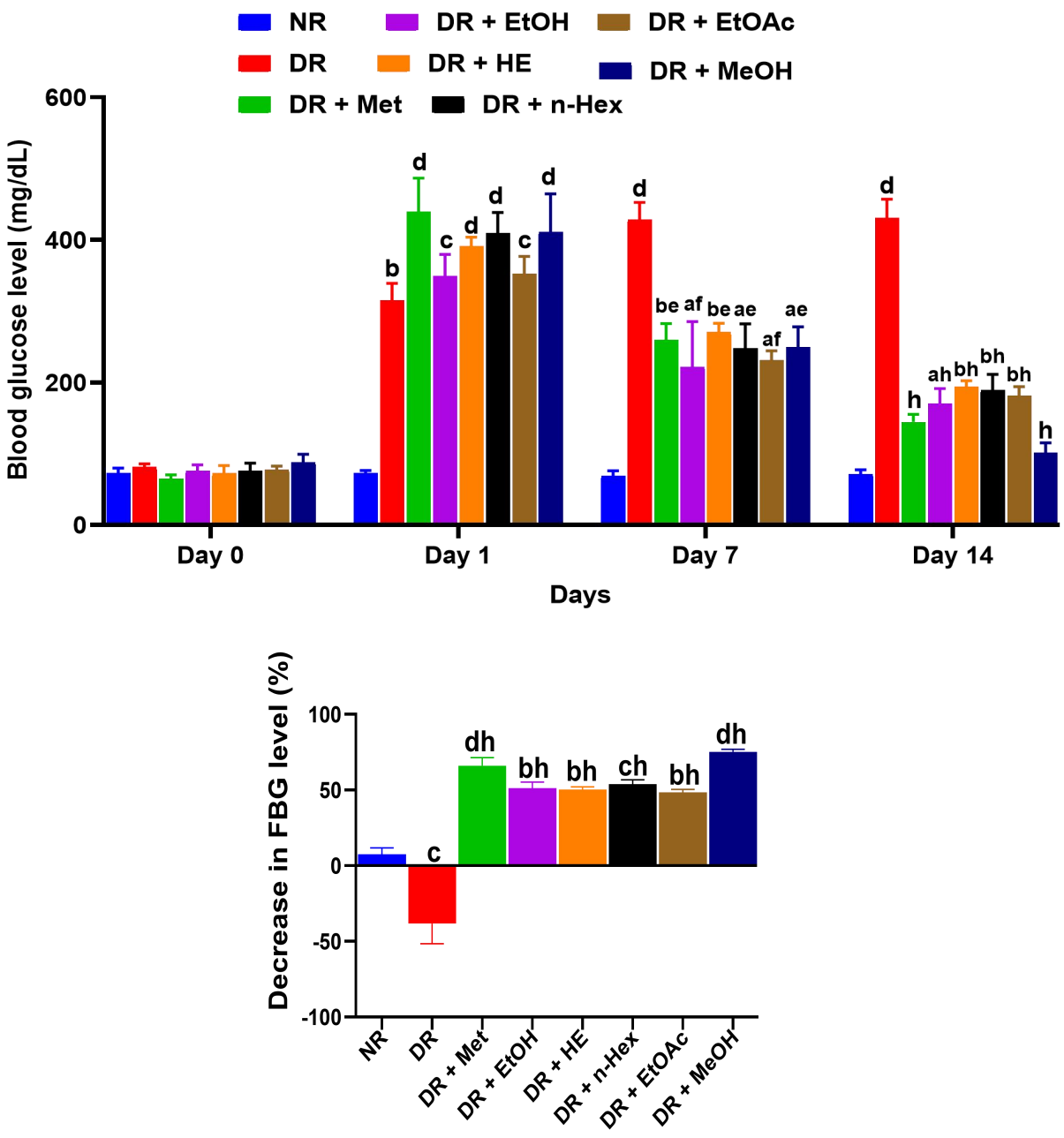

Figure 4.2: Effects of *C. albidum* stem bark extract/fractions on plasma glucose in STZ-induced diabetic rats after 14 days of treatment. Values are expressed as mean ± SEM (n=7). $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$, $^{d}p < 0.0001$, $^{\#}p > 0.05$ in comparison with normal control. $^{e}p < 0.05$, $^{f}p < 0.01$, $^{g}p < 0.001$, $^{h}p < 0.0001$ when compared with the diabetic untreated rats.

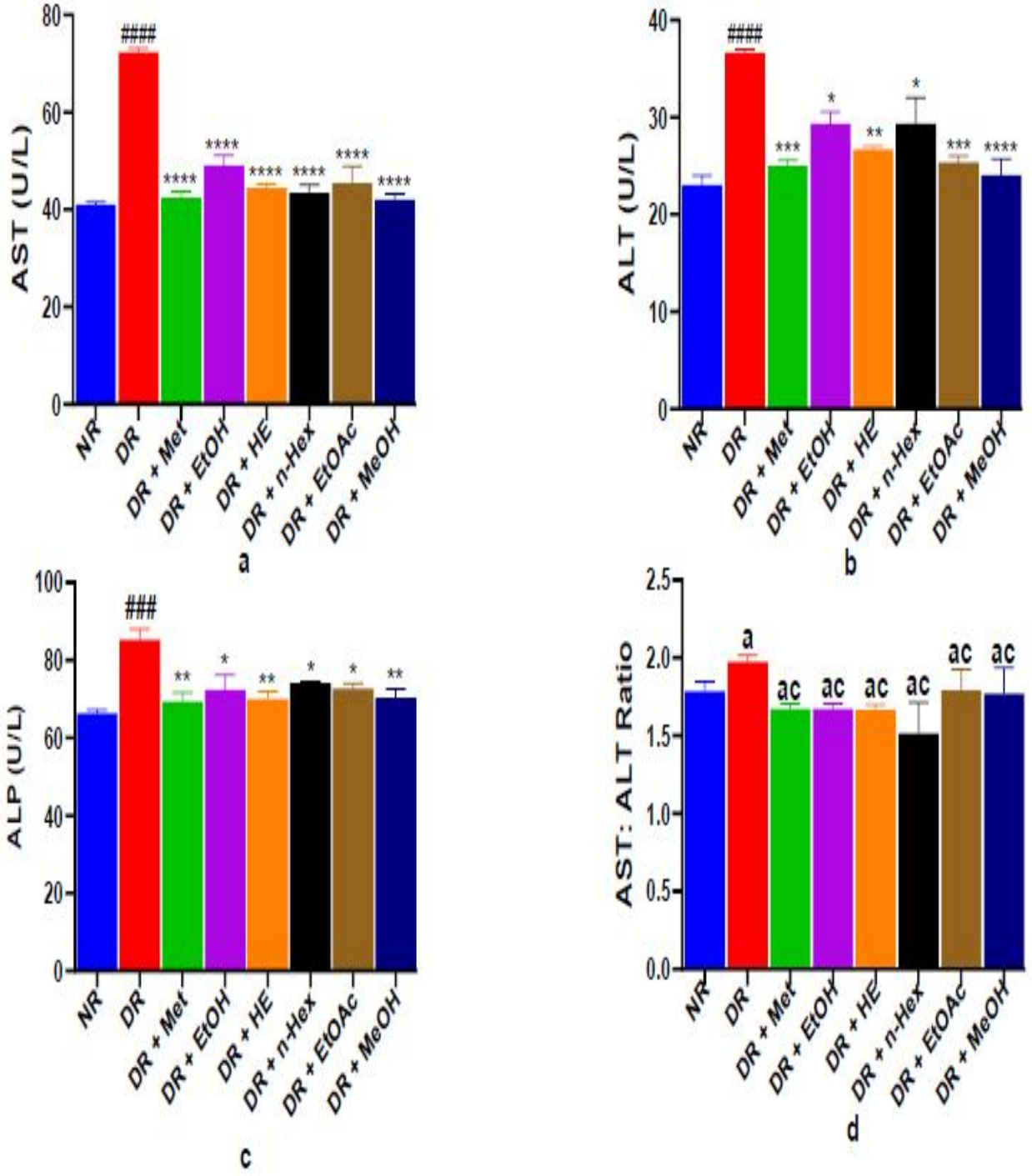

Figure 4.3: Effects of *C. albidum* stem bark extract/fractions on liver enzymes in STZ-induced diabetic rats after 14 days of treatment. Values are expressed as mean ± SEM (n=7). $^{\#}p<0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, $^{\#\#\#\#}p < 0.0001$, $^{a}p > 0.05$ in comparison with control. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, $^{c}p > 0.05$ when compared with diabetic untreated rats.

4.6 Effects of *Chrysophyllum albidum* Stem Bark Extract/Fractions on Liver Function Indices

Figure 4.4 showed the effects of *C. albidum* stem bark extract/fractions on total protein (TP), albumin (ALB), globulin (GLB), total bilirubin and direct bilirubin levels in STZ-induced diabetic rats. Results showed marked decreases in plasma TP, ALB, and GLB levels, but considerably elevated total bilirubin and direct bilirubin levels in diabetic untreated group when compared with normal control ($p < 0.05$). Oral administration of ethanol extract or methanol fraction of *C. albidum* stem bark significantly restored the levels of TP and ALB to near normal ($p < 0.05$). No significant increase was seen in hydroethanol, n-hexane, and ethyl acetate fractions when compared with diabetic untreated group ($p > 0.05$). In addition, treatment with *C. albidum* extract/fractions markedly restored the levels of total bilirubin and direct bilirubin ($p < 0.05$). The highest reduction was observed in the methanol fraction. Similar trend was seen in the group that was treated with metformin.

4.7 Effects of *Chrysophyllum albidum* Stem Bark Extract/Fractions on Lipid Profile and Atherogenic Index of Rats

Figure 4.5 showed the effects of *C. albidum* stem bark extract/fractions on total cholesterol (TC), triacylglycerol (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and atherogenic index in STZ-induced diabetic rats. The lipid profile results showed marked increases in TC, TG, LDL-C, and atherogenic index, while significant decrease in HDL-C was seen in diabetic untreated group when compared with control ($p < 0.05$). However, administration of *C. albidum* extract/fractions significantly decreased TC, TG, LDL-C, and atherogenic index, but it elevated HDL-C ($p < 0.05$). Treatment with metformin followed a similar trend, but methanol fraction produced the best response.

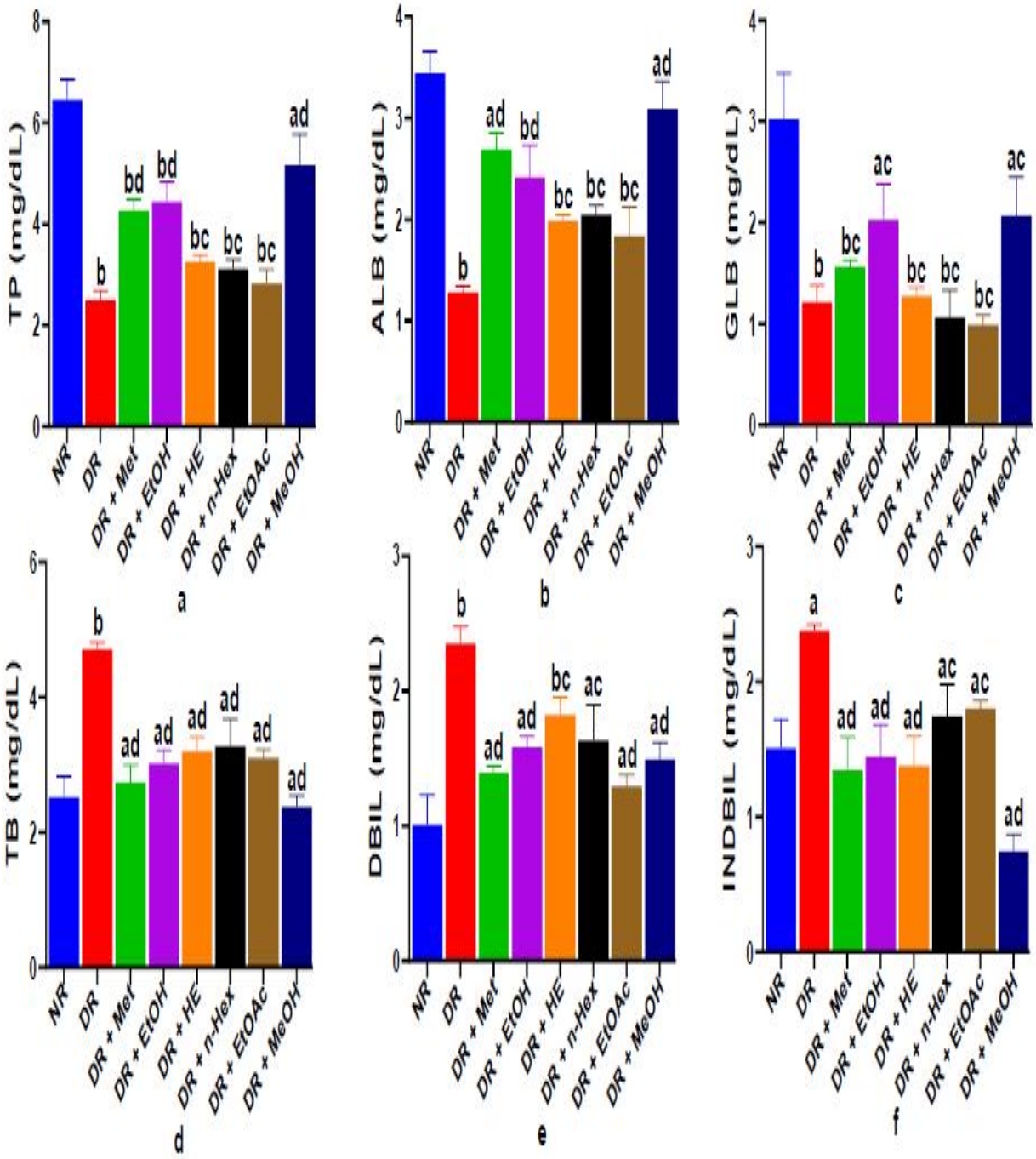

Figure 4.4: Effects of *C. albidum* stem bark extract/fractions on liver function indices in STZ-induced diabetic rats. Values are expressed as mean ± SEM (n = 7). ^{b}p <0.05, ^{a}p>0.05 in comparison with normal rats. $^{d}p < 0.05$, $^{c}p > 0.05$ when compared with diabetic untreated rats.

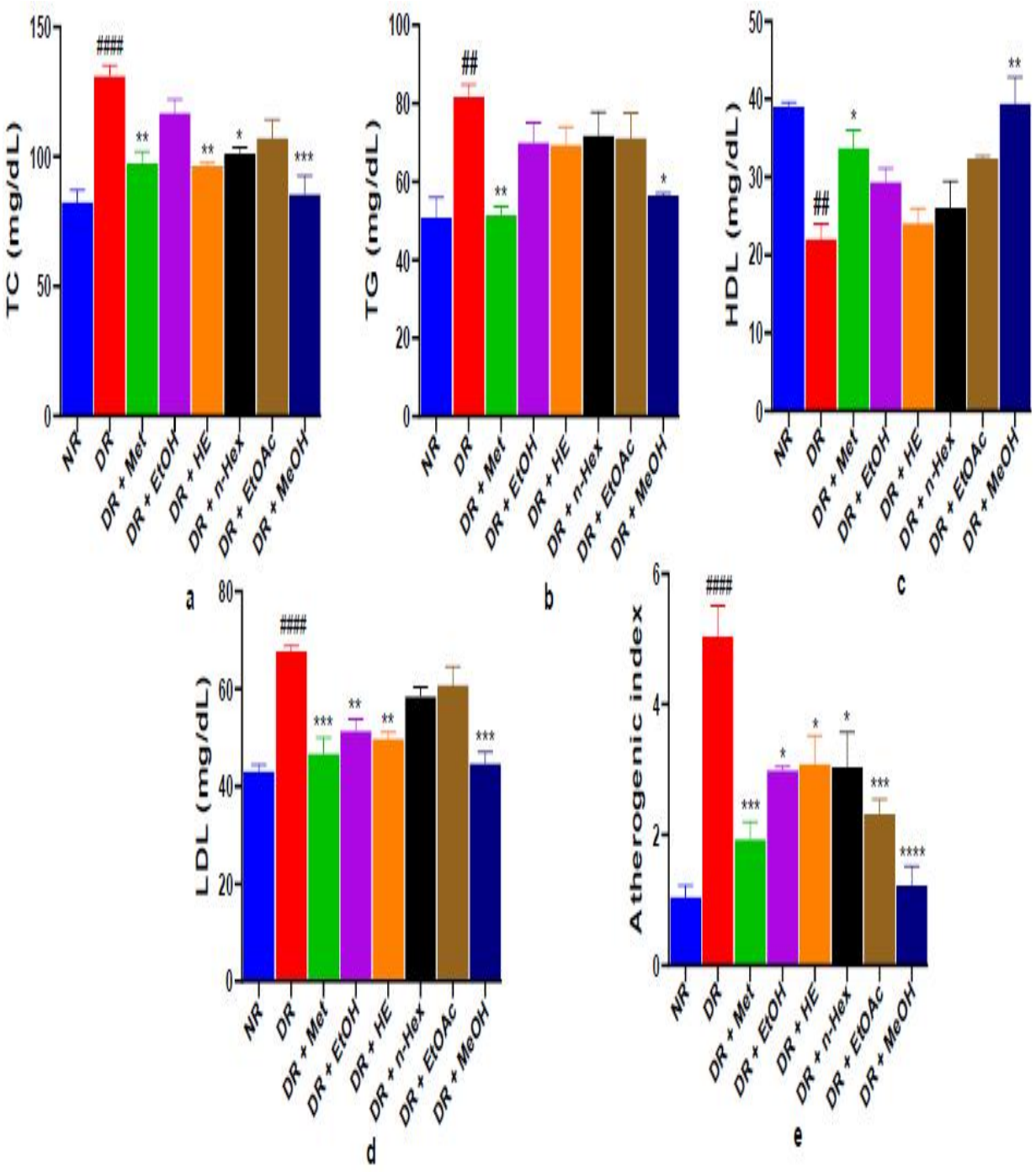

Figure 4.5: Effects of *C. albidum* extract/fractions on lipid profile and atherogenic index in STZ-induced diabetic rats. Values are expressed as mean ± SEM (n=7). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$,

$^{###}p < 0.001$, $^{####}p < 0.0001$ in comparison with normal control rats. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ when compared with diabetic untreated rats.

4.8 Effects of *Chrysophyllum albidum* Stem Bark Extract/Fractions on Rats Renal Function Indices

Figure 4.6 showed the effects of *C. albidum* stem bark extract/fractions on renal function markers in STZ-induced diabetic rats. The results revealed marked increases in urea, creatinine, and blood-urea-nitrogen (BUN) levels of diabetic untreated rats when compared with normal rats ($p < 0.05$). Oral administration of *C. albidum* extract/fractions or metformin significantly restored the alterations in renal function parameters to near normal values ($p < 0.05$). There were significant decreases in sodium (Na^+), potassium (K^+) and bicarbonate (HCO_3^-) levels in diabetic rats when compared with control ($p < 0.05$). Treatment with *C. albidum* extract/fractions/metformin considerably restored the alterations in electrolytes to near normal values when compared with diabetic untreated rats (($p < 0.05$).

4.9 Hemostatic Effects of *Chrysophyllum albidum* Stem Bark Extract/Fractions in STZ-Induced Diabetic Rats

Figure 4.6 showed that administration of 50 mg/kg bwt STZ significantly decreased red blood cell (RBC), haemoglobin (Hb), haematocrit (HCT), and platelet count when compared with normal rats ($p < 0.05$). No marked decrease was observed in mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) in diabetic control rats when compared with normal rats ($p > 0.05$). Treatment with *C. albidum* extract/fractions significantly alleviated the effect of STZ on RBC, Hb, HCT and platelet counts. Same trend was observed in diabetic group treated with standard drug (metformin). Furthermore, no marked increase was

observed in MCHC and MCH in diabetic rats treated with *C. albidum* extract/fractions when compared with diabetic untreated rats ($p > 0.05$).

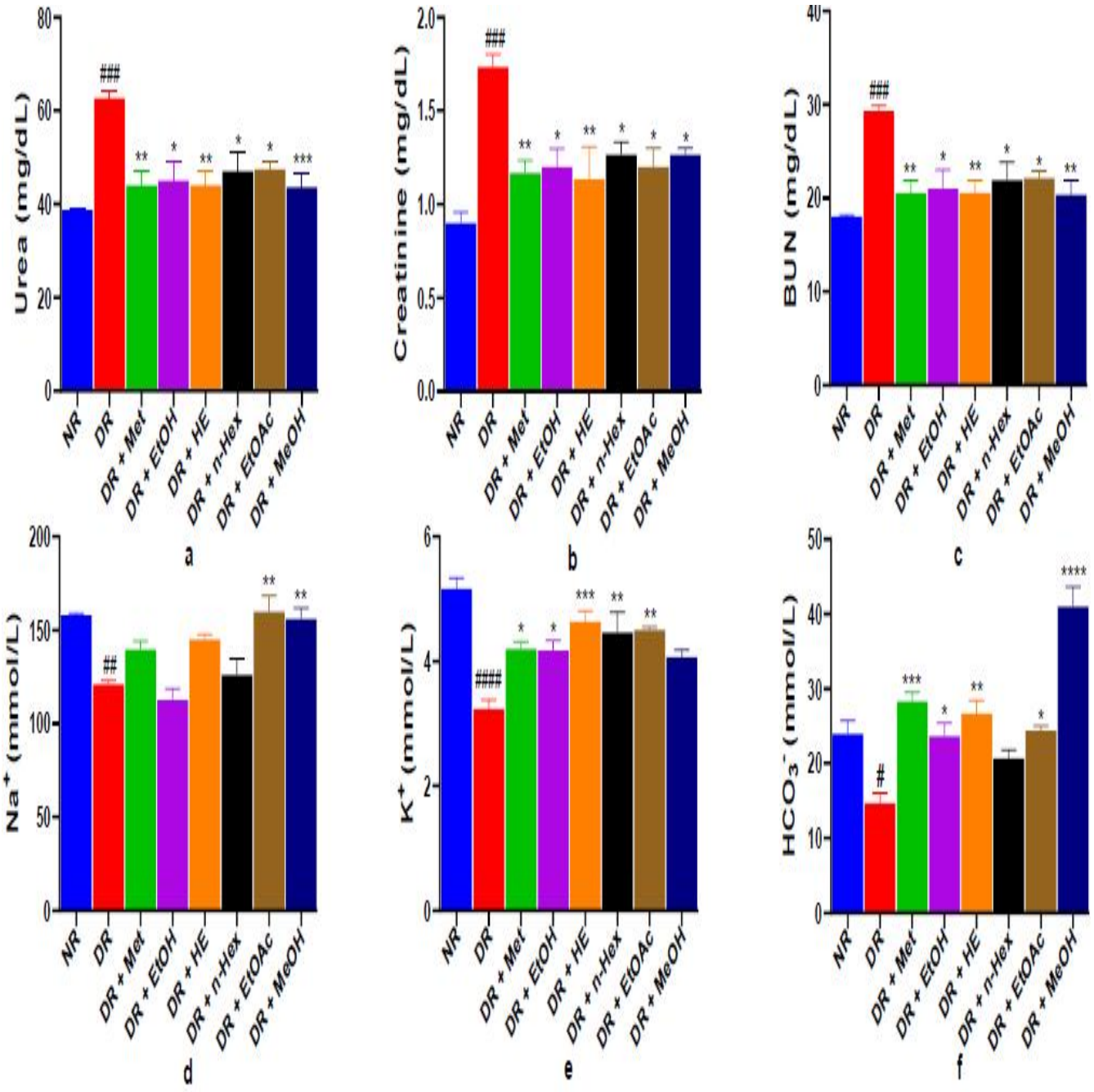

Figure 4.6: Effects of *C. albidum* stem bark extract/fractions on renal function markers in STZ-induced diabetic rats. Values are expressed as mean ± SEM (n=7). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$,

$^{###}p < 0.001$, $^{####}p < 0.0001$ in comparison with control. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ when compared with diabetic untreated rats.

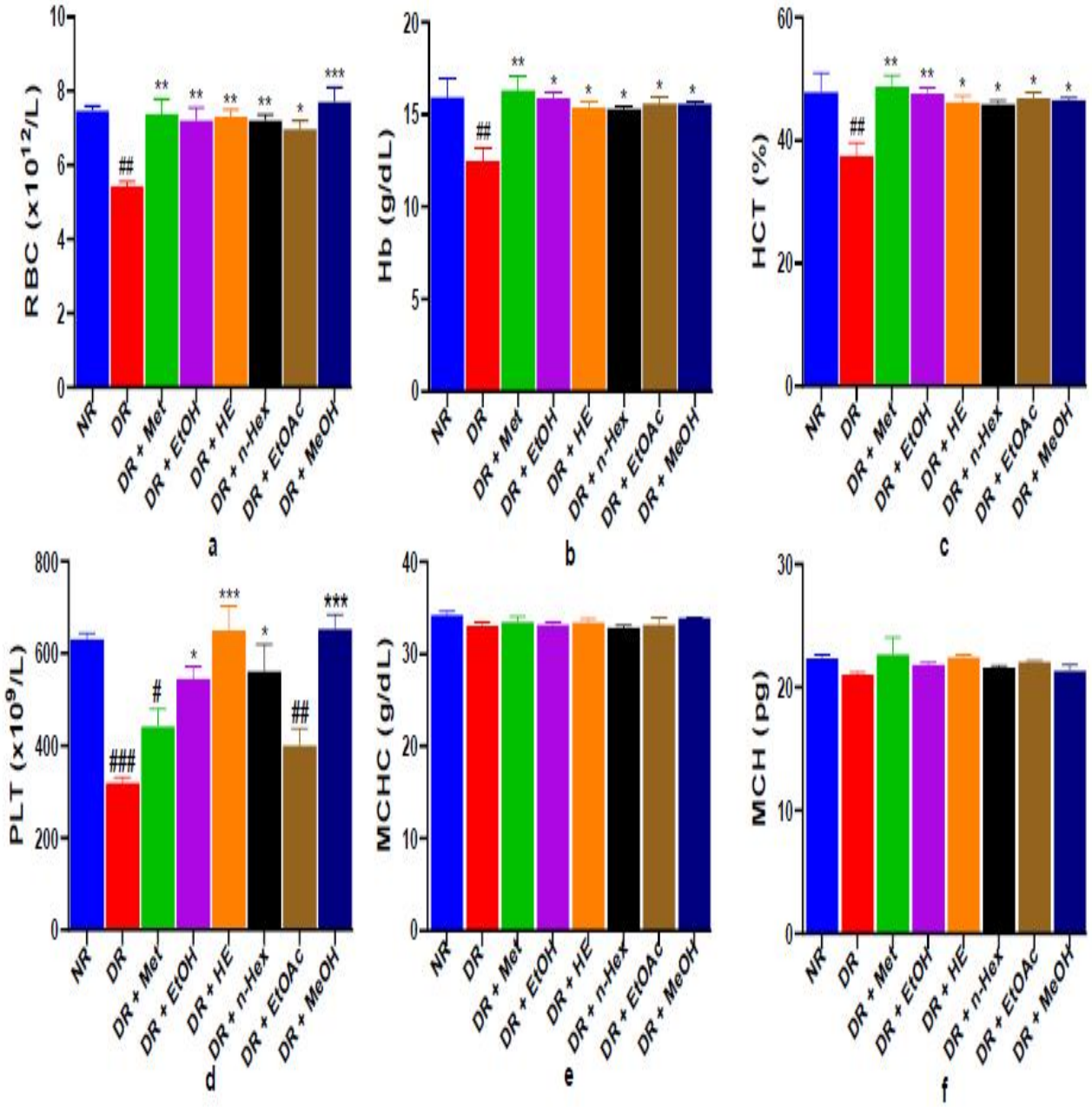

Figure 4.7: Effects of *C. albidum* extract/fractions on blood parameters in STZ-induced diabetic rats. Values are expressed as mean ± SEM (n=7). $^{#}p<0.05$, $^{##}p<0.01$ in comparison with normal rats. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ when compared with diabetic untreated rats.

4.10 Effects of *Chrysophyllum albidum* Stem Bark Extract/Fractions on White Blood Cells in STZ-Induced Diabetic Rats

Figure 4.8 showed the effects of *C. albidum* stem bark extract/fractions on white blood cell count in STZ-induced diabetic rats. The results revealed considerably increase in white blood cell (WBC), lymphocyte, granulocyte, and mid-cell (monocyte + basophil + eosinophil) (MID) levels of diabetic untreated rats when compared with normal group ($p < 0.05$). Oral administration of *C. albidum* extract/fractions/metformin markedly reduced their elevated levels ($p < 0.05$). The methanol fraction produced the best response when compared with other fractions and metformin.

4.11 Effects of *Chrysophyllum albidum* Stem Bark Extract/Fractions on Tissue MDA and Antioxidant Parameters of Rats

Figures 4.9 and 4.10 showed the effects of *C. albidum* stem bark extract/fractions on tissue MDA, and antioxidant parameters in STZ-induced diabetic rats. Results presented in Figure 4.9 (a-d) revealed marked increases in plasma, hepatic, renal, and pancreatic MDA levels while significant decreases in catalase (Figure 4.9 e-g), GPx and SOD (Figure 4.10 a-g) activities were observed in diabetic untreated group when compared with normal rats ($p < 0.05$). Treatment with *C. albidum* extract/fractions markedly alleviated the observed STZ-induced oxidative stress in rat tissues by reducing the level of MDA and increasing the activities of antioxidant enzymes (Figure 4.9 – 4.10). Results obtained with the standard drug, metformin, followed a similar trend.

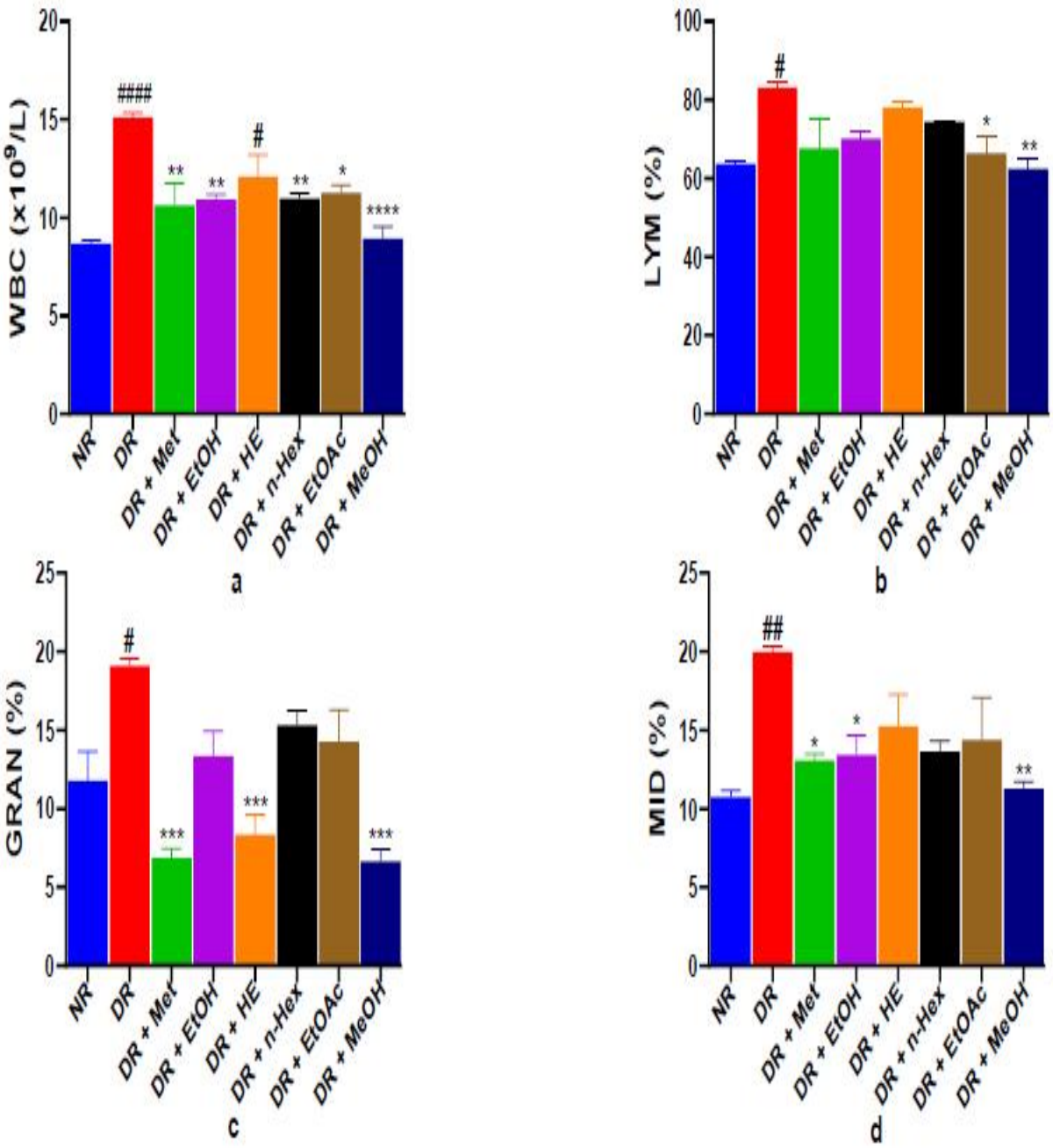

Figure 4.8: Effects of *C. albidum* extract/fractions on white blood cell count in STZ-induced diabetic rats. Values are expressed as mean ± SEM (n=7). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, $^{\#\#\#\#}p < 0.0001$ in comparison with normal rats. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ when compared with diabetic untreated rats.

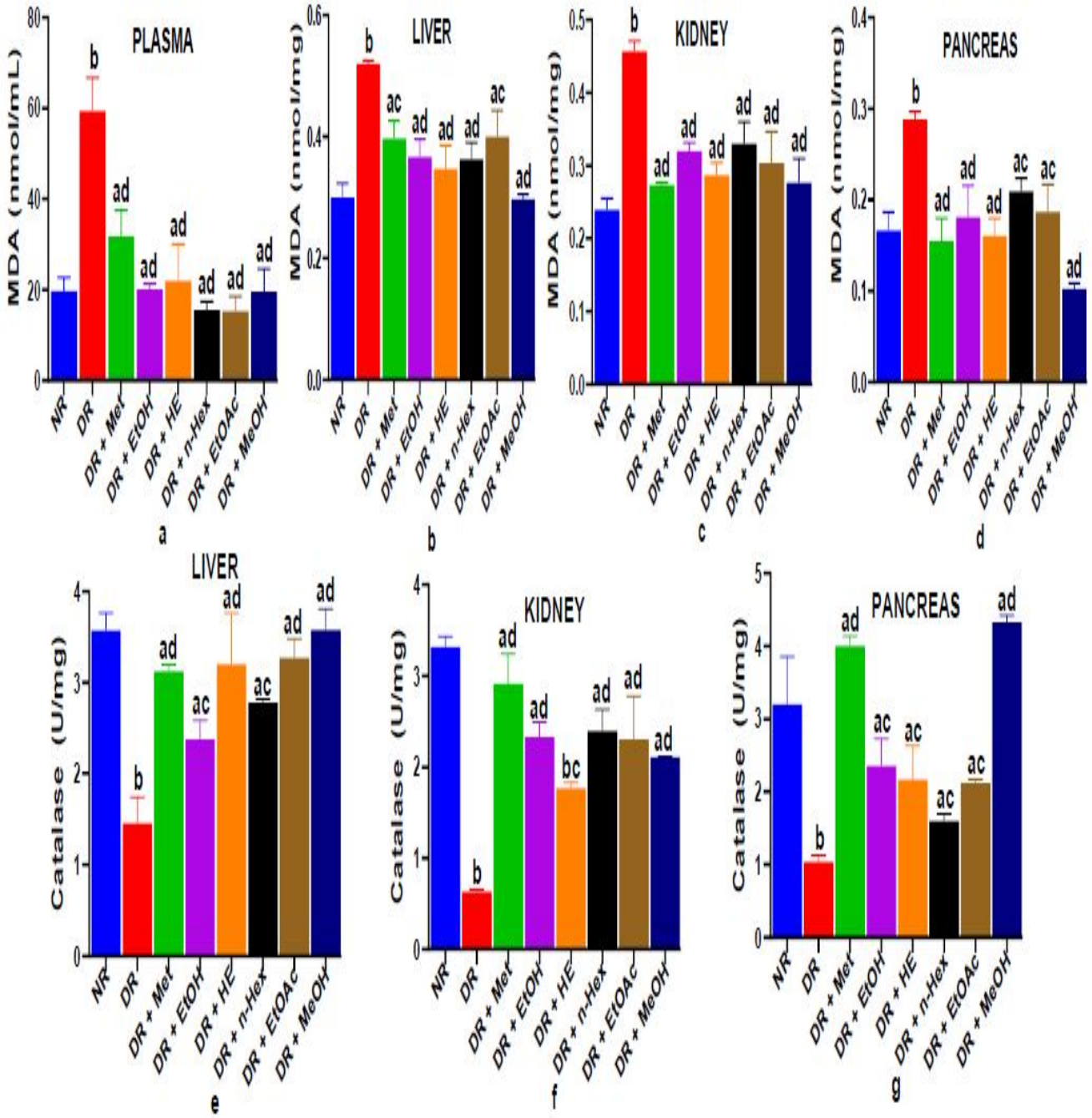

Figure 4.9: Effects of *C. albidum* extract/fractions on plasma, hepatic, renal and pancreatic MDA and antioxidant parameters in STZ-induced diabetic rats. Values are expressed as mean ± SEM (n=7). $^{b}p < 0.05$, $^{a}p > 0.05$ in comparison with control. $^{d}p < 0.05$, $^{c}p > 0.05$ in comparison with diabetic untreated rats.

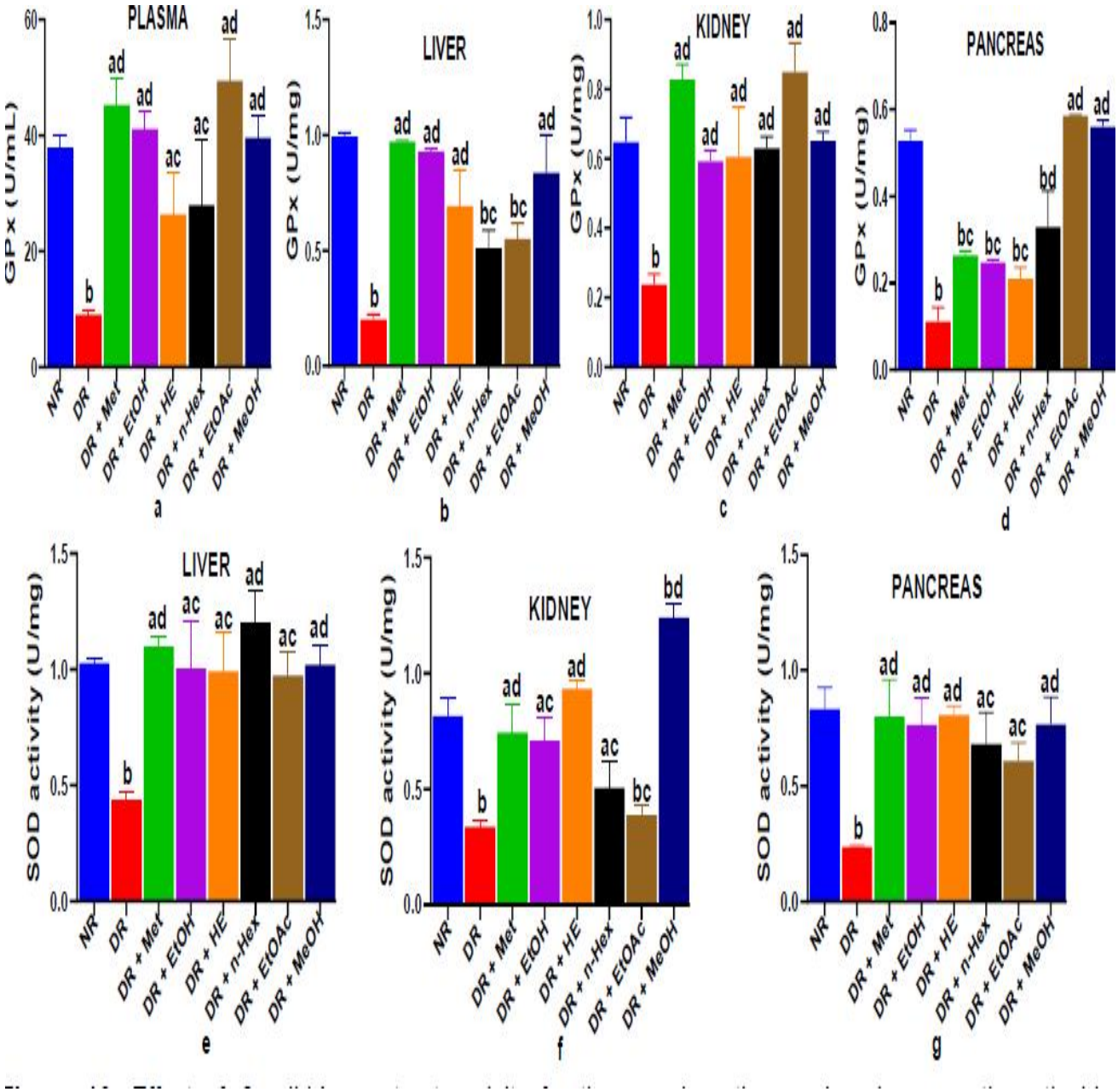

Figure 4.10: Effects of *C. albidum* extract and its fractions on hepatic, renal and pancreatic antioxidant parameters in STZ-induced diabetic rats. Values are expressed as mean ± SEM (n=7). $^{b}p < 0.05$, $^{a}p > 0.05$ in comparison with normal rats. $^{d}p < 0.05$, $^{c}p > 0.05$ in comparison with diabetic untreated rats.

4.12 Results of Histopathological Examination of Pancreatic and Hepatic Tissues

Figures 4.11 and 4.12 revealed the histological examination of pancreatic/hepatic tissues of STZ-induced diabetic rats treated with *C. albidum* stem bark extract/fractions. Results presented in Figure 4.11 revealed severe necrosis, cell reduction of the islet of Langerhan and pancreatic acini, indicating severe pancreatic damage due to STZ toxicity in diabetic untreated group in comparison with normal rats. Treatment with *C. albidum* extract/fractions showed structural improvement with minor reduction in the number of islet of Langerhans and pancreatic acini when compared with diabetic control. Similarly, treatment with metformin (50 mg/kg bwt) restored the islets of Langerhan and pancreatic acini. Histological examination of the hepatic tissue in diabetic untreated rats revealed marked congestion of the central vein and loss of normal hepatocytic architecture when compared with control (Figure 4.12). Treatment with *C. albidum* extract/fractions/metformin restored the central vein and hepatocytes structure as shown in Figure 4.12 (D - H).

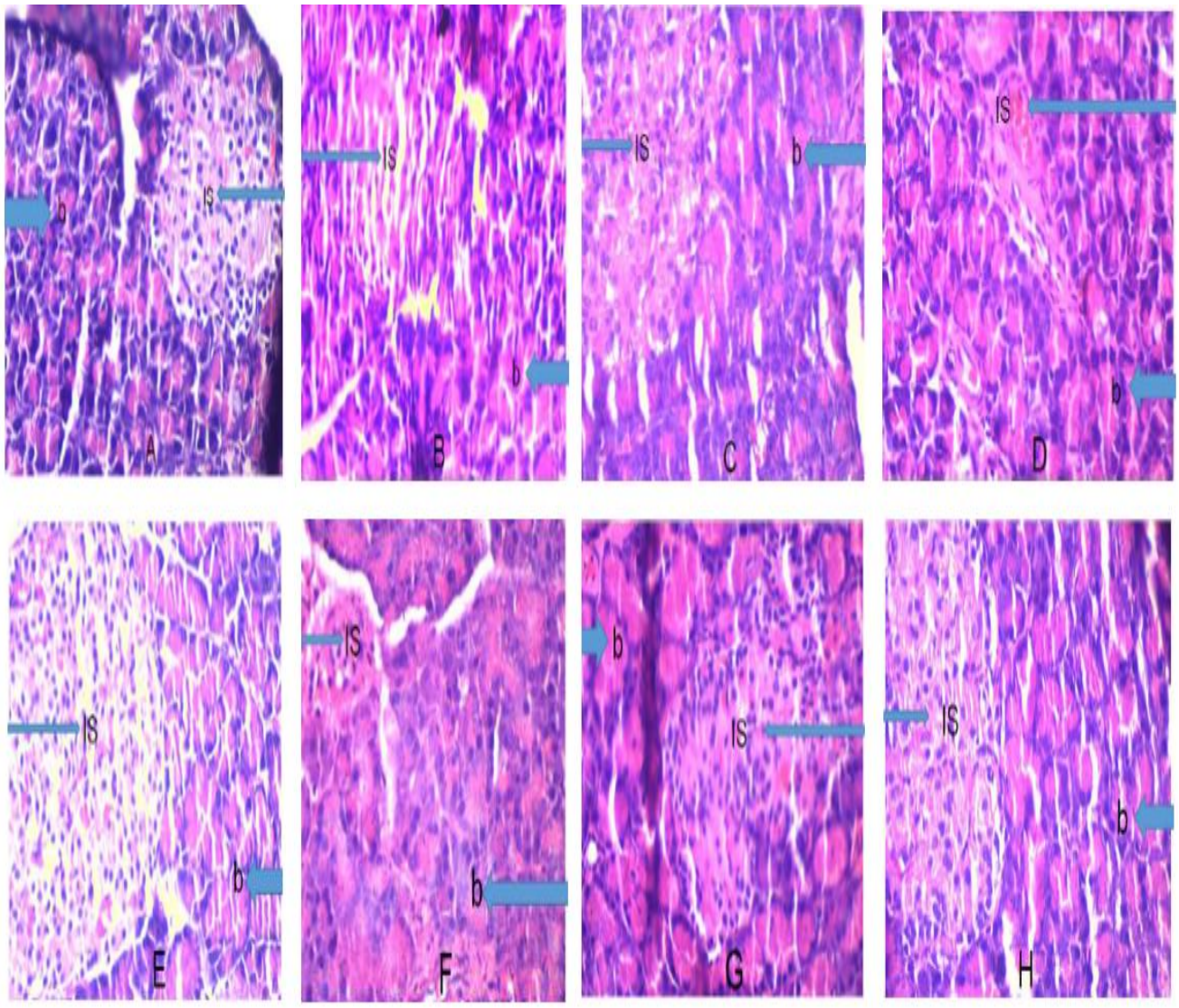

Figure 4.11: Photomicrographs of pancreatic tissue of STZ-induced diabetic rats treated with *C. albidum* stem bark extract/fractions (H&E ×400). Control (A): Showed normal Islets of Langerhan (IS) and pancreatic acini (b), no lesion was observed; Diabetic untreated (B): Revealed severe necrosis, cell reduction of the Islets of Langerhan (IS) and pancreatic acini (b); Diabetic treated with metformin (C): Indicated mild inflammation of the interstitial space, restoration of Islets of Langerhan (IS) and normal pancreatic acini (b) and D - H (*C. albidum* extract and its fractions) showed structural improvement with minor reduction in the number of islets of Langerhan (IS) and normal pancreatic acini (b).

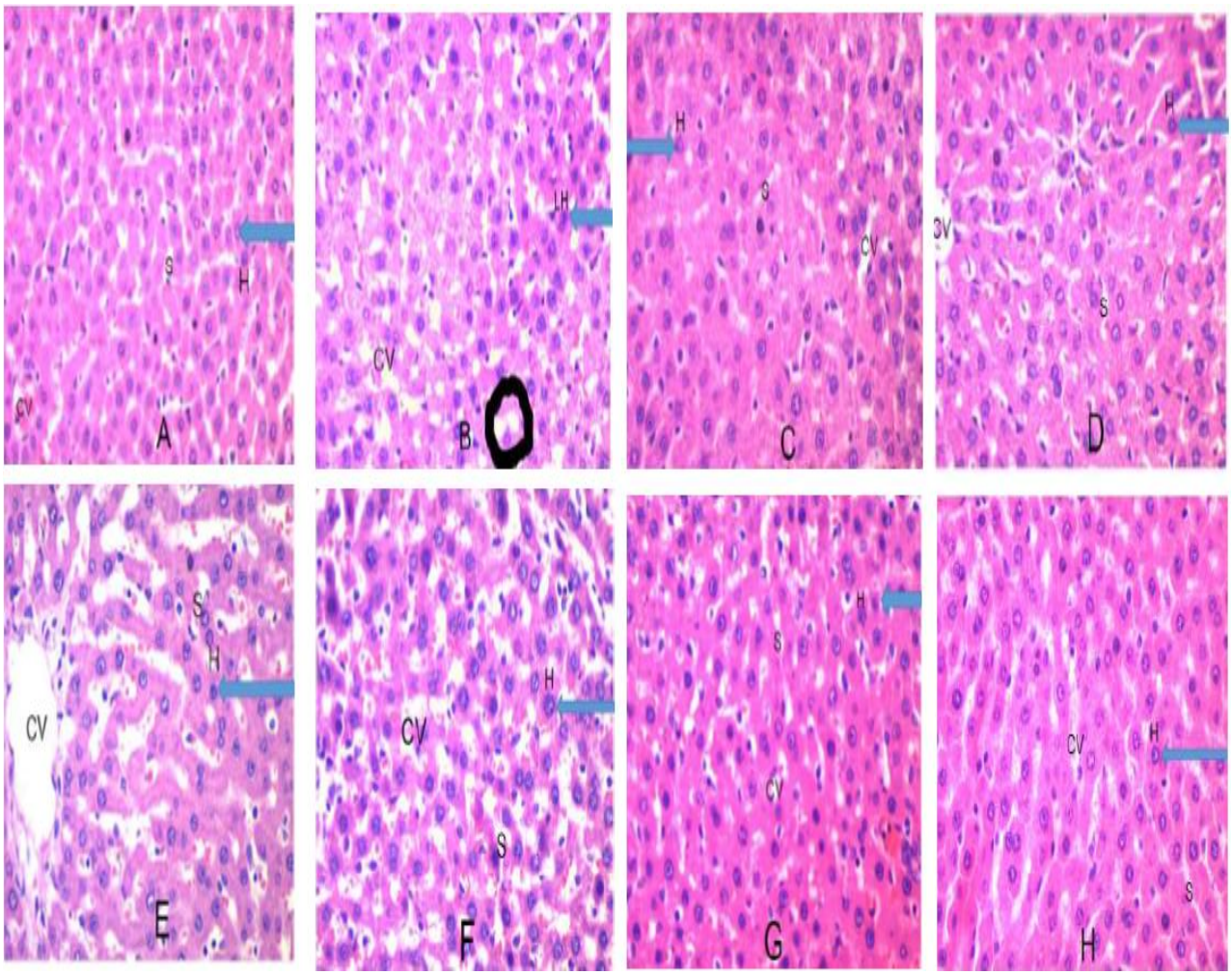

Figure 4.12: Photomicrographs of hepatic tissue in STZ-induced diabetic rats treated with *C. albidum* stem bark extract/fractions (H&E ×400). Control (A): Revealed the normal hepatocytes structure with normal vessiculated nuclei (H), central vein (CV) and sinusoids (S); Diabetic untreated (B): Revealed a loss in the normal hepatocytic architecture (LH), presence of vacuoles (black circle) and congestion of central vein (CV); Diabetic treated with metformin (C): Indicated structural restoration of the central vein (CV) with normal hepatocytes structure; Diabetic treated with *C. albidum* extract and its fractions (D-H): Showed that histological alterations were markedly reduced. However, there was minor congestion of central vein (CV).

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

Diabetes mellitus (DM) is a metabolic disease marked by abnormal glucose metabolism. It produces high rates of morbidity and mortality (Singh *et al.,* 2022). Drugs commonly used for the management of DM (biguanides/sulphonylureas) are limited by severe side effects. As a result, natural products/medicinal plants have received huge attention globally as better alternatives for management of the disease (Abolfathi *et al.,* 2012; Seino *et al.,* 2012). In Nigeria, *C. albidum* fruit is widely consumed by locals because of its numerous benefits, including nutritional and medicinal benefits (Amusa *et al.,* 2013). Different parts of the plant have been reported to possess various biological/pharmacological properties (Ibrahim *et al.,* 2017). The leaves, stem bark, and fruit are reportedly used for the management of DM, high blood pressure, malaria, and ulcer (Ibrahim *et al.,* 2017). The present study evaluated the effects of ethanol extract of *C. albidum* stem bark and its fractions on biochemical status in STZ-induced diabetic rats.

The results revealed marked decrease in body weight of diabetic untreated rats when compared with the control group. Streptozotocin-induced DM causes loss of body weight due to increased muscle wasting and loss of tissue proteins (Shirwaikar *et al.,* 2006). The reduction in body weights of diabetic untreated rats are in accordance with the findings of Antai *et al.* (2010). However, treatment with *C. albidum* extract/fractions markedly restored the body weight loss (Table 4.2). Similarly, administration of metformin (50 mg/kg bwt), significantly increased rats body weight in comparison with diabetic control rats. Hydroethanol fraction showed the highest body weight increase when compared with ethanol extract, metformin and other fractions.

Increase in weight of diabetic rats treated with the extract/fractions provided more evidence that the extract/fractions could promote structural protein biosynthesis by restoring glycemic control via insulin synthesis and release. Negative body weight gain was seen in diabetic untreated rats when compared with normal rats (Figure 4.1). However, there was marked increase in body weight gain of diabetic rats treated with *C. albidum* extract/fractions when compared with diabetic control. The percentage body weight gain of rats treated with ethanol extract, and hydroethanol, n-hexane, ethyl acetate, and methanol fraction were 32.12, 37.83, 35.96, 34.43, and 28.49 %, respectively. Similarly, the standard drug, metformin, significantly increased the percentage body weight gain (23.38 %) in comparison with diabetic untreated group. Hydroethanol fraction showed the highest percentage body weight gain when compared with extract, metformin and other fractions. The results presented in Table 4.3 showed marked increase in relative organ weight for liver and kidneys of diabetic untreated rats while a reduction was seen in pancreas when compared with the normal rats. These results align with those previously reported by Ojeaburu and Nathan (2024). Liver enlargements (fatty liver) in diabetic condition have been attributed to triacylglycerol accumulation (Habibuddin *et al.,* 2008). Kidney enlargements have been ascribed to increased Activin beta A in tubular epithelial cells (Ren *et al.,* 2009). The reduction in pancreas weight/body weight in diabetic untreated rats could be due to the destruction and disappearance of pancreatic islets of Langerhan (Heidari *et al.,* 2008). Treatment with *C. albidum* extract/fractions or metformin, considerably reversed the relative organ weights (liver, kidney). Oral administration of *C. albidum* extract/fractions restored the relative organ weight (pancreas) at a non-significant level. Similar trend was seen in the group treated with metformin, when compared with diabetic untreated group.

Streptozotocin (STZ) is a diabetogenic agent that is widely used to induce DM in rats. Results of this study revealed that intraperitoneal injection of STZ (50 mg/kg bwt) significantly increased the blood glucose level of rats when compared with the normal control group (Figure 4.2). The results agree with those previously reported by Olasehinde and Ojeaburu (2025). Streptozotocin exerts its diabetogenic effect by selectively destroying pancreatic β-cells, thereby reducing the number of active cells (Chaudhry *et al.,* 2013). Oral administration of *C. albidum* extract/fractions/metformin showed significant reduction in plasma glucose levels when compared with diabetic untreated rats. These results align with previous study by Yusuf *et al.* (2020). Reduction in blood glucose level could be as a result of stimulation of insulin release from surviving and regenerated β-cells of the islets of Langerhans by the extract/fractions, and/or promotion of glucose uptake into peripheral tissues (Msomi *et al.,* 2019). The glycemic changes calculated for ethanol extract and hydroethanol, n-hexane, ethyl acetate, and methanol fractions were 51.27, 50.27, 53.98, 48.37, and 75.28 %, respectively (Figure 4.2). Similarly, the glycemic change calculated for standard drug, metformin, was 66.15 % (Figure 4.2). The methanol fraction of *C. albidum* stem bark showed the highest glycemic change when compared with other fractions and metformin. The considerably reduction in blood glucose level (≥ 50 %) in diabetic rats treated with *C. albidum* extract/fractions appears to suggest its potential as an anti-hyperglycemic/anti-diabetic agent.

Results obtained from the present study revealed marked increase in plasma activities of AST, ALT, ALP, and AST:ALT in diabetic untreated group when compared with normal rats (Figure 4.3). The ratio of AST to ALT has been reported to be a marker of non-alcoholic liver damage (Shreevastva *et al.,* 2017). Serum/plasma activities of AST, ALT and ALP are used for the examination of liver diseases. Increased AST, ALT, and ALP activity in the plasma of diabetic

untreated rats may be due to enzyme leakage from the liver cytosol into the systemic circulation, suggesting hepatic damage and loss of functional integrity (Mirmohammadlu *et al.,* 2015). Diabetes mellitus (DM) can lead to liver damage due to the harmful effects of hyperglycemia, which include inhibition of complex III, ROS generation, and alteration of membrane permeability (Harrison, 2006; Hickman and Macdonald, 2007). Their elevation in diabetic rat plasma could be as a result of necrotic action of STZ on the hepatocyte. Administration of *C. albidum* extract/fractions or metformin considerably reduced the activities of these enzymes. These results support the findings of the study conducted on the root bark of *C. albidum* which revealed the capacity of the plant to decrease the activities of serum liver enzymes in alloxan-induced diabetic rat model (Onyeka *et al.,* 2013). Flavonoids, triterpenoids, saponins, tannins, and alkaloids are among the phytochemicals that have been associated with liver protection. The presence of flavonoids, phenols, tannins, and alkaloids was revealed in the phytochemical screening of *C. albidum* extract as reported by Ibrahim *et al.* (2017), indicating that *C. albidum* extract/fractions may have hepatoprotective impact against diabetes-related liver damage. Figure 4.4 revealed marked reductions in plasma total protein, albumin, and globulin levels, but significantly elevated total bilirubin and direct bilirubin levels in diabetic untreated group when compared with control. The marked reduction in protein concentration indicates depletion in hepatic protein reserves and thus suggestive of liver injury/damage. These results agree with findings of previous study reported by Gatsing *et al.* (2005). Treatment with ethanol extract or methanol fraction of *C. albidum* stem bark significantly restored the levels of total protein and albumin to near normal. No marked increase was observed in hydroethanol, n-hexane, and ethyl acetate fractions when compared with diabetic untreated rats. Furthermore, oral administration of *C. albidum* extract/fractions markedly restored the concentrations of total bilirubin and direct

bilirubin. The highest reduction was seen in the methanol fraction (Figure 4.4). Similar trend was seen in the group that was treated with metformin. These results align with the previous study reported by Abiodun *et al.* (2011).

The lipid profile results in Figure 4.5 revealed marked increase in total cholesterol, triacylglycerol, low-density lipoprotein cholesterol and atherogenic index while significant decrease in high-density lipoprotein cholesterol was seen in diabetic untreated group in comparison with normal group. Diabetic cardiovascular complications such as hypertension and atherosclerosis occur as a result of alterations in lipid profile of diabetic patients (Petrie *et al.,* 2018). The results of this study agree with the findings reported by Abu *et al.* (2022). However, administration of *C. albidum* extract/fractions significantly reduced TC, TG, LDL-C, and atherogenic index, but it elevated HDL-C level to near normal values. These results are in agreement with those of previous study reported by Asagba *et al.* (2019). Similarly, treatment with metformin restored TC, TG, LDL-C, atherogenic index, and HDL-C when compared with diabetic untreated group. Methanol fraction produced the best response. High-density lipoprotein cholesterol transports cholesterol esters and cholesterol to the liver for metabolism into bile acids. According to Tosheska and Topuzovska (2017), this route is crucial for lowering blood and peripheral tissue cholesterol levels and preventing the development of atherosclerotic plaque in the aorta. Accordingly, *C. albidum* may provide protection against hypercholesterolemia-related cardiovascular diseases. The antihyperlipidemic effect in diabetic conditions may be due to the antioxidant and free radical scavenging properties of *C. albidum* extract (Omotosho *et al.,* 2013). This effect could be achieved by blocking the activity of hepatic 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, an enzyme that is crucial for cholesterol synthesis (Jung *et al.,* 2006).

Since kidneys function in blood filtration, therefore, measuring the concentrations of excretory products in the serum/plasma is one way of evaluating its functionality (Treacy *et al.,* 2019). Results presented in Figure 4.6 revealed significant increase in the levels of urea, creatinine, and BUN in diabetic untreated group when compared with normal group. Elimination of urea and creatinine from the blood is one of the functions of kidney and is normally used for the assessment of renal competence (Koppe *et al.* 2016). Possible causes of elevated urea levels of diabetic rats include STZ-induced increased muscle proteolysis and deamination of glucogenic amino acids. The findings of this study with respect to urea level is in line with those reported by Adebayo *et al.* (2010) who attributed elevated serum urea and/or creatinine of diabetic subjects to beta cell dysfunction. Oral administration of *C. albidum* extract/fractions or metformin markedly restored the alterations in renal function parameters to near normal values. There was a significant decrease in sodium (Na^+), potassium (K^+) and bicarbonate (HCO_3^-) levels in diabetic untreated rats when compared with control. Electrolyte imbalances are the early biochemical events responsible for long-term diabetic complications, as some electrolytes play crucial roles in intermediary metabolism and cellular functions including osmosis and acid-base balance (Ogunleye and Asaolu, 2016). Diabetes mellitus causes electrolyte imbalance due to insulin resistance, hyperglycemia, and excess ketones (Kitabchi *et al.,* 2006). In uncontrolled DM, the kidneys lose electrolytes by osmotic diuresis (Eteng *et al.,* 2008) and impaired kidney function (Ikpi *et al.,* 2009). Possible causes of low electrolyte levels in DM include fluid loss from dehydration and osmotic diuresis in the kidneys (Eteng *et al.,* 2008). Changes in osmotic fluids caused by hyperglycemia or osmotic diuresis are linked to their insufficiency. In this study, administration of *C. albidum* extract/fractions significantly restored the alterations in electrolytes to near normal values when compared with diabetic untreated rats. Same trend was seen in

diabetic rats treated with metformin. Normalization of electrolytes by *C. albidum* extract and its fractions implies that it might prevent or ameliorate hyperglycemia-induced nephropathy linked to diabetic complications.

Haematological results in Figure 4.7 showed that intraperitoneal injection of STZ (50 mg/kg bwt) considerably decreased red blood cells, haemoglobin, haematocrit, and platelet count when compared with the normal/control group. Neither the mean corpuscular haemoglobin concentration nor the mean corpuscular haemoglobin of diabetic untreated rats showed marked decrease when compared with the normal group. These results are in agreement with the findings of Ibrahim *et al.* (2019). The observed reduction in red blood cell and its indices in diabetic untreated rats may be linked to destruction of mature red blood cells (Muhammad and Oloyede, 2009). Oral administration of *C. albidum* extract/fractions significantly reversed the effect of STZ on haematological indices. Same trend was observed in diabetic rats treated with standard drug, metformin. Oral administration of *C. albidum* extract/fractions showed no marked elevation in mean corpuscular haemoglobin concentration/mean corpuscular haemoglobin when compared with diabetic untreated rats. Results of Figure 4.8 showed significant increase in white blood cells, lymphocytes, granulocytes, and mid-cell (MID) levels of diabetic untreated rats in comparison to normal rats. Findings of this study agree with those reported by Ibrahim *et al.* (2019). Streptozotozin-induced oxidative stress, angiotensin II and pro-inflammatory cytokines could be the cause of significant increase in white blood cell, granulocyte, lymphocyte, and MID levels (Ogbe *et al.,* 2010). Treatment of diabetic rats with *C. albidum* extract/fractions considerably reduced the elevated white blood cell, granulocyte, lymphocyte and mid-cell levels. Similar trend was observed in diabetic rats treated with metformin. The methanol fraction showed the highest decrease in the elevated white blood cell, granulocyte, lymphocyte and mid-

cell levels when compared with other fractions and metformin. These results align with previous report of Yakubu *et al.* (2007). The reduction in white blood cell, granulocyte, lymphocyte and mid-cell levels could be attributed to the capacity of *C. albidum* extract/fractions to restore insulin excitation, reduce advanced glycation end products generation, and decrease oxidative stress within blood cells.

The results in Figure 4.9 (a-d) revealed marked increases in plasma, hepatic, renal, and pancreatic MDA levels while significant decrease in catalase (Figure 4.9 e-g), GPx and SOD (Figure 4.10 a-g) activities were seen in diabetic untreated rats when compared with normal rats. Increased oxidative stress, one of the major causes of DM, is linked to auto-oxidation of glucose, protein glycation, lipid peroxidation, and lowered activities of enzymatic and non-enzymatic antioxidants (Lapena *et al.,* 2018; Liguori *et al.,* 2018). The results obtained in this study align with previous report of Abu *et al.* (2023). Administration of *C. albidum* extract/fractions or metformin markedly alleviated the observed oxidative stress in plasma, pancreatic, renal, and hepatic tissues of diabetic control by reducing the level of MDA and elevating the activities of antioxidant enzymes (Figure 4.9 – 4.10). The reversal of oxidative stress by the extract or fractions is an indication that *C. albidum* stem bark extract/fractions may possess antioxidant properties and this is likely to be one of the mechanism by which they alleviate the diabetogenic effect of STZ. The results of this study are consistent with reported antioxidant activities of *C. albidum* extract from other diabetic studies (Abayomi *et al.,* 2021).

Histological assessment of pancreatic tissue further validated the biochemical findings. The results in Figure 4.11 revealed severe necrosis and cell reduction of the islet of Langerhans and pancreatic acini in diabetic untreated rats when compared with the normal rats. The result align with previous report of Ibrahim *et al.* (2019). However, oral administration of *C. albidum* extract

and its fractions showed pancreatic β-cell restoration, as seen in mild reduction in the number of islets of Langerhans and pancreatic acini when compared with diabetic untreated rats. Similarly, diabetic rats treated with metformin (50 mg/kg bwt) showed restoration in the islet of Langerhans and pancreatic acini. This suggests that *C. albidum* extract and its fractions not only helped in glucose regulation but also provided a protective effect on pancreatic tissue. Histological examination of the hepatic tissue in diabetic untreated rats revealed marked congestion of the central vein and loss of normal hepatocytic architecture when compared with control (Figure 4.12 B). However, treatment with *C. albidum*/fractions restored the central vein and hepatocytes structure as shown in Figure 4.12 (D-H). Similar trend was also observed in diabetic rats treated with metformin. These results align with previous report of Abayomi *et al.* (2021).

5.2 Recommendations

Going forward it is recommended that further work/attempt should be carried out to elucidate the precise molecular mechanism underlying the potential antidiabetic effect of *C. albidum* stem bark extract and fractions. Analytical techniques such as high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) may be employed to identify precise phytochemical compound(s) responsible for the perceived anti-hyperglycemic/anti-diabetic property of the plant extract. In addition, studies aimed at isolation, identification, and characterization of specific bioactive compounds of *C. albidum* stem bark with potential therapeutic use in the management of DM should be encouraged.

5.3 Conclusion

The results obtained in this study have shown that ethanol extract of *C. albidum* stem bark extract/fractions can markedly reduce typical derangements associated with STZ-induced

diabetes mellitus (that is, hyperglycemia, hyperlipidemia, hepatotoxicity, nephrotoxicity, and oxidative stress). The crude extract/fractions of the medicinal plant have the capacity to ameliorate STZ-induced oxidative damage to hepatic, renal and pancreatic tissues.

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APPENDIX

AST

	NR	DR	DR + Met	DR + EtOH	DR + HE	DR + n-Hex	DR + EtOAc	DR + MeOH
Number of values	5	5	5	5	5	5	5	5
Minimum	40.00	71.00	40.00	46.00	43.00	40.00	40.00	40.00
25% Percentile	40.00	71.00	40.00	46.00	43.00	40.00	40.00	40.00
Median	41.00	72.00	42.00	48.00	44.00	44.00	44.00	42.00
75% Percentile	42.00	74.00	45.00	53.00	46.00	46.00	52.00	44.00
Maximum	42.00	74.00	45.00	53.00	46.00	46.00	52.00	44.00
Mean	41.00	72.33	42.33	49.00	44.33	43.33	45.33	42.00
Std. Deviation	1.000	1.528	2.517	3.606	1.528	3.055	6.110	2.000
Std. Error of Mean	0.5774	0.8819	1.453	2.082	0.8819	1.764	3.528	1.155
Lower 95% CI	38.52	68.54	36.08	40.04	40.54	35.74	30.16	37.03
Upper 95% CI	43.48	76.13	48.58	57.96	48.13	50.92	60.51	46.97

ALT

	NR	DR	DR + Met	DR + EtOH	DR + HE	DR + n-Hex	DR + EtOAc	DR + MeOH
Number of values	5	5	5	5	5	5	5	5
Minimum	21.00	36.00	24.00	27.00	26.00	24.00	24.00	21.00
25% Percentile	21.00	36.00	24.00	27.00	26.00	24.00	24.00	21.00
Median	24.00	37.00	25.00	30.00	27.00	31.00	26.00	24.00
75% Percentile	24.00	37.00	26.00	31.00	27.00	33.00	26.00	27.00
Maximum	24.00	37.00	26.00	31.00	27.00	33.00	26.00	27.00
Mean	23.00	36.67	25.00	29.33	26.67	29.33	25.33	24.00
Std. Deviation	1.732	0.5774	1.000	2.082	0.5774	4.726	1.155	3.000
Std. Error of Mean	1.000	0.3333	0.5774	1.202	0.3333	2.728	0.6667	1.732

Lower 95% CI	18.70	35.23	22.52	24.16	25.23	17.59	22.46	16.55
Upper 95% CI	27.30	38.10	27.48	34.50	28.10	41.07	28.20	31.45

ALP

	NR	**DR**	**DR + Met**	**DR + EtOH**	**DR + HE**	**DR + n-Hex**	**DR + EtOAc**	**DR + MeOH**
Number of values	5	5	5	5	5	5	5	5
Minimum	65.00	80.00	66.00	65.00	66.00	73.00	70.00	66.00
25% Percentile	65.00	80.00	66.00	65.00	66.00	73.00	70.00	66.00
Median	66.00	88.00	68.00	74.00	71.00	74.00	74.00	71.00
75% Percentile	68.00	88.00	74.00	78.00	73.00	75.00	74.00	74.00
Maximum	68.00	88.00	74.00	78.00	73.00	75.00	74.00	74.00
Mean	66.33	85.33	69.33	72.33	70.00	74.00	72.67	70.33
Std. Deviation	1.528	4.619	4.163	6.658	3.606	1.000	2.309	4.041
Std. Error of Mean	0.8819	2.667	2.404	3.844	2.082	0.5774	1.333	2.333
Lower 95% CI	62.54	73.86	58.99	55.79	61.04	71.52	66.93	60.29
Upper 95% CI	70.13	96.81	79.68	88.87	78.96	76.48	78.40	80.37

APPENDIX II

Urea

	NR	DR	DR + Met	DR + EtOH	DR + HE	DR + n-Hex	DR + EtOAc	DR + MeOH
Number of values	5	5	5	5	5	5	5	5
Minimum	38.00	60.00	39.00	38.00	39.00	39.00	44.00	38.00
25% Percentile	38.00	60.00	39.00	38.00	39.00	39.00	44.00	38.00
Median	39.00	64.00	44.00	44.00	44.00	49.00	49.00	44.00
75% Percentile	39.00	64.00	49.00	53.00	49.00	53.00	49.00	49.00
Maximum	39.00	64.00	49.00	53.00	49.00	53.00	49.00	49.00
Mean	38.67	62.67	44.00	45.00	44.00	47.00	47.33	43.67
Std. Deviation	0.5774	2.309	5.000	7.550	5.000	7.211	2.887	5.508
Std. Error of Mean	0.3333	1.333	2.887	4.359	2.887	4.163	1.667	3.180
Lower 95% CI	37.23	56.93	31.58	26.25	31.58	29.09	40.16	29.99
Upper 95% CI	40.10	68.40	56.42	63.75	56.42	64.91	54.50	57.35

Creatinine

	NR	DR	DR + Met	DR + EtOH	DR + HE	DR + n-Hex	DR + EtOAc	DR +MeOH
Number of values	5	5	5	5	5	5	5	5
Minimum	0.8000	1.600	1.100	1.100	0.8000	1.200	1.000	1.200
25% Percentile	0.8000	1.600	1.100	1.100	0.8000	1.200	1.000	1.200
Median	0.9000	1.800	1.100	1.100	1.200	1.200	1.300	1.300
75% Percentile	1.000	1.800	1.300	1.400	1.400	1.400	1.300	1.300
Maximum	1.000	1.800	1.300	1.400	1.400	1.400	1.300	1.300

Mean	0.9000	1.733	1.167	1.200	1.133	1.267	1.200	1.267
Std. Deviation	0.1000	0.1155	0.1155	0.1732	0.3055	0.1155	0.1732	0.05774
Std. Error of Mean	0.05774	0.06667	0.06667	0.1000	0.1764	0.06667	0.1000	0.03333
Lower 95% CI	0.6516	1.446	0.8798	0.7697	0.3744	0.9798	0.7697	1.123
Upper 95% CI	1.148	2.020	1.454	1.630	1.892	1.554	1.630	1.410

APPENDIX III

Total Cholesterol (TC)

	NR	**DR**	**DR + Met**	**DR + EtOH**	**DR + HE**	**DR + n-Hex**	**DR + EtOAc**	**DR + MeOH**
Number of values	5	5	5	5	5	5	5	5
Minimum	71.00	123.0	91.00	106.0	95.00	98.00	95.00	71.00
25% Percentile	71.00	123.0	91.00	106.0	95.00	98.00	95.00	71.00
Median	73.00	134.0	95.00	120.0	95.00	99.00	106.0	91.00
75% Percentile	94.00	136.0	106.0	124.0	99.00	106.0	120.0	94.00
Maximum	94.00	136.0	106.0	124.0	99.00	106.0	120.0	94.00
Mean	79.33	131.0	97.33	116.7	96.33	101.0	107.0	85.33
Std. Deviation	12.74	7.000	7.767	9.452	2.309	4.359	12.53	12.50
Std. Error of Mean	7.356	4.041	4.485	5.457	1.333	2.517	7.234	7.219
Lower 95% CI	47.68	113.6	78.04	93.19	90.60	90.17	75.87	54.27
Upper 95% CI	111.0	148.4	116.6	140.1	102.1	111.8	138.1	116.4

Triacylglycerol (TG)

	NR	**DR**	**DR + Met**	**DR + EtOH**	**DR + HE**	**DR + n-Hex**	**DR + EtOAc**	**DR + MeOH**
Number of values	5	5	5	5	5	5	5	5
Minimum	40.00	76.00	48.00	60.00	60.00	60.00	58.00	55.00
25% Percentile	40.00	76.00	48.00	60.00	60.00	60.00	58.00	55.00
Median	55.00	82.00	50.00	75.00	73.00	75.00	75.00	56.00
75% Percentile	57.00	87.00	56.00	75.00	75.00	80.00	80.00	58.00
Maximum	57.00	87.00	56.00	75.00	75.00	80.00	80.00	58.00

Mean	50.67	81.67	51.33	70.00	69.33	71.67	71.00	56.33
Std. Deviation	9.292	5.508	4.163	8.660	8.145	10.41	11.53	1.528
Std. Error of Mean	5.364	3.180	2.404	5.000	4.702	6.009	6.658	0.8819
Lower 95% CI	27.59	67.99	40.99	48.49	49.10	45.81	42.35	52.54
Upper 95% CI	73.75	95.35	61.68	91.51	89.57	97.52	99.65	60.13

High-Density Lipoprotein Cholesterol (HDL-C)

	NR	DR	DR + Met	DR + EtOH	DR + HE	DR + n-Hex	DR + EtOAc	DR + MeOH
Number of values	5	5	5	5	5	5	5	5
Minimum	38.00	20.00	30.00	26.00	20.00	20.00	32.00	33.00
25% Percentile	38.00	20.00	30.00	26.00	20.00	20.00	32.00	33.00
Median	39.00	20.00	33.00	30.00	26.00	26.00	32.00	40.00
75% Percentile	40.00	26.00	38.00	32.00	26.00	32.00	33.00	45.00
Maximum	40.00	26.00	38.00	32.00	26.00	32.00	33.00	45.00
Mean	39.00	22.00	33.67	29.33	24.00	26.00	32.33	39.33
Std. Deviation	1.000	3.464	4.041	3.055	3.464	6.000	0.5774	6.028
Std. Error of Mean	0.5774	2.000	2.333	1.764	2.000	3.464	0.3333	3.480
Lower 95% CI	36.52	13.39	23.63	21.74	15.39	11.10	30.90	24.36
Upper 95% CI	41.48	30.61	43.71	36.92	32.61	40.90	33.77	54.31

Low-Density Lipoprotein Cholesterol (LDL-C)

	NR	DR	DR + Met	DR + EtOH	DR + HE	DR + n-Hex	DR + EtOAc	DR + MeOH
Number of values	5	5	5	5	5	5	5	5
Minimum	40.00	66.00	42.00	48.00	47.00	55.00	53.00	40.00
25% Percentile	40.00	66.00	42.00	48.00	47.00	55.00	53.00	40.00
Median	44.00	67.00	45.00	50.00	50.00	58.00	63.00	46.00
75% Percentile	45.00	70.00	53.00	56.00	52.00	62.00	66.00	48.00
Maximum	45.00	70.00	53.00	56.00	52.00	62.00	66.00	48.00

Mean	43.00	67.67	46.67	51.33	49.67	58.33	60.67	44.67
Std. Deviation	2.646	2.082	5.686	4.163	2.517	3.512	6.807	4.163
Std. Error of Mean	1.528	1.202	3.283	2.404	1.453	2.028	3.930	2.404
Lower 95% CI	36.43	62.50	32.54	40.99	43.42	49.61	43.76	34.32
Upper 95% CI	49.57	72.84	60.79	61.68	55.92	67.06	77.58	55.01