HYPOGLYCEMIC EFFECT OF *Eleusine coracana*, *Sorghum bicolor* AND *Moringa oleifera* SEEDS FORMULATED FEED ON ALLOXAN INDUCED DIABETIC RATS

BY

BAWALA, Aaron Enogela
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DEPARTMENT OF BIOCHEMISTRY
FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGER STATE

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ABSTRACT

Diabetes mellitus is a metabolic syndrome characterized by hyperglycemia, polyurea, polydypsia and polyphagia. The aim of this research is to study the effect feed constituting; *Eleusine coracana, Sorghum bicolor* with a fixed ratio of *Moringa oleifera* seeds on Alloxan induced diabetic albino rats. Feeds were formulated at various concentration using *Moringa oleifera seeds, Sorghum bicolor and Eleusine coracana.* The milled samples were made into various ratios/concentrations and pelletized. The experimental rats were randomly allotted to 7 cages with each group having three rats each. Alloxan monohydrates were administered to rats in groups A to F. Rats with glucose level of 110mg/dl and above were considered Diabetic. Group A to D were fed with various concentration of the formulated feed, while group E was treated with the standard drug, group F was the negative control group and received 1ml normal saline, while group G was the normal control group. Group E to G were fed with the normal rats pellets. The blood glucose was monitored twice in a week for 21 days after which animals were euthanized. The results for the proximate analysis shows that Group D (30 % *Eleusine coracana*, 50 % *Sorghum bicolor* and 20 % *Moringa oleifera* seeds) had the highest fibre content of 6.335 % ± 0.005 followed by Group A (50 % *Eleusine coracana*, 30 % *Sorghum bicolor* and 20 % *Moringa oleifera* seeds) with 5.655 % ± 0.005. Group B (45 % *Eleusine coracana*, 35 % *Sorghum bicolor* and 20 % *Moringa oleifera* seeds) had highest fat content of 7.785 % ± 0.005 followed by Group A with 7.775 % ± 0.005 while Group D had the highest protein content of 21.495 % ± 0.005. The rats in Group A had the highest blood glucose reduction of 21.74 % compared to Group B, Group C (40 % *Eleusine coracana*, 40 % *Sorghum bicolor* and 20 % *Moringa oleifera* seeds) and D. All the treatment groups (A, B, C and D) shows significant ($p<0.05$) reduction of blood glucose by 21.74 %, 20.97 %, 20.9 % and 16.07 % respectfully in the diabetic rats, with an exception of the negative control (group F) showing little significant ($p<0.05$) reduction of 4.04 %. The positive control (group E) which was treated with the standard drug was the most effective with the highest percentage of blood glucose (23.08 %). The Amino acid profile shows that Group A formulated feed has the highest Lysine (3.685 g/100 g ± 0.005), Isoleucine (5.085 g/100 g ± 0.005), Phenylalanine (4.485 g/100 g ± 0.005), Tryptophan (1.265 g/100 g ± 0.005), Valine (5.095 g/100 g ± 0.005), Methionine (1.955 g/100 g ± 0.005), Proline (8.165 g/100 g ± 0.005), Arginine (5.995 g/100 g ± 0.005), Histidine (2.545 g/100 g ± 0.005), Glycine (4.105 g/100 g ± 0.005), Threonine (3.565 g/100 g ± 0.005), and Serine (4.325 g/100 g ± 0.005) which triggers and forms significant components of insulin. The result from this research suggests that 50 % *Eleusine coracana*, 30 % *Sorghum bicolor* and 20 % *Moringa oleifera* seeds could be effective for management of *Diabetes mellitus*.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

Diabetes mellitus is a disease that affects the metabolism where the body is unable to use the available glucose which results to an increase in blood glucose concentration above the expected or normal level or limits (Yadav *et al.,* 2015). The incidences of diabetes are become highly alarming globally with prevalence rate of about 2.8% (Esser *et al.,* 2014). It is categorized into two (2) types: Type 1 diabetes due to insulin deficiency and type 2 diabetes due to insulin resistance (Esser *et al.,* 2014). The preponderance of diabetes is basically attributed to or caused by wide range of factors; Lifestyle, Obesity, Genetic makeup, and ethnicity (Sivaprasad *et al.,* 2012).

A number of herbs and other plant ingredients are used in diabetes treatment (Srinivasan, 2008). Diet plays a significant function in the management of diabetes mellitus. The health benefits of dietary fiber and antioxidants from food sources of plants were thoroughly examined in this research context. The major sources of dietary fiber, minerals and phytochemicals with antioxidant activity are whole-grain cereals. Consumption of whole grains has been linked to a lower incidence of diabetes and cardiovascular disease (Liu *et al.,* 1999). The aim of this research is to study the effect of feed formulated from varying ratios of Finger millet and Sorghum, with a fixed ratio of Moringa seeds on how it could help in the management of diabetes disease.

Moringa oleifera is a species of Moringa, the most commonly used of the 13 members of its family. The plant is grown in all tropical and subtropical areas such as Pakistan, Arabia, Central America, North and South Philippines, Cambodia, the Caribbean Islands and

Africa (Morton, 1991). Aside from the protein and carbohydrate content, *Moringa oleifera* seed is reported to have vitamins A and B1 (Mbah *et al.,* 2012). Minerals, micronutrients, and bioactive metabolites such as flavonoids, saponins, sterols, phytates, and trypsin are all found in them. The rich lipid content of *Moringa oleifera* seeds categorizes it as oilseeds ranging from 13% to 46% in addition to its rich fiber content (Compaoré *et al.,* 2011).

Finger millet, commonly known as Ragi or Tambar, is a member of the millets family (Ramashia *et al.,* 2018), so named because of its panicle form of development which takes the finger-like form. Finger millet grains are free of gluten and do not form acid, easily digested with low glycemic index food (Manjula and Visvanathan, 2014). Foods having a low glycemic index are said to be a healthy choice for celiac disease sufferers (a condition induced by the consumption of gluten-containing cereal protein) and diabetes as grain intake helps regulate blood glucose levels (Jideani and Jideani, 2011). The grains consist of nutrients such as; dietary fibre, vitamins and high concentration calcium relative to other grains of cereals (Sood *et al.,* 2016). The grains of finger millet are also rich in magnesium and phosphorus. Chronic illnesses such as low blood pressure, ischemic stroke, cardiovascular disease, cancer, obesity, and type II diabetes are reduced when these nutrients are absorbed and used properly in the human body (Kaur *et al.,* 2014). Finger millet grains have polyphenols and phytates, according to Krishnan *et al.,* (2012), which are thought to impact mineral content.

Sorghum bicolor is a major grain crop, especially in the semi-arid tropics of the world. It is a significant food crop in Sub-Saharan parts of Africa and Southern Asia, as well as the main diet available to majority of people in the world who are food insecure. After wheat, rice, maize, and barley, it is the world's most significant grain (FAO, 2010). Sorghum is

cultivated on 40 million hectares in over 105 countries in Africa, Asia, Oceania, and the Americas, with 60 percent of this land in Africa, where it continues to play an essential role in food security (Mutegi *et al.,* 2011). Ethiopia is sorghum's origin and heterogeneity center. It is widely located across Ethiopia, and because of its drought resistant nature, it is the most significant cereal crop in the lowlands (Kebede, 1991).

1.2 Statement of the Research Problem

The prevalence of diabetes mellitus is growing rapidly, especially of type 2. It was estimated that 30 million people suffered this condition in 1985, and by 2006 ending, the number had risen to 230 million and 80 per cent of this number is in the developing world (Roglic *et al.,* 2005). Diabetes is responsible for 3.8 million deaths worldwide per annum, a similar number in proportion to HIV/AIDS mortality (WHO, 2007). In 2018, 34.2 million people in America which represent 10.5% of the USA population, had diabetes with 26.8 % prevalence (American Diabetes Association, 2018). In Nigeria however, the pooled Diabetes mellitus prevalence of 5.77 % observed in our meta-analysis suggests that 11.2 million Nigerians (representing 1 out of every 17 adults) are living with the disease (i.e. going by the UN 193.3 million of Nigerian population) (Musa *et al.,* 2018). Nigeria is Africa's most populated country, with an estimated population of 170 million people, including 76 million adults and 3.1 million persons with Diabetes mellitus disease which is quite significant. Diabetes mellitus is a very expensive disease to manage as well as many other co-morbid conditions that may arise which makes it difficult for the poor and vulnerable population in Nigeria. The drugs are very expensive with side effects. Diet such as Acha, have been reported to help in managing Diabetes (Liu *et al.,* 1999) but has now grown very expensive and scarce in supply forcing the fate of the poor common man to

resort back to their usual carbohydrate diets thereby putting them at risk of extreme condition of this disease. This study therefore seeks to explore alternatives in feed combination of finger millet, Moringa seeds and corn based on availability in various proportions to see which is more effective in managing Diabetes mellitus using the animal model.

1.3 Justification for the Study

The available therapeutics and diet management of Diabetes mellitus are either very expensive, limited or ineffective. Also, most management available are drug-based and have side effects, are monotonous and sometimes not readily available hence the need to provide cheaper alternative for the poor and the vulnerable who are left with no choice but to go with poor carbohydrates diets thus putting them at risk. This research therefore seeks to explore a new option and knowledge into Diabetes mellitus management, thereby contributing to the available literatures on the disease.

1.4 Aim and Objectives of the Study

1.4.1 Aim

This research is aimed to study the effect of feed formulated from varying ratio of Finger millet and Sorghum, with a fixed ratio of Moringa seeds on Alloxan induced diabetic albino rats.

1.4.3 Objectives of the study

The objectives of this research are to determine;

i. The proximate, mineral, vitamin and amino acids compositions of *Eleusine coracana*, *Sorghum bicolor* and *Moringa oleifera* seeds;

ii. The proximate, mineral, vitamin and amino acids constituents of the formulated diets;

iii. The effects of alloxan induction on blood sugar level of the Wistar albino rats before feed administration;

iv. The effects of the formulated feed on blood glucose of Alloxan induced Wistar rats.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Diabetes Mellitus

Diabetes is a chronic, complicated disease with advancing metabolic disorder marked by persistent hyperglycemia which is caused by errors in carbohydrate, lipid, and protein metabolism. Damages, malfunction and organs degeneration, which include; the eyes, kidneys, nerves, heart, and blood vessels among others, are linked to persistent hyperglycemia (Bardsley *et al.,* 2004). Diabetes mellitus is likely one of humanity's known earliest illnesses. It was initially written around 3000 years ago in an Egyptian text. (Ahmed, 2002). In 1988, Type 2 Diabetes mellitus was identified as a component of the metabolic syndrome for the first time (Patlak, 2002).

Diabetes mellitus has a wide range of causes and etiologies, however usually involves anomalies in insulin secretion or action, or even both, at some stage throughout the disease's advancement. Affecting the majority of diabetic individuals are; Type 1Diabetes (which is immune-supported or unexplained) or Type 2 diabetes (also known as non-insulin-reliant diabetes) is the most prevalent type of diabetes (Vinay *et al.,* 2005). The relativity of genetic, environmental, and behavioral risk factors causes type 2 diabetes mellitus (WHO, 1999).

2.2 Classification of Diabetes Mellitus

If there is one quality that may characterize the new goals for the categorization of Diabetes mellitus, it is the need to bring together the changes in existing viewpoints on the disease. Insulin-dependent diabetes mellitus (IDDM) is an ancient and ambiguous phrase and non-insulin-dependent diabetes mellitus (NIDDM) proposed by WHO in 1980 and

1985 have vanished, and the new categorization identifies four kinds of diabetes mellitus: type1, type2, "other specific types," and gestational diabetes (WHO, 1999).

2.2.1 Type 1 diabetes mellitus

The antibody mechanism which causes type 1 diabetes mellitus (juvenile diabetes) is distinguished by the destruction of beta cells, resulting in absolute insulin insufficiency. Antibodies to anti-glutamic acid decarboxylase, islet cell, or insulin, which suggest autoimmune processes that lead to the death of beta cells, are commonly seen in Type 1 diabetes patients (Ozougwu *et al.,* 2013).

2.2.2 Type 2 diabetes mellitus

Type 2 diabetes is far more common, and it is caused by a combination of anomalies in insulin production and insulin action, with one of these issues gaining precedence over the other. Extracellular insulin is not needed to control blood sugar in patients with type 2 diabetes, but it may be necessary if diet and oral hypoglycemic medicines are ineffective. Ninety percent to ninety five percent of diabetics have this kind of diabetes (Defronzo, 1999). All forms of diabetes are defined by diabetes mellitus and the development of diabetes-specific micro-vascular pathology in the retina, renal glomerulus, and peripheral nerve. Due to its micro-vascular pathology, diabetes is a leading cause of vision loss, later part renal disease, and a variety of severe neuropathies. When islet beta-cell function is impaired, insulin secretion is inadequate, resulting in glucose over secretion by the liver and glucose underutilization in surface tissue (Jamaly, 2020).

Type 2 diabetes is split into various categories, each with a different amount of insulin impedance and β-cell dysfunction, but all of them resulting to diabetes (American Diabetes Association, 2001). Single gene disorders affect the pancreatic beta-ability cell's to

generate insulin at both ends of the spectrum (Owen and Hattersley, 2001) or the ability of muscle, fat, and straight cells to respond to insulin activity (Taylor and Arioglu, 1999).

2.2.3 Gestational diabetes mellitus (GDM)

Females that develop diabetes during pregnancy are classified as having gestational diabetes mellitus, which is a functional classification rather than a patho-physiologic illness. Females that develop Type 1 diabetes mellitus during pregnancy or who have undiagnosed asymptomatic Type 2 diabetes mellitus that is discovered in pregnancy are referred to as having gestational hyperglycemia. The condition generally begins in the final three months of pregnancy in the majority of women who acquire gestational diabetes mellitus (WHO, 1999).

2.2.4 Genetic defects of beta–cell function

Several types of hyperglycemia can be linked to monogenic abnormalities in β-cell activity, which are often characterized by the start of moderate hyperglycemia at a young age (usually prior to age 25 years). They are generally hereditary in a dominant autosomal manner. These types of diabetic clients (previously known as maturity onset diabetes of the young (MODY)), have reduced insulin production but little or non-insulin action deficiency (Byrne *et al.,* 1996).

Three-genetic sites on separate chromosomes have been identified as abnormal. The majority frequent type is linked to mutation in HNF1, a nuclear hepatic transcription factor on chromosome 12" (Yamagata, 2014). Glucokinase gene mutation on chromosome 7p is linked to a second type (Osbak*et al.,* 2009). Glucokinase transforms glucose to glucose–6–phosphate, which promotes beta cell insulin production via metabolism. As a result, glucokinase acts as the beta cell's "glucose sensor."Increased glucose levels are required to

elicit normal amounts of insulin secretion due to glucokinase gene abnormalities. A third type is caused by HNF4" gene mutation on chromosome 20q (Yagamata., 2014). HNF4" is a transcription factor that controls HNF1 expression. A fourth variation has recently been linked to mutations in another transcription factor gene, IPF–1, that causes complete pancreatic agenesis in its homozygous form (Kofent and Spagnoli, 2016). Other people with a similar clinical appearance are presently being studied for specific genetic abnormalities.

Diabetes mellitus and deafness have both been linked to point mutations in mitochondrial DNA (Schaefer *et al.,* 2013). The most frequent mutation in the tRNA leucine gene is an A to G change at position 3243. The MELAS syndrome (Mitochondrial myopathy, Encephalopathy, Lactic Acidosis, and Stroke–like syndrome) has a similar lesion; however, diabetes is not a feature of this disease, suggesting that alternative phenotypic manifestations of this genetic lesion exist for unknown reasons (García-Ávila, 2018).

In a few families, genetic defects that translate in the lack of ability to generate insulin from pro-insulin have been discovered. Carbohydrate intolerance is generally moderate since such characteristics are inherited in an autosomal dominant manner (Akinci *et al.,* 2018). In a few families, mutant insulin molecules with reduced the binding of receptors have also been discovered. These are similarly linked to autosomal inheritance and glucose metabolism that is either normal or moderately affected (Ikle & Gloyn, 2021).

2.2.5 Incidence and prevalence of diabetes mellitus

The use of epidemiology to the study of diabetes mellitus has given valuable insights into the disease's historical development and occurrence, prevalence, incidence, morbidity, and mortality in a range of populations across the world. Identification of the illness's cause and

potential preventative measures that might be used to stop or postpone the spread of this condition, which has reached epidemic proportions in both developed and developing countries (Shapira *et al.,* 2010). Unfortunately, advances in outcomes for individual diabetic individuals have not translated into equivalent benefits in public health.

The global prevalence of diabetes has continued to rise at an alarming rate. Every nation is seeing an increase in the number of individuals with type 2 diabetes, with 80 percent of those affected residing in low- and middle-income countries. There are little figures on the incidence of type 2 diabetes mellitus in Africa overall, that's from a literature study. According to a 2008 World Fact Book study, the incidence of diabetes mellitus in Africa was 3.2 percent, with 40,895 people (2.0 percent) in Ethiopia (CIA, 2008).

Even though Type 2 Diabetes mellitus is often diagnosed in adults, it has also become much more common in children over the last two decades which has been traced back to genes inherited from parents. In the pediatric population, the prevalence of Type 2 Diabetes mellitus is greater in females than in males (Rosenbloom *et al.,* 1999).

The average age at which Type 2 Diabetes mellitus begins is between the age ranges from 12-16 years old; this occurs around the time of puberty, when a metabolic condition of insulin resistance emerges. Type 2 diabetes mellitus occurs if insufficient beta-cell activity is combined with additional threats in this physiologic state (e.g. obesity) (Grinstein *et al.,* 2013).

According to some sources, Type 1 Diabetes Mellitus is the most popular type of diabetes in the majority of the globe. Diverse populations' frequencies of occurrence vary greatly; the lowest rate (0.1 per 105 per year) is in China, while the highest rate is in Finland (37

per 105 per year). In most populations, both girls and boys are affected equally. In general, the incidence rises with age, with the peak occurring during puberty. The incidence rate in young women declines substantially after pubertal years, while it stays rather high in young adult males up to the age of 29-35 years (Soltesz *et al.,* 2007)

2.3 Clinical Features of Diabetes Mellitus

2.3.1 General symptoms

Most of the symptoms are identical in all diabetes forms, although they differ in severity and advance more quickly in type 1 diabetes.

2.3.2 Clinical features of type I diabetes

Loss of weight, polydipsia, polyurea, polyphagia, impaired eyesight, tiredness, constipation, cramps, and candidiasis are some of the symptoms (Bearse *et al.,* 2004). Patients with long-term type 1 diabetes may develop micro-vascular problems as well as macro-vascular illness (coronary artery or heart diseases, as well as peripheral vascular disease) (Pittas, 2009).

2.3.3 Clinical features of type II diabetes

The majority of instances are discovered as a result of problems or because they have a high risk of big artery atherosclerosis, which is often related to hypertension, hyper-lipidemia, and obesity. Cardiovascular problems and end-stage renal disease are the leading causes of death in people with Type 2 diabetes. Geographical variation can influence the severity of issues as well as overall morbidity and death rates (Mensah *et al.,* 2017).

2.4 Pathogenesis and Pathophysiology of Diabetes Mellitus

Hyperglycemia and physiological and behavioral reactions are inextricably linked. When the brain recognizes hyperglycemia, nerve signals are sent to the pancreas and other organs to minimize the effect (Patidar, 2011).

2.4.1 Type 1 diabetes mellitus

CD4+ and CD8+ T lymphocytes, as well as macrophages invades the islets, destroy cells producing insulin in the pancreas, resulting in Type 1 Diabetes (Ifeanyi, 2018). As an autoimmune illness, type 1 diabetes mellitus has certain characteristics (Hussain and Vincent 2007):

a. Infiltrated pancreatic islets have immune-competent and accessory cells.

b. The major histo-compatibility complex's class II (immune response) genes are linked to illness vulnerability (MHC; human leucocyte antigens HLA).

c. Islet cell-specific auto-antibodies are present.

d. Immune-regulatory changes mediated by T cells, particularly in the CD4+ T cell compartment.

e. Monokines and TH1 cells that produce interleukins have a role in the illness process.

f. Immunotherapy response and

g. Other organ-specific auto-immune disorders are frequently seen in afflicted people or their family members.

Around 85 percent of patients have circulating islet cell antibodies before commencing insulin treatment, and the most of them also have detectable anti-insulin antibodies. The enzyme glutamic acid decarboxylase (GAD) present in pancreatic Beta-cells is the target of the bulk of islet cell antibodies (Raju and Raju, 2010).

The lack of insulin production caused by the autoimmune death of pancreatic beta-cells leads to the metabolic abnormalities associated with Type 1 Diabetes. The activity of pancreatic beta-cells is abnormal in Type 1 Diabetes mellitus patients, and there is excessive synthesis of glucagons in addition to the lack of insulin secretion. Hyperglycemia typically inhibits glucagon release; however, in those with Type 1 Diabetes, hyperglycemia has no effect on glucagon secretion (Holt, 2004). The metabolic problems that result from insulin deficiency are worsened by the unusually high glucagon levels that follow as illustrated in figure 2.1 below. Although insulin deficiency is the most serious defect in Type 1 Diabetes, the immune system is also compromised.

Insulin insufficiency leads to uncontrolled lipolysis and high plasma levels of free fatty acids, which prevents glucose from being used in peripheral tissues like skeletal muscle (Holt, 2004). Insulin insufficiency affects glucose consumption and lowers the expression of a number of genes required for target tissues to respond properly to insulin, including glucokinase in the liver and the GLUT 4 class of glucose transporters in adipose tissue (Holt, 2004) stated that decreased glucose, lipid, and protein metabolism are the main metabolic derangements which occur from insulin insufficiency in Type 1 Diabetes mellitus.

2.4.2 Type 2 diabetes mellitus

These processes fail in type 2 diabetes, resulting in two major clinical defects: reduced insulin production due to pancreatic-cell malfunction and impaired insulin action due to insulin resistance (Forouhi and Wareham, 2010). When insulin resistance is prevalent; the bulk of beta-cells undergo a change that increases insulin supply while adjusting for the excessive and abnormal demand. Although the concentration insulin in plasma (both

fasting and meal stimulated) is typically raised in absolute terms, it is insufficient to sustain normal glucose level "relative" to the degree of insulin resistance. Given the close link between hormone action sensitivity and insulin secretion in the complex management of homeostatic glucose, it's nearly difficult to separate their contributions to the etiopathogenesis of Diabetes mellitus (Baynes, 2015).

Insulin resistance and hyperinsulinemia contribute to reduce glucose tolerance in the long run and increases the quantity of free fatty acids in blood circulation as illustrated in figure 2.2 below (Rifa'i and Widodo, 2014). With the exception of maturity onset diabetes in the young, the mechanism of inheritance for type 2 diabetes mellitus remains unclear. MODY is inherited as an autosomal dominant disease and is caused by mutations in the glucokinase gene on chromosome 7p. If islet cell antibodies (ICA) are negative, hyperglycemia detected before the age of twenty-five years can be controlled without insulin for more than five years (Mensah *et al.,*2017).

2.4.3 Insulin resistance

An early insulin secretion shortfalls, as well as relative insulin shortage in many people, are believed to be the main events, together with peripheral insulin resistance (Botero and Wolfsdorf, 2005). As illustrated in figure 2.3, insulin resistance results in reduced insulin-mediated glucose absorption in the peripheral (by muscle and fat), insufficient hepatic glucose output reduction, and impaired fat triglyceride uptake. To overcome insulin resistance, islet cells will increase the amount of insulin released. Endogenous glucose production is enhanced in those with type 2 diabetes or impaired fasting blood sugar. Because it occurs in the setting of hyperinsulinemia, hepatic insulin resistance is the

driving force of hyperglycemia in type 2 diabetes, at least in the early and middle stages of the condition.

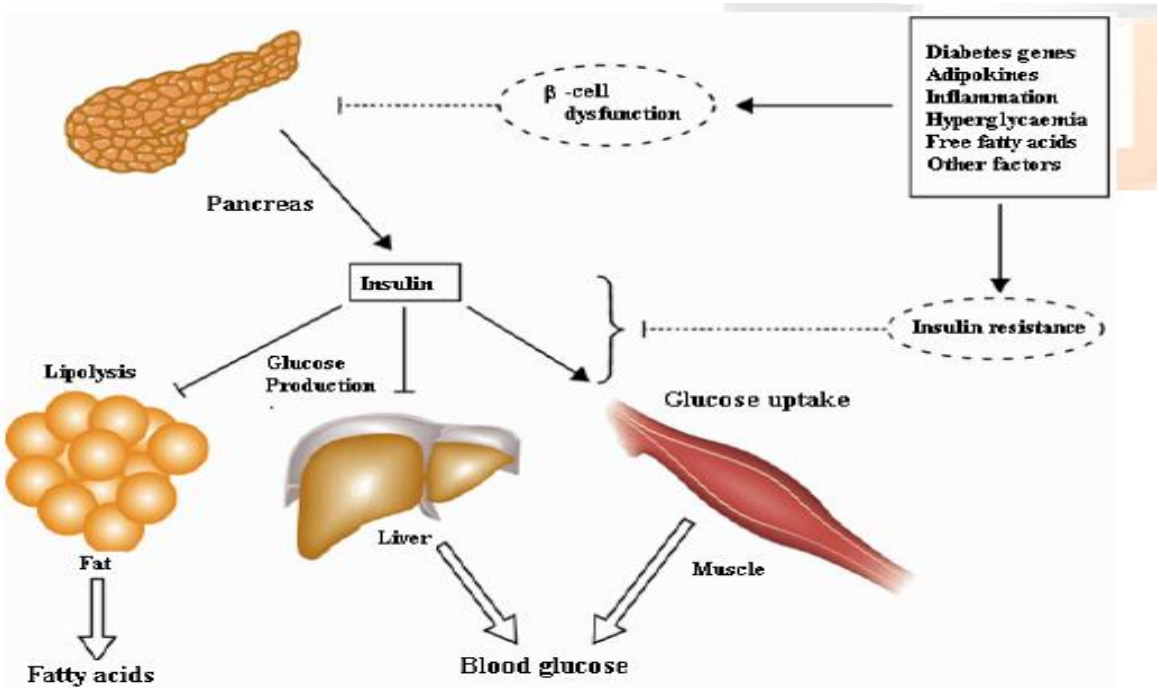

Figure 2.2: Pathophysiology of Hyperglycemia and Increased Circulating Fatty Acids in Type 2 Diabetes.

Sourced from Pittas, Mensah *et al.,* 2017

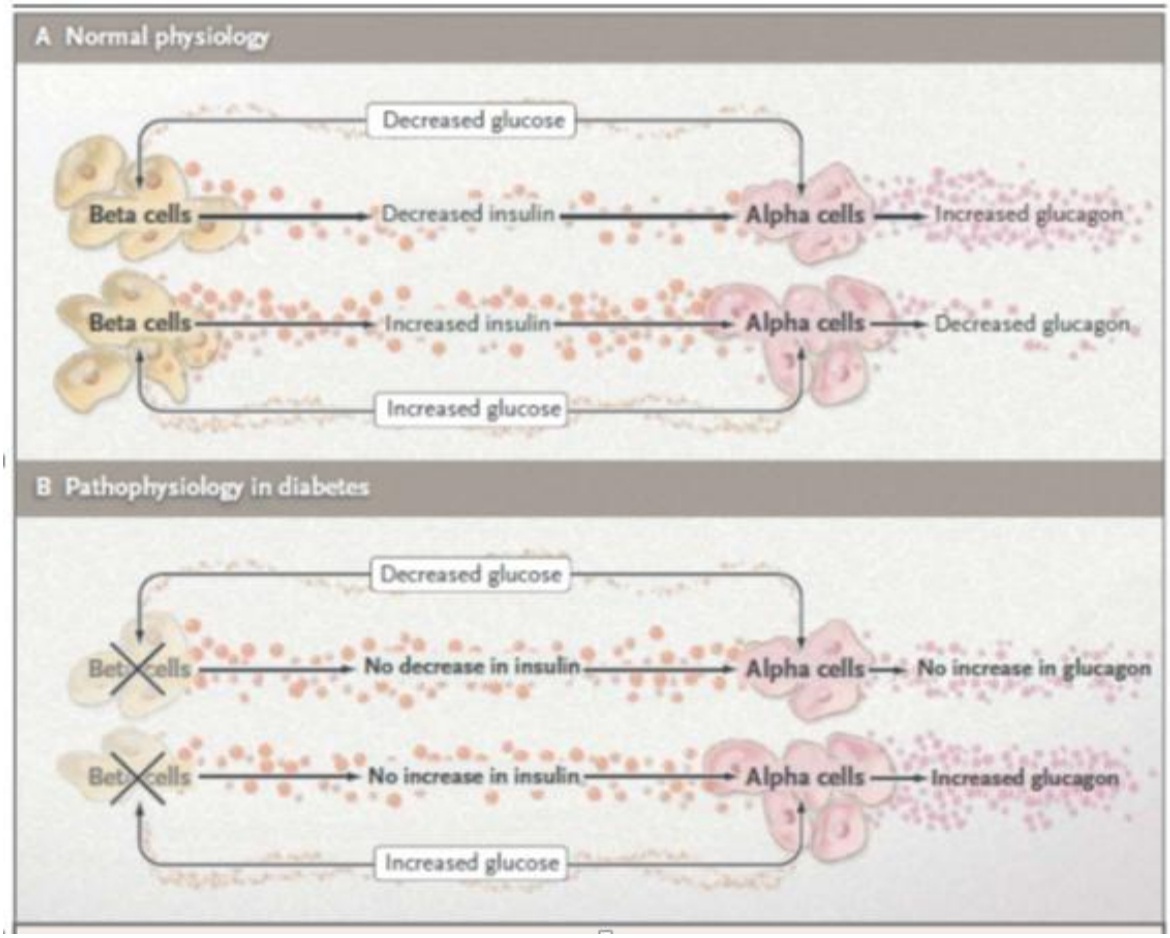

Figure 2.3: Pathophysiology of Blocked Beta cells Actions

Sourced from Forbes and Cooper, 2013

Panel A illustrates the physiological effect of a reduction in insulin combined with a low glucose levels in spurring alpha-cell glucose release, whereas Panel B illustrates the pathophysiological impact of beta-cell malfunction and the consequent loss of both a reduction in insulin release and a boost in alpha-cell glucagon secretion, despite relatively low glucose levels.

2.5 Diagnosis of Diabetes Mellitus

Screening for diabetes or pre-diabetes allows for earlier intervention, perhaps decreasing future complication rates; nevertheless, randomized trials are required to establish benefit clearly. Obesity, hypertension, and a family history of diabetes are risk factors for the

patient depicted in the vignette, and she should be tested (Cryer, 2012; Forbes and Cooper, 2013). At the time of diagnosis, around 25% of individuals with type 2 diabetes mellitus already have micro-vascular problems, indicating that they have had the condition for more than 5 years (American Diabetic Association, 2011). As a result, multiple methods for diagnosing diabetes exist for different people. Fasting Blood Sugar (FBS) is the emphasis of the 1997 American Diabetes Association (ADA) recommendations for diabetes mellitus diagnosis, but the Oral Glucose Tolerance Test (OGTT) is the focus of the WHO recommendations (Gillett, 2009).

2.5.1 Random blood sugar test

• The most basic test, which does not need fasting before to administration.

• Blood glucose levels of 200 mg/dl or higher are likely to suggest diabetes, although this must be verified.

2.5.2 Fasting blood glucose test:

• Before taking this exam, you should fast for at least eight hours. A diabetes diagnosis is confirmed by blood glucose levels of higher than 126 mg/dl on two or more independent tests (Gillett, 2009).

2.5.3 Oral glucose tolerance test

• This test is performed when the random plasma glucose level is 160-200 mg/dl and the fasting plasma level is 110-125 mg/dl (Lloyd *et al.,* 2008).

• This blood test determines how the body reacts to glucose. A fast of at least eight hours but no more than sixteen hours is required for this test.

• Following the determination of the fasting glucose level, 75 gm of glucose is administered, with 100 gm administered to pregnant women.

• This test is normal if the glucose level is below 140 mg/dl after two hours. A fasting glucose level of 126 mg/dl or higher, as well as a two-hour glucose level of 200 mg/dl or above, confirms a diabetes diagnosis (Gillett, 2009).

2.5.4 Glycated proteins

Glycated derivatives are formed when proteins react spontaneously with glucose in the blood. The amount of glucose in the blood and the number of reactive amino groups in the protein that are accessible for interaction with glucose dictate the level of glycation. Glycation occurs in all proteins with reactive sites, and also the amount of glycated proteins found in blood is a marker for changes in blood glucose concentration over time. Glycated proteins with a longer blood life time are important in clinical diagnosis because they indicate longer periods of glucose exposure.

2.5.5 Glycated hemoglobin

Hemoglobin has a 90-120-day life lifetime in the body. Glycated hemoglobin A, a ketoamine molecule produced by the interaction of hemoglobin A and glucose, occurs during this time. Several glycated hemoglobin sub-fractions have been identified. The most intriguing of these is the glycated hemoglobin A portion HbA1C, which acts as a retroactive indicator of average glucose level. HbA1C is indicated as a critical biomarker for blood glucose management monitoring (Selvin *et al.,* 2010).

2.5.6 Fructosamine test

Plasma proteins are mostly composed of albumin. Albumin includes free amino groups, which causes a non-enzymatic interaction with glucose in plasma. As a result, glycated albumin could also be used to monitor the concentration of blood glucose. Glycated

albumin is often used to provide a one-to three-week retrospective estimate of average blood glucose concentration205-285 μmol/L is the reference range.

2.5.7 Diagnosis of gestational diabetes mellitus

At least 6 weeks after the pregnancy ends, the woman should have an oral glucose tolerance test to determine if she has diabetes, normal glucose tolerance, impaired glucose tolerance, or impaired fasting glucose. If you have a positive family history, a history of gestational diabetes, are obese, or belonging to a high-risk ethnic group, you should get tested as soon as possible. They should be checked again in 24-48 weeks if the initial screening is negative. Gestational diabetes mellitus is diagnosed when two or more of the plasma glucose values are attained or exceeded (Hussain and Vincent, 2007).

It's unclear whether the likelihood of diabetic complications varies depending on whether the condition was detected only by fasting plasma glucose testing or solely through glycated hemoglobin testing. The glycated hemoglobin level, which combines fasting and postprandial glucose levels over a longer period, may be a stronger predictor of some problems, particularly cardiovascular disease, according to preliminary results from a large, community-based prospective cohort research (Rathmann *et al.,* 2010). It's also unclear if the risk of diabetes changes between individuals who have been diagnosed with pre-diabetes using glycated hemoglobin testing and those who have been diagnosed using fasting plasma glucose testing. Depending on whatever test is used to establish the final diagnosis, such risks are likely to vary. The usefulness of risk scores that incorporate not just hyperglycemia measures but also other biomarkers and risk factors is currently being investigated (American Diabetes Association, 2012).

Impaired fasting glucose is defined as a fasting plasma glucose level of 100 to 125 mg/dl (5.6 to 6.9 mmol/L). Increased glycated hemoglobin is defined as a glycated hemoglobin level of 5.7 to 6.4 percent. A repeat test on a different day or an alternative test (for example, glycated hemoglobin instead of FPG or vice versa) on the same or other day validates the diabetes diagnosis. If the repeat test results are in the pre-diabetic range, the patient should be counseled or treated for pre-diabetes. Rescreening in 6 months should be considered if the repeat test results are completely normal (which is improbable).

2.6 Diabetic Complications and their Pathogenesis

2.6.1 Acute complications

Diabetic keto-acidosis (DKA) and non-ketotic hyper-osmolar condition are two examples (NKHS). While the first is more regular in persons who have type 1 diabetes, the latter is more common in those who have type 2 diabetes. Both illnesses are connected to absolute or relative insulin deficiency, volume depletion, and disrupted mental state. Insulin insufficiency is coupled with an overabundance of counter-regulatory hormones in diabetic keto-acidosis (glucagon, catecholamines, cortisol, and growth hormone).The lower insulin-to-glucagon ratio stimulates gluconeogenesis, glycogenolysis, and ketone body production in the liver, and also increased transport of free fatty acids and amino acids from fat and muscle to the liver. Ketosis is caused by an increase in the release of free fatty acids from adipocytes as a result of enhanced lipolysis. Nausea and vomiting are common in diabetic keto acidoses. In severe diabetic keto-acidoses, lethargy and CNS depression might progress to coma. Cerebral edema, a potentially fatal consequence, is most commonly found in youngsters (Keenan *et al.,* 2007).

Non-ketotic hyper-osmolar state (NKHS) is more common in persons over the age of 50 who have type 2 diabetes. The most noticeable symptoms are polyuria, orthostatic hypotension, and a variety of neurological symptoms such as altered mental state, lethargy, obtundation, seizure, and perhaps coma. The fundamental causes of Non-Ketotic hyper-osmolar condition include insulin insufficiency and insufficient fluid consumption (NKHS). Insulin insufficiency causes hyperglycemia, which causes osmotic diuresis and a significant loss of intravascular volume (Keenan *et al.,* 2007).

2.6.2 Chronic complications

Diabetes mellitus has long-term effects on a range of organ systems and is responsible for the majority of morbidity and mortality. There are two types of chronic complications: vascular and nonvascular. Microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular (coronary artery disease, peripheral vascular disease, and cerebrovascular disease) problems are the two types of vascular complications (Keenan *et al.,* 2007).

Gastroporesis, sexual dysfunction, and skin abnormalities are examples of nonvascular consequences. Diabetes mellitus is the most frequent cause of adult blindness, a range of severe neuropathies, and cardiac and brain problems due to its chronic consequences. Diabetes complications are typically more expensive to treat than the disease itself. Early in the course of diabetes, intracellular hyperglycemia causes erratic blood flow and increased vascular permeability. Reduced nitric oxide activity, increased activity of vasoconstrictors such angiotensin II and endothelin-1, and elaboration of permeability factors like vascular endothelial growth factor are all variables contributing to this.Insulin resistance specific to the phosphotidylinositol-3-OH kinase pathway and hyperglycemia appear to be the cause of endothelial dysfunction in diabetic arteries (Keenan *et al.,* 2007).

2.6.3 Diabetic retinopathy

Diabetic retinopathy, the most frequent cause of blindness, affects 3/4 of all people who have had diabetes for more than 15 years. There is an increase in the severity of retinal vascular lesions, culminating in new vessels development.

Diabetic retinopathy is divided into two types: non-proliferative and proliferative. Retinal vascular microneurisms, blot hemorrhages, and cotton-wool spots, as well as the loss of retinal pericytes, increased retinal vascular permeability, changes in regional blood flow, and abnormal retinal microvasculature, all lead to retinal ischemia in the non-proliferative stage of the disease.

Neo-vascularization arises as a response to retinal hypoxia in proliferative retinopathy. The newly formed vessels may form near the optic nerve and/or macula, where they might easily burst, resulting in vitreous hemorrhage, fibrosis, and retinal detachment (Keenan *et al.,* 2007).

2.6.4 Neuropathy

Neuropathy affects around half of all diabetics, and it can range from polyneuropathy to mononeuropathy to autonomic neuropathy. Polyneuropathy produces a loss of peripheral sensation, which, when paired with a malfunctioning micro- and macro-vascular junction in the periphery, can result in non-healing ulcers, the leading cause of non-traumatic amputation. In small myelinated or non-myelinated C-fibers, axon thickening, microfilament reduction, and capillary constriction occur. Endothelial cell activation, pericyte deterioration, basement membrane thickening, and monocyte adhesion can all be caused by direct hyperglycemia-induced damage to the nerve parenchyma, as well as neuronal ischemia causing endothelial cell activation, pericyte degeneration, basement

membrane thickening, and monocyte adhesion. Mono-neuropathy is a kind of polyneuropathy in which specific cranial or peripheral nerves fail. It is not as common as polyneuropathy. Autonomic neuropathy can impact a variety of systems, including the cardiovascular, gastrointestinal, genitourinary, sudomotor, and metabolic systems (Cheshire *et al.,* 2021).

2.6.5 Nephropathy

This is one of the most prominent causes of end-stage renal failure. Micro-albuminuria is a symptom of glomerular hemodynamic irregularities that contribute to glomerular hyperfiltration and glomerular injury. Overt proteinuria, a low glomerular filtration rate, and end-stage renal failure are all present. Micro-albuminuria is a symptom of glomerular filtration dysfunction, which is caused by alterations in the production and catabolism of different glomerular basement membrane polymers such as collagen and proteoglycans, resulting in glomerular basement thickening. Because VEGF is both an angiogenic and a permeability agent, a rise in renal VEGF levels reported in preclinical models of diabetes might explain the increase in glomerulus permeability (Dou and Jourde-Chiche, 2019).

2.6.6 Cardiovascular morbidity and mortality

Diabetes mellitus increases the risk of sudden death by five times, as well as the risk of peripheral vascular disease, congestive heart failure, coronary artery disease, and myocardial infarction by one to five times. Individuals with diabetes frequently experience the lack of chest discomfort (silent ischemia), and a comprehensive cardiac assessment is recommended for those having significant surgical operations. Despite evidence that better glycemic management lowers micro-vascular problems in diabetes mellitus, it's possible that such interventions have no effect on macro-vascular complications or possibly

aggravate them. The lipid profiles of the intensive group improved (lower total and low-density lipoprotein cholesterol, lower triglycerides), suggesting that intensive therapy might reduce the risk of coronary vascular mortality. Cerebrovascular disease, as well as coronary artery disease, is more frequent in those with diabetes (threefold increase in stroke). Diabetes mellitus patients are more likely to develop congestive heart failure (diabetic cardiomyopathy).The pathogenesis of this anomaly is likely complex, with variables such as atherosclerosis-induced myocardial ischemia, hypertension, and myocardial cell failure due to chronic hyperglycemia all contributing. Despite the fact that diabetes does not raise LDL levels, low density lipoprotein particles seen in type 2 diabetes are more atherogenic, more readily glycated, and more sensitive to oxidation (Orekhov *et al.,* 2014).

2.6.7 Hypertension

Additional diabetes issues, such as cardiovascular disease and nephropathy, might be accelerated by hypertension. Antihypertensive medications should be chosen based on the therapeutic agent's advantages and disadvantages in relation to the patient's risk factor profile (Orekhov *et al.,* 2014). Some diabetes-related considerations are as follows:

1. Insulin resistance is reduced and the lipid profile is improved somewhat with a-adrenergic blockers. A-blockers and thiazide diuretics can increase insulin resistance, impair lipid profiles, and somewhat increase the risk of type 2 diabetes.

2. B-blockers are effective because they can mask hypoglycemia symptoms, and hypoglycemic episodes are rare when cardio-selective b1 medicines are used.

3. Lipid and glucose neutral central adrenergic antagonists and vasodilators

4. Orthostatic hypotension in diabetics with autonomic neuropathy may be linked to sympathetic inhibitors and a-adrenergic blockers.

5. Calcium-channel blockers are glucose and lipid-unaffected, and they have been shown to reduce cardiovascular morbidity and mortality in patients with type 2 diabetes, particularly in those over the age of 65 who have systolic hypertension.

2.6.8 Infections

Infections are more common and severe in people with diabetes. Unidentified anomalies in cell-mediated immunity and phagocyte function associated to hyperglycemia, as well as decreased vascularization owing to long-term diabetes, are two possible reasons. Many common infections are more common and severe in diabetes, whereas a few unusual diseases affect diabetics almost exclusively (examples include rhinocerebral mucormycosis and malignant otitis externa, which is usually secondary to *P. aeruginosa* infection in the soft tissue surrounding the external auditory canal). Pneumonia, urinary tract infections, and skin and soft tissue infections are all more common among diabetics. In diabetics, Gram-negative bacteria including *Staph aureus* and *Mycobacterium tuberculosis* are more prevalent. Diabetic individuals had a higher rate of *S. aureus* colonization in skin folds and nares, as well as a higher risk of postoperative wound infections (American Diabetes Association, 1999).

2.7 Mechanisms of Hyperglycemia-Induced Damage

Many ideas regarding how hyperglycemia promotes diabetes complications have resulted in a significant quantity of data and various therapeutic studies with particular inhibitors of these pathways. The key concepts include Aldose Reductase, Advanced Glycation End

Product (AGE) theory, Protein Kinase C (PKC) isoform activation theory, Increased Hexosamine Pathway Flux theory, and Reactive Oxygen Intermediate theory.

2.7.1 Aldose reductase

This is the first enzyme in the polyol pathway. In the presence of NADPH, it is a cytosolic monomeric oxido-reductase that catalyzes the reduction of a wide variety of carbonyl compounds, including glucose. In a hyperglycemic state, rising intracellular glucose causes greater enzymatic conversion to the polyalcohol sorbitol, resulting in lower NADPH levels (Tripathi and Srivastava, 2006). In the polyol route, the enzyme sorbitol dehydrogenase transforms sorbitol to fructose, while NAD^+ is transformed to NADH. In diabetes and galactosemia, excess sorbitol generated by the action of aldose reductase on glucose or galactose accumulates in the lens, promoting cataract formation.

Several techniques have been used to explain the probable deleterious effects of hyperglycemia-induced increases in polyol pathway flux. Reduced (Na^+ + K^+) ATPase activity, increased cytosolic NADH/NAD^+, and decreased cytosolic NADPH are all instances of sorbitol-induced osmotic stress. PKC activation by hyperglycemia boosts the activity of cytosolic phospholipase A2, which boosts the production of two Na^+ K^+ ATPase inhibitors, arachidonate and PGE2. It's also been claimed that when NADPH converts glucose to sorbitol, it eats it. Intracellular oxidative stress may be produced or worsened because NADPH is required for the regeneration of reduced glutathione (GSH) (Tripathi and Srivastava, 2006).

2.7.2 Advanced glycation end products

Advanced Glycation End Products (AGE) is found in greater concentrations in diabetic retinal vasculature and renal glomeruli (Daroux *et al.,* 2010). AGE inhibitors partly

protected different functional and anatomical signs of diabetic microvascular diseases in the retina, kidney, and nerve. An inhibitor of Advanced Glycation End Products (AGEs), amino guanidine, decreased total urine protein and delayed the onset of neuropathy (Yamagishi *et al.,* 2008).Intracellular Advanced Glycation End product precursors cause three types of damage to target cells: Advanced Glycation End Products (AGE) have changed the activity of intracellular proteins; Advanced Glycation End Products (AGE) precursor-modified extracellular matrix components interact inappropriately with other matrix components and cell receptors for matrix proteins (integrins); Intracellular proteins changed by Advanced Glycation End product precursors interact inappropriately with other matrix components and cell receptors for matrix proteins (integrins) (Nowotny *et al.,* 2015).

When the Advanced Glycation End product receptor is ligated, pleiotropic transcription factors are activated, causing pathogenic alterations in gene expression as well as other cellular signaling events such as activation of mitogen-activated protein (MAP) kinase or Protein Kinase C, which can lead to cellular dysfunction (Fukami *et al.,* 2014).

2.7.3 Diacyl-glycerol (DAG) and protein kinase C (PKC)

These are key intracellular signaling molecules that regulate vascular permeability, vasodilator release, endothelial activation, and growth factor signaling, among other things. There are at least eleven Protein Kinase C isoforms, nine of which are activated by Diacyl-glycerol, a lipid second messenger (DAG). Intracellular hyperglycemia increases the amount of Diacyl-glycerol in cultivated microvascular cells, as well as the retina and renal glomeruli of diabetic animals (Paul *et al.,* 2020). Protein Kinase C isoforms are activated by increased de novo DAG synthesis, which has been related to abnormalities in retinal and renal blood flow. Protein Kinase C is activated when glucose levels rise, causing smooth

muscle cells to generate the permeability-enhancing factor VEGF. Protein Kinase C activity in the retina and renal glomeruli was dramatically reduced in diabetic mice treated with a Protein Kinase C inhibitor (Koya *et al.,* 2000).At the same time, the treatment reduced diabetes-related increases in retinal mean circulation time, normalized glomerular filtration rate increases, and partially rectified urinary albumin excretion.

2.7.4 Hexosamine pathway

Excess intracellular glucose shunting into the hexosamine system might result in a variety of diabetes problems (Pang *et al.,* 2020). This pathway diverts fructose 6-phosphate from glycolysis to provide substrates for activities that need UDP-N-acetylglucosamine, such as the formation of proteoglycans and the synthesis of O-linked glycoproteins. When the rate-limiting enzyme in the conversion of glucose to glucosamine-glutamine, fructose-6-phosphate amido-transferase (GFAT), is hindered, hyperglycemia-induced increases in TGF a, TGF b, and PAI-1 transcriptions are prevented. Insulin resistance induced by hyperglycemia and obesity is also a result of this pathway.

2.7.5 Reactive oxygen intermediate theory

Both enzymatic and non-enzymatic mechanisms can cause hyperglycemia to enhance oxidative stress. The glycolytic and TCA cycles provide reducing equivalents, which are used to power mitochondrial ATP synthesis via oxidative phosphorylation. Byproducts of mitochondrial oxidation, such as superoxide anion, increase when glucose levels rise. Free radicals are formed when glucose is oxidized, and they damage cellular proteins and mitochondrial DNA (Pang *et al.,* 2020).

Increased oxidative stress reduces the barrier function of the endothelium by lowering nitric oxide levels, degrading cellular proteins, and increasing leukocyte adherence to the

endothelium. GSH, vitamin C, and vitamin E levels have been found to be lower in diabetics, whereas oxidative stress markers such as oxidized low-density lipoprotein cholesterol are being found to be greater. As a result, there are two approaches to planning therapy for hyperglycemia-related issues. The first is to find and normalize the activity of a shared signaling route that both glucose and glucotoxins use to exert their effects, such as reactive oxygen species (ROS) or advanced glycation end products (AGEs). Both of these approaches are now being explored in clinical trials.

2.8 Therapeutics for Diabetes Mellitus

Therapeutics for Diabetes mellitus is classified into two major groups which include (1) Non-pharmacological management of diabetes mellitus, which is subdivided into dietary management and physical exercise, (2) clinical or drug management.

2.8.1 Diet

The majority of individuals with non-insulin-dependent diabetes mellitus are overweight or obese, which is now widely acknowledged as a key determinant in insulin resistance. As a result, losing weight is an important part of managing non-insulin-dependent diabetes. When extreme caloric restriction and/or rapid weight loss are needed, an extremely low calorie diet or a protein-sparing modified fast may be utilized.

2.8.1.1 *Macronutrients*

In individuals with non-insulin-dependent diabetes mellitus, the optimum carbohydrate, protein, or fat consumption balance is currently being debated. It was only recently discovered that a high-carbohydrate (60%) but low-sugar diet may predispose to the development of dyslipidemia (Garg *et al.,* 1988). Carbohydrates should be primarily complex and high in soluble fiber; low glycemic index meals are preferred, although a

modest quantity of sucrose does not seem to be detrimental (Tripathi & Srivastava, 2006). Protein intake should not exceed the daily need, since too much protein might damage renal function (Marckmann *et al.,* 2015).

2.8.1.2 *Dietary fibers*

Several trials reviewed by Hoewitz *et al.,* (1990) have demonstrated that adding certain kinds of soluble fiber, notably guar gum and pectin, to individuals with non-insulin dependent Diabetes mellitus can result in considerable reductions in postprandial glucose and insulin levels.

2.8.1.3 *Fish oils*

According to some evidence, fish oils or fish-derived omega-3 fatty acids may help to avoid atherosclerotic vascular disease by decreasing plasma triglyceride and lipoprotein levels (Usman, 2013). There is proof, nevertheless, that in non-insulin-dependent diabetes mellitus, the decrease in plasma triglyceride levels is counteracted by negative effects on blood glucose or LDL cholesterol (Hocht *et al.,* 2017).

2.8.2 Physical activity

Recent clinical research has provided insight on the mechanism by which exercise can aid in the management of high blood glucose levels (Tripathi and Srivastava, 2006). Furthermore, there is substantial evidence that regular exercise has a positive influence on a number of cardiovascular risk factors that aggravate type 2 diabetes diagnoses (American Diabetes Association, 1990). However, exercise may be detrimental to a tiny percentage of individuals and should not be advised. Regular exercise enhances insulin sensitivity, which may lead to better glucose tolerance (Akins *et al.,* 2019).These advantages are the consequence of skeletal muscle enzymatic adaptation, which is considered to be

responsible for improved maximal oxygen absorption, as well as a decrease in body weight, body fat, and perhaps cell size. These advantages are beneficial to type 2 diabetes patients because they increase job capacity and quality of life while potentially reducing the requirement for insulin or oral hypoglycemic medicines.

2.8.3 Drug targets for insulin resistance diabetes mellitus

Current treatment methods were primarily established in the lack of precise molecular targets or a thorough understanding of illness etiology. A vast number of molecular pharmacological targets involving diverse metabolic processes have been identified in recent years. These are predicated on their expected roles in regulating one or more critical elements of diabetes and metabolic syndrome etiology. These are the following:

a) Reducing the liver's excessive glucose synthesis

b) Beta-cells targeting

c) Insulin signaling pathways targeting and

d) Targeting lipid metabolism.

2.8.3.1 *Reducing excessive hepatic glucose production*

The liver controls endogenous glucose production via gluconeogenesis and glycogenolysis, the creation and degradation of glycogen. The development of overt hyperglycemia is primarily due to increased rates of hepatic glucose production. The activation of the gluconeogenesis and glycogenolytic pathways by glucagon leads to hyperglycemia (Shah *et al.,* 2000). Its receptor, a G protein receptor with seven trans-membrane domains, might be a target for small-molecule antagonists (Felsing *et al.,* 2019). In addition, several enzymes that regulate rate-controlling steps in the gluconeogenesis or glycogenolytic pathways might be used as therapeutic targets. One such enzyme that is inhibited is hepatic

glycogen phosphorylase (Treadway *et al.,* 2001), which catalyzes the release of glucose from glycogen. Other enzymes include fructose-1,6-bisphosphatase and glucose-6-bisphosphatase (Moller, 2001). Inhibition of fructose-1,6-bisphosphatase will indeed selectively block gluconeogenesis by disrupting the transformation of fructose-1,6-biphosphate to fructose-6-phosphate, whilst inhibition of glucose-6-phosphatase will indeed attenuate the final step in hepatic glucose production that both the gluconeogenic and glycogenolytic pathways share.

2.8.3.2 *Treating β-cells*

Glucagon-likepeptide-1 (GLP-1) and gastric inhibitory peptide (GIP), two gut-derived peptide hormones, operate on β-cells via G protein-coupled receptors to augment glucose-stimulated insulin release (Drucker, 2001). Humans can be given either of these two hormones to increase insulin secretion. Because both hormones are rapidly degraded at the amino terminus by dipeptidyl peptidase-IV (DP-IV), modified GLP-1 peptide agonists that are resistant to this enzyme are advised. DP-IV deficient mice had higher levels of circulating active GLP-1, as well as increased insulin production and an otherwise healthy phenotype (Marguet *et al.,* 2000).As a result, the growth of GLP-1 analogues and DP-IV inhibitors is anticipated to result in significant fresh treatment methods that avoid the risks of hypoglycemia, weight gain, and subsequent failurerelated with sulphonylurea usage.

2.8.3.3 *Targeting the insulin signaling pathways*

Reduced insulin receptor-tyrosine kinase activating and insulin-stimulated phosphatidylinositol-3-OH kinase activities are two signaling issues that can lead to insulin resistance (PI-3-K). Several molecular markers are now being investigated as potential methods to enhance insulin-mediated signal transduction. PTP 1B expression has been

shown to be higher in insulin-resistant individuals (Torkarz *et al.,* 2018). Overexpression of this enzyme prevents insulin receptor kinase activation. A PTP 1B knockout mouse's insulin sensitivity was greater than that of control littermates. As a result, inhibiting PTP 1B is a promising target for therapeutic development (Vieira, 2017). In experimental models of insulin resistance, serine kinases may phosphorylate IRS 1 and therefore block its tyrosine phosphorylation. Another promising avenue for anti-diabetic treatment is the identification of these kinases and their particular inhibitors. PI-3-kinase products, on the other hand, are crucial for insulin action and may be reduced during insulin resistance. Other potential treatment targets for putative insulin signaling negative controllers have recently been discovered.

Glycogen synthase kinase-3 (GSK-3) suppresses the stimulation of glycogen synthase and, as a result, the accumulation of glycogen in muscle, mitigating the effects of insulin. Recent discoveries in vivo using GSK-3 activity inhibitors show that insulin action might be enhanced (Henriksen *et al.,* 2001). SH2 domain containing inositol 5-phophatase type 2 (SHIP 2) has the potential to dephosphorylate key phospholipids such phosphatidyl inositol phosphate, which is generated by insulin-mediated PI-3-K activation. The enhanced insulin sensitivity of heterozygous null mice was recently described, showing that this enzyme is a diabetes target. Increased muscle PKC q activity has been discovered in the context of fatty acid-induced insulin resistance, implying that it might also be a therapeutic target (Shulman, 2002).

2.8.3.4 ***Targeting lipid metabolism***

Reduced appetite and/or increased energy expenditure will be useful in the treatment of type 2 diabetes since obesity is connected to the progression of insulin resistance. Obesity and type 2 diabetes patients may benefit from the melanocortin-4 receptor (MCR-4). As a result, either enhanced expression of a natural MCR-4 antagonist or receptor deletion can result in a robust phenotype with a variety of metabolic syndrome symptoms (Richard and Picard, 2011). The central restriction of fatty acid synthase as a means of reducing hunger is a unique goal. cAMP triggered protein kinase, acetyl CoA carboxylase, adipocyte-related complementing protein 30, PPAR-g, and PPAR a are some of the mechanisms which might be employed to repair or prevent overweight lipotoxicity. (Willson *et al.,* 2000). PPARs are nuclear receptors that are ligand-activated transcription factors. They are a possible therapeutic target for metabolic syndrome. Insulin-sensitizing thiazolidinedione (TZD) medications commonly target PPAR-g as a molecular target (Wilson *et al.,* 2000; Moller and Greene, 2001). PPAR-g activates adipocyte genes including lipoprotein lipase and fatty acid transporter-1, leading in enhanced insulin action and lower triglyceride and FFA levels (Moller and Greene, 2001). PPAR-a, a nuclear receptor that is very similar to PPAR, is the molecular target for the fibrate class of lipid-modulating medications. Willson and associates (Willson *et al.,* 2000). PPAR-a agonists have an autonomous insulin-sensitizing effect due to the decrease in muscle lipid content (Ye *et al.,* 2001).

2.8.4 Pharmacological management of diabetes mellitus

In people with diabetes mellitus, hyperglycemia is always the result of a mismatch between the quantity of insulin necessary to regulate metabolic processes and the amount of insulin generated by the person's beta-cells. Patients with type 1 diabetes or insulin-sensitive type 2 diabetes that have normal insulin activity have an absolute insulin deficit. Insulin

resistance generally starts as a relative insulin deficit and progresses to an absolute insulin insufficiency over time.

Insulin resistance can be reduced with oral anti-hyperglycemic drugs like thiazolidinediones or metformin, postprandial insulin demands can be lowered with -glucosidase antagonists, insulin secretagogues can help to increase endogenous insulin secretion, and exogenous insulin secretion can be replaced with insulin and its analogues.

2.8.4.1 Drug therapy of non-insulin dependent diabetes mellitus

Drug therapy for non-insulin-dependent Diabetes mellitus individuals must be considered when diet, patient education, and increased physical activity have failed to fulfill specific treatment goals.

2.8.4.2 *Antidiabetic drugs*

Effective hyperglycemia management is definitely a goal for type 2 diabetes patients in order to prevent macro- and microvascular complications (Arambewela *et al.,* 2018). The conventional method starts with food, exercise, and healthy-living recommendations tailored to help obese people lose weight. More than four-fifths of newly diagnosed type 2 diabetes patients fail to respond to these treatments, and the progressive nature of the disease demands pharmaceutical treatment for the majority of patients. Sulphonylureas, metformin, and acarbose are common monotherapies, and a new class of TZDs (thiazolidinediones) was just authorized. When oral monotherapy fails to offer adequate glucose control, two different types of oral medicines are used together (Bailey and Krentz, 2016).

2.8.5 Treatment of insulin resistance and type 2 diabetes mellitus

Insulin resistance is a frequent early and ubiquitous feature of type 2 diabetes and other syndrome components, therefore it's surprising that it's not often recognized as a clinical entity deserving of its own therapy. Giving more insulin to make the signaling pathways work harder or decreasing insulin levels to reduce the consequences of hyper-insulinemia are not simple strategies to treat insulin resistance. Both approaches have drawbacks. More insulin can improve the effects of insulin that are hampered by signal transduction bottlenecks. Averting the intended impact on a highly damaged insulin pathway (e.g., impaired glucose transport) might, however, result in an overabundance of other less desirable insulin activities (e.g. lipogenesis, leading to hypertriglyceridemia and obesity, or sodium retention, promoting hypertension). Excess insulin enhanced insulin resistance at both the receptor and post-receptor levels. Insulin treatment can thereby exacerbate the negative consequences of hyperinsulinemia, with the added danger of excess insulin precipitating hypoglycemic episodes. In type 2 diabetes, lowering insulin concentrations is a particular challenge (Oldham, 2002).

2.8.5.1 *Sulphonylureas*

Sulphonylureas are commonly regarded as a fine-line pharmacological therapy for individuals with non-insulin-dependent diabetes who are not morbidly obese (Oldham, 2002). Sulphonylureas and repaglinide, a novel short-acting insulin releaser, operate directly on the islet b cells, closing ATP-sensitive K^+ channels and stimulating insulin production (Meneses *et al.,* 2015). The effectiveness of these medicines is contingent on the presence of a sufficient number of b cells with appropriate functional reserve. In advanced stages of type 2 diabetes, however, the endogenous insulin response to glucose is generally reduced.As a result, modest drug-induced increases in insulin secretion,

particularly post-prandially, can help with glycemic management. When oral medications are insufficient, insulin treatment typically provides excellent glucose control (Weir and Bonner-Weir, 2004). The most serious side effect of sulphonylureas is hypoglycemia, which is more common in the elderly and those with renal failure. Interactions with a variety of medications, including alcohol (ethanol), aspirin, phenylbutazone, and oxidase inhibitors, might aggravate sulphonylurea-induced hypoglycemia.

2.8.5.2 *Biguanides*

Metformin is the only insulin-resistance-targeting anti-diabetic drug that has been proved to work. The main reasons of its glucose-lowering effect are decreased hepatic glucose synthesis (gluconeogenesis and glycogenolysis) and increased insulin-stimulated glucose absorption and glycogenesis in skeletal muscle (Meneses *et al.,* 2015). Metformin improves insulin action by increasing phosphorylation and tyrosine kinase activity in insulin-stimulated insulin receptors, an action that occurs in insulin-responsive tissues. (Turban *et al.,* 2012). Metformin also helps to rectify the glucose-fatty acid cycle imbalance by reducing fatty acid oxidation in an insulin-independent manner. Metformin improves insulin sensitivity in both lined and skeletal muscle whilst having little effect on blood sugar levels. Insulin levels, in reality, tend to fall following long-term therapy (Arambewela *et al.,* 2018).

Metformin also enhances insulin action in adipose tissue; however obesity is counterbalanced by increased glucose turnover and lower insulin levels. Metformin has a number of benefits when it comes to fighting insulin resistance, and different parts of syndrome X associated with metformin-based type 2 diabetes treatment regimens show a

particularly favorable long-term reduction in morbidity and mortality from micro- and macrovascular complications (Arambewela *et al.,* 2018).

2.8.5.3 *α-Glucosidase inhibitors*

The intraluminal synthesis of monosaccharide, notably glucose, is slowed by acarbose and similar substances. Acarbose suppresses α-glucosidases that are involved in the breakdown of complex polysaccharides and sucrose and are associated with the small intestine's brush border membrane (Riddle, 2005). By delaying carbohydrate digestion, this lowers post-prandial hyperglycemia. Although insulin resistance is not directly addressed, the blood glucose lowering effect with reduced gluco-toxicity neither raising, and perhaps diminishing, insulin levels reduces at least a component of insulin suppression.

2.8.5.4 *Thiazolidinediones (TZDs)*

PPARs (peroxisome proliferator-activated receptors), which are ligand-activated transcription factors, can be used to treat metabolic syndrome (members of the nuclear receptor family). The mechanism that can reduce lipotoxicity is similar to the known beneficial effects of PPAR ligands. PPAR-g is the most frequent molecular target for insulin-sensitizing thiazolidinedione (TZD) medications (Wilson *et al.,* 2000). New compounds with much enhanced efficacy and selectivity for the receptor have recently been discovered (Moller and Greene, 2001). This new class of anti-diabetic medicines targets PPAR-g, a nuclear protein that promotes insulin-sensitive gene transcription. As a result, TZDs provide an unique insulin resistance treatment alternative (Arambewela *et al.,* 2018). Their blood glucose-lowering effect appears to be increased in the presence of normal circulating insulin levels, despite the fact that their long-term therapeutic efficacy is still being investigated. As a result, whether used in conjunction with insulin therapy or an

insulin releaser, efficacy is improved. The two groups of medicines can be taken together to produce additive blood glucose-lowering effect, according to preliminary clinical studies, which is aligned with the different circular processes of TZDs and metformin.

Troglitazone, roziglitazone, and pioglitazone are three TZDs that have recently been authorized to treat type 2 diabetes as insulin sensitizers. These compounds improve insulin sensitivity by binding to a nuclear receptor known as peroxisome proliferator-activated receptor-g (PPAR-g), which de-represses the retinoid X receptor (RXR) and increases transcription of insulin-sensitive genes in adipose tissue, such as lipoprotein lipase (LPL), fatty acid transporter protein (FATP), and adipocyte fatty acid binding protein (AFABP). TZDs may reduce renal issues and extend the granulation of functionally injured cells, according to preliminary preclinical findings. Troglitazone was a success in type 2 diabetes, but it was taken off the market because of idiosyncratic hepatotoxicity; however, neither rosiglitazone nor pioglitazone have had this problem (Saleh*,* 1999). TZDs are most commonly used in combination with insulin to reduce insulin dosage and improve glycemic control in people with type 2 diabetes, although they can also be used with other anti-diabetic medications (Patel *et al.,* 2001).

2.8.5.5 *Insulin*

The discovery of insulin by Banting and Best in 1922 marked a turning point in diabetic therapy. Diabetics, whether type I or type II, have a long life expectancy thanks to insulin (Roth *et al.,* 2021). Insulin treatment, on the other hand, should only be used after a complete failure of diet, fitness, and oral anti-diabetics. Insulin treatment can help individuals with type 2 diabetes mellitus improve or repair various metabolic problems. Insulin suppresses hepatic glucose synthesis, increases postprandial glucose utilization, and

improves the aberrant lipoprotein composition found in individuals with insulin resistance, all of which help to lower glucose levels. Insulin treatment can also help to reduce or eliminate the symptoms of glucose toxicity by improving insulin sensitivity and b-cell secretary function by lowering hyperglycemia. It helps to prevent or postpone diabetes consequences by suppressing ketosis. Diabetes was first treated with injectable bovine or bovine-porcine combinations. It was unable to reproduce the usual process of nutrient-related and basal insulin secretion because to significant inter- and intra-subject heterogeneity in subcutaneous absorption (Roth *et al.,* 2021).

Recombinant DNA technology was used to produce insulin analogues in order to get around these limitations. The later availability of rapid-acting (insulin lispro, insulin aspart) and long-acting (insulin glargine and detemir insulin) insulin analogues for meal and basal requirements provides individual and societal benefits. As a consequence of further advances in insulin delivery, external continuous subcutaneous insulin infusion pumps were created, providing for improved metabolic control and a decreased risk of hypoglycemia. Oral, buccal, rectal, cutaneous, nasal, and pulmonary modes of administration have all been studied, but with limited success because to varying bioavailability. A better understanding of the effects of aerosol particle size, inspiratory flow rate, and inhaled volume on insulin delivery into the alveolar surface of the lung using a liquid aerosol formulation has assisted in enhancing insulin delivery into the alveolar surface of the lung (Roth *et al.,* 2021).

Islet cell implantation is one of the alternative possibilities. Despite the recent availability of a new long-acting insulin analogue (insulin glargine) in conjunction with a rapid-acting analogue, intrapulmonary insulin delivery has a good chance of becoming the first non-

subcutaneous mode of administration. Advances in cell biology and genetics, on the other hand, may give the final opportunity for insulin independence (Roth *et al.,* 2021).

2.8.6 Combined oral therapy

Combination therapy may be essential in individuals whose condition is not effectively managed by diet and hypoglycemic therapy single-drug.

2.8.6.1 *Sulphonylureas and biguanides*

For more than 30 years, this combination treatment has been utilized. Addition of metformin to sulphonylurea medication may give sufficient control for several years in individuals who have failed to respond to sulphonylurea therapy, although sulphonylureas to metformin monotherapy are used less frequently (Vaccaro *et al.,* 2017).

2.8.6.2 *Sulphonylureas and acarbose*

Several placebo-controlled trials have shown that sulphonylurea compounds with acarbose improved diabetic management in individuals with non-insulin-dependent diabetes mellitus (Lefèbvre and Sebeen, 2019). After one week of treatment with placebo or acarbose 100 mg three times daily, six non-insulin dependent Diabetes mellitus sufferers were received a clear dose of glybenclamide (glyburide) 5 mg immediately before a standardized breakfast in a randomized crossover study conducted by Gerard *et al.,* (1984). Acarbose resulted in a considerable decrease in plasma insulin levels as well as a progressive adjustment in blood glucose profile. Acarbose had no influence on the pharmacokinetics of glybenclamide, which is surprising. In 12 patients with non-insulin-dependent diabetes mellitus who were poorly managed by diet plus sulphonylureas, Reaven *et al.,* (1990) found that adding acarbose resulted in substantial decreases in fasting and postprandial blood glucose, HbA1c, and plasma triglyceride levels.

2.8.6.3 *Biguanides and acarbose*

Combination treatment with biguanides and acarbose is uncommon, owing to the danger of gastrointestinal side effects with these two kinds of medications. HbA1c levels were considerably decreased in metformin treated individuals with acarbose, according to a preliminary investigation (Adak *et al.,* 2018). If such a combination is taken, it is important to remember that acarbose has been shown to alter the pharmacokinetics of metformin substantially (Kumar and Sinha, 2012).

2.8.7 Insulin therapy of non-insulin dependent diabetes mellitus

Insulin therapy is generally begins when diet and oral medication (either monotherapy or combination therapy) fail to achieve adequate glycemic control in non-insulin dependent Diabetes mellitus patients.

2.8.7.1 *Insulin and sulphonylureas*

A number of articles have been published on the efficacy of combining insulin and sulphonylurea therapy in people with non-insulin-dependent diabetic mellitus (Tripathi and Srivastava, 2006). The majority of researches looking into the active mechanism have found that the positive benefits (better blood glucose management, lower HbA1c levels, lower daily insulin need) are mostly attributable to stimulation of remaining insulin production, with little or no effect on insulin kindliness. Though, some researchers believe that sulphonylureas may reduce the pace at which insulin is cleared from the body's metabolic system (Khursheed *et al.,* 2019).

2.8.7.2 *Insulin and biguanides*

Despite lowering circulating insulin levels, biguanides enhance diabetic management in obese people with type 2 diabetes (Blaslov *et al.,* 2018). Metformin increases both

peripheral and hepatic (Pernicova and Korbonits, 2014) insulin sensitivity in individuals with non-insulin-dependent diabetes mellitus, according to several investigations. However, no studies appear to have simply demonstrated the benefits of combining insulin and biguanides throughout chronic therapy in overweight type 2 diabetes patients.

2.8.7.3 *Insulin and acarbose*

Insulin needs reduced with acarbose therapy in numerous trials in patients with type 1 diabetes (Cheshire *et al.,* 2021). This has also been found in diabetics who are not insulin-dependent. During acarbose therapy, metabolic regulation was improved, with a minor but considerable decrease in insulin needs. When rapid-acting insulin administered before meals fails to control an excessive postprandial rise in blood glucose in insulin-requiring non-insulin dependent Diabetes mellitus patients, the addition of acarbose to insulin should be considered.

2.9 *Moringa oleifera*

Originally, this plant was grown for its leaves, which have a high nutritional value and are used to combat hunger (Ndong, *et al.,* 2007). Leaf extracts also have hypo-cholesterolemic and hypotensive properties (Mehta, *et al.,* 2003). The seeds have antibacterial, anticancer, and anti-inflammatory properties against fungus and bacteria (Bharali *et al.,* 2003). The purifying of water and the extraction of oil are heavily emphasized in seed research. The oil has high cooking stability and frying technical capabilities (Abdulkarim *et al.,* 2005). However, research on the nutritional content and functional characteristics of defatted *M. oleifera* flour is limited, indicating that it is a valuable underutilized protein source (Govardhan *et al.,* 2011).

2.9.1 Nutritional potential of *Moringa oleifera* seeds

M. oleifera seeds include vitamins A and B1 in addition to the intriguing presence of proteins, lipids, and carbs (Mbah *et al.,* 2012). Flavonoids, saponins, sterols, phytates, and trypsin inhibitors are among the minerals, micronutrients, and bioactive compounds found in them. Because the seed's lipid content ranges from 13 to 46 percent, it may be classified as an oilseed. *M. oleifera* seeds are thus not just a source of protein, but also of fats and fibers (Compaoré *et al.,* 2011).

2.9.1.1 *Proteins*

Proteins are the primary supply of nitrogen in human diet, providing necessary amino acids for body tissue building and repair (Biesalski and Grimm, 2010). Protein is found in *Moringa oleifera* seeds, and it is the second most abundant component after lipids. Protein content has been observed to range between 18.6% and 37.2% in recent research (Bridgemohan *et al.,* 2014). Defatted *M. oleifera* seed protein content, on the other hand, ranged from 32 percent to 62.8 percent (Anwar and Rashid, 2007; Govardhan *et al.,* 2011). With the exclusion of methionine, lysine, valine, and tryptophan, which are considered limiting amino acids, the protein content of the seeds can only fulfill human demands for a few essential and semi-essential amino acids (histidine, threonine, tyrosine, leucine, isoleucine, phenylalanine).

Moringa seeds' nutritional value can be enhanced by combining them with other meals high in sulfur amino acids or lysine. *M. oleifera* seed can be mixed with grains (rice, corn, sorghum, and millet) to create complementary meals with balanced proteins in this regard.

2.9.1.2 *Carbohydrates*

The carbohydrate content of *Moringa oleifera* seeds ranges from 9.17 percent to 53.36 percent (Compaoré *et al.,* 2011; Ijarotimi *et al.,* 2013). Fibers make up about 24% of the dry weight of non-dehulled seed and 3% of the dry weight of dehulled seed, which are non-digestible carbohydrates for the organism (Bridgemohan *et al.,* 2014). *M. oleifera* seeds had low amounts of glucose (2.57 g/100 g dw), fructose (0.03 g/100 g dw), and sucrose (2.91 g/100 g dw) when compared to pulps of Adansonia digitata (6.96, 4.03, 21.63 g/100 g dw) and Parkia biglobosa (13.55, 18.51, 24.07 g/ *M. oleifera* seeds, according to Compaoré *et al.,* (2011), might be used in diabetic meals.

2.9.1.3 *Vitamins and minerals*

Mbah *et al.,* (2012) identified provitamin A (2.04 percent) and vitamin B group, namely B1 or thiamin, in *M. oleifera* seeds (0.94 percent). Vitamin A is vital for vision and contains antioxidant properties in the form of -carotene, which inhibits oxidation of substances such as vitamin E. *M. oleifera* seed oil contains vitamin E in the forms of alpha-tocopherol, gamma-tocopherol, and delta-tocopherol.

The most prevalent minerals discovered in *Moringa oleifera* seeds are potassium, phosphorus, sodium, zinc, magnesium, and calcium. *M. oleifera* seeds exhibit a Ca/P ratio larger than 1 and a Na/K ratio greater than the permissible threshold (0.60), according to Ijarotimi *et al.,* (2013). The mineral content of *M. oleifera* seeds, on the other hand, varies substantially from one location to the next (Nieman *et al.,* 1992).

2.9.1.4 *Lipids*

Lipid levels in *M. oleifera* seeds were found to range between 14 and 46 percent. Polyunsaturated fatty acids contribute for up to 75–79 percent of total fatty acids in the

lipids, which are low in monounsaturated and saturated fatty acids (Ijarotimi *et al.,* 2013). The oil, on the other hand, is a source of a few small chemicals (phytosterols and tocopherols). Abdulkarim *et al.,* 2005; Compaoré *et al.,* 2011) looked at the function of *M. oleifera* seed oil in human nutrition, including the oil's physicochemical properties and biological value.

2.9.2 Biological activity of *Moringa oleifera* seed

Few researches on *M. oleifera* seed as a protein source have been published. Consumption of *M. oleifera* crude seeds had detrimental consequences in Wistar rats, according to Oliveira *et al.,* (1999). The seeds' toxicity was related to a lectin (hemagglutinin) previously discovered in *M. oleifera* seeds. According to Ben and Makkar (2009), defatted Moringa seed added in meal at levels up to 4 g/day had positive effects on rumen fermentation, digestion, and lamb performance. At a 6 g/day feeding level, the scientists saw a reduction in the lambs' overall performance, which they attributed to the presence of glucosinolates. Furthermore, Igwilo *et al.,* (2013) found that soaking *M. oleifera* seeds for 30 minutes did neither improve development or damage the liver of Wistar albinos rats fed for 21 days.

2.10 *Eleusine coracana* (Finger Millet)

Finger millet grains are globular in shape and range in diameter from 1.0 to 1.5 mm. (Siwela, 2009). The brown (purna) variety of finger millet grains is the most common, with white (hamsa) (Ramashia, 2018) and red cultivars also present. According to Sood *et al.,* (2016), finger millet grains have a distinctive grain feature of a utricle rather than a genuine caryopsis, causing the pericarp to be partially united with the testa. Other millets' structures, such as pearl, foxtail, fonio, and teff millet, are also classified as caryopses.The name

caryopses refers to a fruit of single-seed with a brick red seed coat and a fruit coat or pericarp that surrounds the grain and clings closely to the grain coat (Patel *et al.,* 2014).

Finger millet grains are processed using both traditional and contemporary technologies. Value-added goods such as soaked, cooked, malted, papad, fermented, popped or puffed, extruded, and multi-grain flour can be made using the conventional technique of processing. The traditional method of preparing Finger mill*et al*so entails spreading and drying the grains in the sun for one week. The grains are dried and stored in a bag before being processed and manufactured into a variety of foods. Finger millet grains may be dried and kept for up to 10 years, however the grains are extremely small and difficult to handle. The grains are disease and insect resistant, although fungal disease can readily infect those (Sood *et al.,* 2016). In comparison to traditional cereal grains such as maize, sorghum, rice, and wheat, there is less study and innovation on Finger millet grains/flours, despite their utility and health benefits. As a result, this study discusses the processing, nutritional, and health benefits of Finger millet, as well as its use in value-added products.

When rubbing the grains with a mortar and pestle, the pericarp may be readily removed due to the peculiarity of the Finger millet grain. Another distinctive feature of Finger millet grain is its five layered-testa, which has been proposed as one of the reasons for the grain's high dietary fibre (Shobana *et al.,* 2013). The pericarp, germ, and endosperm are the three main anatomical components of Finger millet grains. The pericarp, also known as the glume, is a thin covering that covers the grain. The grain pericarp is made up of three (3) layers: epicarp (outermost layer), mesocarp (middle layer), and endocarp (inner layer) (Siwela, 2009; Ramashia, 2018).

2.10.1 Nutritional, antioxidant and health benefits of finger millet grains

Essential elements such as Ca and P are claimed to be present in finger millet grains (P). When compared to other millet species, the grains have the most calcium, ranging from 162.0 to 358.0 mg/100 g (Manjula *et al.,* 2015). Ca, which is abundant in Finger millet, is important for developing infants, pregnant women, the elderly (Jideani, 2012; Chappalwar *et al.,*2013), as well as those suffering from obesity, diabetes, and malnutrition (Jideani, 2012; Chappalwar *et al.,* 2013). Ca deficiency in the body may be addressed by including Finger millet food items in both young and old people's regular diets (Towo *et al.,* 2006). Phosphorus is another mineral present in Finger millet, and it helps with body tissue development and energy metabolism (Vanithasri *et al.,* 2012; Ramashia *et al.,* 2018). The phosphorus content of Finger millet varies between 130.0 and 283.0 mg/g. Iron, which has a concentration of 3-20% in Finger millet grains, is another mineral present in the grain (Shukla & Srivastava, 2014), and magnesium, which has been linked to lower blood pressure, asthma severity, migraine frequency, and heart attack risk (Verma & Patel, 2013; Prashanth & Muralikrishna, 2014). Finger millet grains are higher in mineral content and proximate composition than other millet species, making them more nourishing (Shobana *et al.,* 2013; Devi *et al.,* 2014), yet the grain is still ignored and underused.

Vitamins, which are important micronutrients required for the human body for healthy growth and maintenance, are also present in Finger millet grains. Vitamins are either fat-soluble or water-soluble, and a lack of vitamins can create vitamin deficiencies, which can lead to health problems (Mahfood and Farouk, 2020).Finger millet grains are high in fat-soluble and water-soluble vitamins, as well as vitamins A and B (Chappalwar *et al.,* 2013; Devi *et al.,* 2014). The dried grain, on the other hand, is devoid of vitamin C. (Siwela,

2009). The essential amino acids methionine, cysteine, and tryptophan (Jideani, 2012; Manjula *et al.,* 2015; Ramashia *et al.,* 2018), lysine, isoleucine, leucine, and phenylalanine, as well as threonine, are found in 44.7 percent of finger millet grains (Singh & Raghuvanshi, 2012), lysine, isole (Thapliyal & Singh, 2015).When compared to other millet species, the grains have the greatest concentration of methionine (194 mg/g) (Singh *et al.,* 2012; Prashanth & Muralikrishna, 2014).

Finger millet grains also include essential fatty acids such as linolenic and palmitic acids, which are required for the development of brain and neural tissue (Muthamilarasan *et al.,* 2016). Finger millet grain has a low fat content (1-2%), which contributes to better storage properties and aids in the prevention of obesity and/or weight control (Singh *et al.,*2012; Verma & Patel, 2013). Other millet grains, on the other hand, have a greater fat content, ranging from 3.5 to 5.2 percent (Shahidi & Chandrasekara, 2013).Low fat content, dietary fiber, and greater levels of carbohydrates in the form of non-starchy polysaccharides are all necessary for nutritional and physiological advantages such as hypo-cholesterolaemic and hypoglycemic effects (Vanithasri *et al.,* 2012). Finger millet grains contain dietary fibre, which is important for its nutritional and physiological value. Dietary fibre, which is divided into cellulose, pectin, arabinoxylan, lignin, and -glucan (Prashanth & Muralikrishna, 2014), helps to determine the end-use quality of cereal-based foods.

Tryptin inhibitors, phytate, phytic acid, tannins, and flavonoids found in finger millet grains have been linked to a reduction in mineral bioavailability, resulting in a decrease in the nutritional quality of the grain (Palanisamy *et al.,* 2012). However, the major polyphenols found in Finger millet are phenolic acids and tannins, with flavonoids believed to be present in tiny quantities. Polyphenols are important antioxidants that perform actions

that support the body's immune system. Polyphenols may be found in a variety of foods, including Finger millet grains (Devi *et al.,* 2014). Tannins in the grain's outer layer operate as a physical barrier against fungal invasion and are important for plant and human biological function (Devi *et al.,* 2014). Their anti-nutritional effects, on the other hand, are partly unfavorable because they impair nutrient digestibility and mineral absorption. Some processing techniques like as malting, fermentation, decortication, soaking, and steaming, have been found to increase the bioavailability of these nutrients in recent research (Krishnan *et al.,* 2012). Tannins also inhibit development through interfering with the pancreas and thyroid glands' functions, as well as producing pathological alterations in the liver. The color, taste, and nutritional quality of Finger millet grains and products have been found to be affected by tannin compounds. The chemical also boosts the antioxidant activity of Finger millet meals, which has been linked to healthy aging and the avoidance of metabolic diseases (Shibairo *et al.,* 2014).

Finger millet grains provide a number of health advantages, including a delayed release of glucose into the bloodstream after digestion (Chappalwar *et al.,* 2013) and the ability to relieve constipation (Vanithasri *et al.,* 2012).T Finger millet grain consumption has also been associated to a lower risk of diabetes, high blood pressure, and cardiovascular disease. Consumption of the grain has also been linked to lower cholesterol and a lower risk of cancer (Asharani *et al.,* 2010). Finger millet starch is used as a binder in the pharmaceutical sector to make granules and capsule dosage forms (Shiihii *et al.,* 2011). Other advantages of Finger millet include body tissue formation and repair, gallstone prevention, breast cancer prevention, postmenopausal cancer prevention, and pediatric cancer prevention (Verma & Patel, 2013). The grains may be eaten whole, are easily digestible, and have a

nice taste (Thapliyal & Singh, 2015). Due to the high vitamin and fatty acid content of finger millet grains, they are one of the most important cereal grains for low-income people, notably in Africa and parts of Asia (Verma & Patel, 2013).

2.10.2 Glycemic response to finger millet diet

Finger millet is beneficial in diabetic management because of the high dietary fiber and phenolic content which play very important roles in blood glucose regulation. It also has a low glycemic index (GI), making it a great late-night snack for satisfying hunger and maintaining blood sugar levels. Finger millet preparations like chapathi (unleavened flat bread), sevai (rice string hoppers), idli (fermented, steamed rice cake), dosai (fermented rice pan cake), and kozhukattai (steamed rice balls) were found to have a significant reduction in type 2 diabetes mellitus (formerly known as non-insulin dependent diabetes mellitus) in a small sample size (n=6) study (Geetha and Parvathi, 1990). As a consequence, consuming finger millet products on a regular basis can drop fasting glucose by 32% and improve insulin resistance by 43%.

Because finger millet dosa and roti have a higher glycemic response than whole finger millet dosa and roti, the malting and fermentation processes improve carbohydrate digestion. During germination, starch is converted to dextrins and maltose, resulting in a higher glycemic response. Polished finger millet in grain form as a legume formulation showed a higher glycemic index of 93.4 when compared to wheat-based formulations with legumes (55.4) (Shobanna *et al.,* 2007).The glycemic index of foxtail millet integrated biscuits was 50.8, compared to 68.0 for both barnyard millet (dehulled) and control refined flour wheat biscuits, and 50.0 and 41.7 for dehulled and heat-treated barnyard millet, respectively (Ugare *et al.,* 2014).

2.10.3 Applications of finger millet grains/flours

Finger millet grains are commonly employed in the production of traditional meals such as alcoholic and non-alcoholic beverages, while its flour is used to produce a variety of Finger millet value-added food products. The grains are also used in geriatric, infant, and health foods, both in their natural and malted forms (Kulkarni *et al.,* 2012). The majority of food items made from Finger millet grain or flour, especially those produced in poor nations, are not marketed. Foods made from sorghum and wheat, on the other hand, are commercialized and sold in supermarkets and retail stores all over the world (Siwela, 2009).Dishes produced with Finger millet grain/flour differ from country to country, and occasionally from region to region, according to Towo *et al.,* (2006). According to Abulude *et al.,* (2005), finger millet grains are not harmful to health at any stage of use; yet, despite their nutritious value, finger millet grains are often overlooked.

The discussion above illustrates the need for more research on finger millet grains, particularly in developed countries, with finger millet research focusing on the rising use of grain products in developed countries (Jideani, 2012). This might help these countries lower the frequency of ischemic strokes, cardiovascular diseases, cancers, obesity, and type 2 diabetes among the elderly (Kaur *et al.,* 2014).

2.11 *Sorghum bicolor* (Guinea Corn)

After wheat, maize, rice, and barley, sorghum is the world's fifth largest and most significant cereal crop (ICRISAT 2015). Sorghum output in the world is expected to be over 60 million tonnes per year (FAOSTAT 2013). Sorghum has a wide range of applications, and there are several in-depth studies accessible (FAO 1995; Taylor 2003). Sorghum is a valuable commodity that is used as a human staple as well as primary animal

feed in intensive farming methods. Sorghum may be one of the crops best adapted to future climate change due to its ability to adapt to conditions such as drought, salt, and high temperatures (ICRISAT 2015).Different races or cultivars of *Sorghum bicolor* are categorized as grain sorghum, fodder sorghum, or sweet sorghum based on their morphology or intended purpose. Sorghum is frequently used as a dual-purpose crop, with cattle grazing the stubble after the grain has been harvested. It has been recognized for its potential as a biofuel crop, and its importance is rising (CGIAR 2015).

2.11.1 Sorghum-based foods

More than 500 million people in 30 African and Asian countries use sorghum as a staple meal (ICRISAT 2015). Sorghum is important for the security of food in Africa because of its drought resistance and ability to endure high temperatures and water-logging. It thrives in the semi-arid and sub-tropical climates of much of Africa, when heavy rainfall occurs often over short periods of time. In contrast to the industrialized production methods utilized in most other parts of the globe, cultivation in Africa is mostly part of subsistence agricultural systems. Africa produces nearly a third of the world's sorghum, but its yields per hectare are the lowest (FAO 1995; Taylor 2003). Over half of all sorghum produced is used for animal feed, while the vast majority of sorghum is grown for human use in some regions, particularly in Sub-Saharan Africa (ICRISAT & FAO 1996).

Sorghum grains are used to make rice-like boiling meals, maize-like roasting or popping, threshing and grinding into flour for breads, porridges, pancakes, muffins, dumplings, morning cereals, or couscous, as well as alcoholic and non-alcoholic beverages (FAO 1999; Taylor 2003; CGIAR 2015; ICRISAT 2015; FAO 2015). Sugar and syrup are made from the stalks of sweet sorghum cultivars with high sugar content (CGIAR 2015).

In Western nations, there is growing interest in expanding sorghum's potential for usage in human meals and drinks, particularly as a gluten-free food source (O'Hara *et al.,* 2013; Norwood 2015). In Australia, gluten-free beer, breakfast cereals, and baked goods are among the few applications in human diet.

2.11.2 Nutritional and medicinal implications of *Sorghum bicolor*

In 2015, De Morais Cardoso L1 and colleagues released a study titled "Sorghum: nutrients, bioactive compounds, and the potential effect on human health," in which they analyzed the composition of this grain and, in particular, the health advantages of these compounds. Sorghum is primarily composed of starch, which is digested more slowly than other low-digestibility cereals, protein, unsaturated fat, vitamins, and minerals. Furthermore, phenolic chemicals, particularly 3–deoxyanthocyanidins and tannins, are abundant in most sorghum cultivars. In vitro and animal research have demonstrated that the gut microbiota and parameters associated to obesity, oxidative stress, inflammation, diabetes, dyslipidemia, hypertension, and cancer are balanced or stabilized by phenolic compounds and soluble chemicals (polycosanols) present in sorghum. Sorghum, in conclusion, would be a source of nutrients and bioactive compounds, notably 3 - deoxyanthocyanidins, tannins, and polycosanols, which have been shown in vitro and in animals to favorably affect the parameters of several non-communicable diseases (De Morais, 2015).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals and reagents

British Drug House (BDH) limited, England, and Sigma Aldrich Chemical Company Incorporation, Milwaukee, Wisconsin, USA, were the grade of chemicals and reagents utilized.

Assay kits for total protein, Creatinine, Bilirubin, Chloride, Albumin and Urea were products of Spectrum diagnostics, Hannover, Germany while assay kits for Potassium andSodium,were products of Teco diagnostics , Lakeview Avenue Anabeim USA while Alkaline phosphate, Aspartate transaminase were products of Agappe diagnostics Switzerland.

3.1.2 Equipment

Amino acids analyzer (applied biosystem PTH aminoacid analyzer model 120A PTH, USA), Pelletizer (SLKL-120 Shully China) mindray Auto Hematology analyzer BC- 5300 model, Atomic absorption spectrometer -6800 shimadzu, shimdazu UV-spectrometer UV-1800, Moisture extraction oven (Gallenkamp,size 2, Brain-weigh B, UK) Hot plate (Biotech India), Genway model 6000 electronic spectrometer, Water bath (Surrey GU 185TA,UK), Rotary evaporator (RE500 Yamato scientific America Incorporations USA, USA), Electronic compact Scale(Labtech BL10001), Water bath (light water, surrey GU185TA, UK),Medium scale grinding engine (Henry west G160, 5.5HP),Crown star thermostat-heating element (model: MC- HP1000) with power of 230 50/60Hz 1000w, Rechargeable LED light (LED-9087B).

3.2 Methods

3.2.1 Sample collection and preparation

Eleusine coracana (Finger millets), *Moringa oleifera* seeds and *Sorghum bicolor* (guinea corn) were purchased from Kure New Modern Market, Minna, Niger state, Nigeria. The *Moringa oleifera* seeds were dehulled to expose the whitish part. The samples (Finger millets, *Sorghum bicolor* and *Moringa oleifera* seeds) were air-dried to constant weight and processed mechanically to flour. The feed was formulated into various ratio diets of the respective four experimental groups.

3.3 Proximate Analysis

Proximate analysis of samples for moisture, total ash, crude fiber, and fat content was performed using Onwuka (2005) method. The nitrogen content was determined using Onwuka (2005)'s micro Kjeldah method, and the nitrogen was then multiplied by 6.25 to convert it to protein. The 'difference' technique was used to compute the total carbohydrate content. All of the numbers in the vicinity were represented as percentages.

3.3.1 Determination of moisture content

Two grams of the substance were weighed in a clean, dry crucible (W1). The crucible was dried for 6-12 hours in an oven at 100-105^{o}C until it achieved an uniform weight. After that, the crucible was placed in the desiccators for 30 minutes to cool. After cooling, the crucible was re-weighed (W2).The moisture percentage was determined using the formula;

$$\text{Percentage Moisture (\%)} = \frac{(W_1 - W_2) \times 100}{W_1} \quad (3.1)$$

Where;
W_1 = Initial crucible weight + Sample weight before drying and
W_2 = Final weight of crucible + Sample weight after drying

3.3.2 Determination of ash content

A clean empty crucible was heated to 550°C in a muffle furnace for an hour, then cooled in desiccators, and the weight of the empty crucible was measured (W1). In the crucible, two grams of the sample were put and weighed as follows: (W2). The crucible was put over a flame with its contents until they were burned. The crucible with the charred contents was then placed in a muffle furnace and ashed for 2-4 hours at 550°C. The presence of gray white ash implies that all organic content in the sample has been completely oxidized. The crucible was cooled and weighed after ashing (W3). The formula was used to compute the percentage of ash.

$$\text{Percentage Ash Content (\%)} = \frac{\text{Weight of ash} \times 100}{\text{Sample weight}} \quad (3.2)$$

W1, W2, and W3 are the weights of the crucible, crucible + sample before ashing, and crucible + sample after ashing, respectively.

3.3.3 Crude protein content determination

The protein content of the sample was determined using the Kjeldahl method. In a digestion flask containing 6 ml of concentrated H_2SO_4 and a speck of kjeldah1 catalyst (combination of 10 g Na_2SO_4+ 5 g $CuSO_4$+ 0.05 g selenium), 0.25 g of dried sample was added to a speck of kjeldah1 catalyst (combination of 10 g Na_2SO_4+ 5 g $CuSO_4$+ 0.05 g selenium). The contents of the flask were thoroughly mixed until the mixtures became transparent before being digested on the digestion block (colourless or greenish in color). After cooling, the digest was transferred to a 100 ml volumetric flask, which was then filled to the required volume with distilled water. The digest was distilled using the Markham Distillation Apparatus. In the distillation tube, ten milliliters of digest were added

first, followed by ten milliliters of 40% NaOH added in the same manner. The NH_3 generated was collected as NH_4OH in a conical flask containing 5 ml of 4 percent boric acid solution and a few drops of methyl red indicator after distillation was conducted for at least 10 minutes. Because of the NH_4OH, a yellowish hue develops during distillation. After that, the distillate was titrated against standard 0.1 N HCI solutions until it became pink. The technique below was used to calculate the percent crude protein content of the fish sample:

percentage Crude Protein = 6.25* x %N (*. Correction factor)

$$\% \text{ Nitrogen} = \frac{(S-B) \times N \times 0.014 \times D \times 100}{\text{Weight of the sample} \times V} \quad (3.3)$$

Where;

S = Sample titration reading B = Blank titration reading

N = Normality of HCI D = Dilution of sample after digestion

V = Volume taken for distillation 0.014 – Milli-equivalent weight of Nitrogen

3.3.4 Crude fat determination

The Soxhlet apparatus was used to evaluate crude fat using the ether extract technique. A 2.0 g moisture-free sample was wrapped on filter paper, placed in a fat-free thimble, and then placed in the extraction tube. A weighed, cleaned, and dried receiving flask filled with petroleum ether was included in the setup. The extract was placed in a clean glass dish and evaporated over a water bath after the Soxhlet apparatus was erected and left to reflux for 6 hours. After that, the dish was cooked for 1 hour at 105-110^{0}C and then dried in desiccators. The crude fat percentage was calculated using the formula below:

$$\% \text{ Crude Fat} = \frac{\text{Weight of ether x } 100}{\text{Weight of sample}} \quad (3.4)$$

3.3.5 Determination of crude fiber content

Two grams of samples were defatted with petroleum ether and boiled under reflux for 30 minutes with 200ml of solution containing 1.25 g of H_2SO_4 per 100 ml. The solution was filtered through several layers of cheese cloth on a fluted funnel, washed with boiling water until the washings were no longer acidic, then transferred to a beaker and boiled for 30 minutes with 200ml of carbonate free NaOH per 100ml, and the final residue was filtered through a thin but close pad of washed and ignited asbestos in a Gooch filter. The weight loss after incineration multiplied by 100 is the percentage crude fiber.

3.3.6 Determination of carbohydrate

Nitrogen free technique described by AOAC (1990) was employed. The carbohydrate content was determined by dividing the weight by 100 and adding the other proximate parameters to get the Nitrogen free Extract (NFE) % carbohydrate content (NFE) = 100-(m+p+F+A+F_2). Where; M=moisture, P=protein, F_1=Fat, A=ash and F_2=crude fiber

3.4 Determination of Minerals

Nielson's procedures, which were explained in detail here, were used to determine the mineral composition of each sample (2002). Two grams (2 g) of the material was weighed into a crucible, and samples were allowed to ash for two hours at 550°C. In a beaker, 15 ml of strong hydrochloric acid and 5 ml of concentrated nitric acid were added to the ash. The acid was fully evaporated when the beaker was placed on a hotplate and heated to about 100^0C.After 10 ml of distilled water was added to the beaker, the sample was filtered into a

100 ml volumetric flask and brought up to mark. The mineral content of the digested sample was determined using an atomic absorption spectrophotometer.

3.5 Determination of Vitamins

The dichlorophenol Indophenol dye reduction technique was used to determine vitamin C content (Smirnoff, 2000). The colorimetric approach was used to determine niacin, whereas the flourometric method was used to determine thiamine and riboflavin (Nudelman and Nudelman, 1976; Hodson and Norris, 1939)

3.6 Amino Acid Analysis

The amino acid profile in the sample was determined using Benitez's method (1989). The material was defatted, hydrolyzed, and evaporated in a rotary evaporator before being fed at a consistent weight into an Applied Biosystems PTH Amino Acid Analyzer.

3.6.1 Defatting sample

A 2:1 mixture of chloroform and methanol was used to defat the substance. 500 mg of the substance was put in an extraction thimble in a soxhlet extraction apparatus and extracted for 15 hours (AOAC, 2006).

3.6.2 Nitrogen determination

115 mg of material is weighed, wrapped with Whitman filter paper (No. 1), and placed in the Kjeldahl digesting flask. A ten-milliliter strong sulphuric acid solution was added. A catalyst mixture including sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$), and selenium oxide (SeO_2) in a 10:5:1 ratio was added to the flask to assist digestion (0.5 g). Six anti-bumping grains were placed in each of the six components.

The flask was then placed in the Kjeldhal digesting apparatus for three hours, until the liquid became pale green. The digested sample was cooled and diluted to 100ml with

distilled water in a standard volumetric flask. An aliquot (10 m1) of the diluted solution was distilled with 10ml of 45 percent sodium hydroxide into 10ml of 2 percent boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70 ml of distillate was produced using the Markham distillation equipment. The distillate was titrated with 0.01 NHCl until a grey-colored end point was achieved.

$$\text{Percentage Nitrogen} = \frac{(a-b) \times 0.01 \times 14 \times V \times 100}{W \times C} \quad (3.5)$$

Where:

a. = Titre value of the digested sample

b. = Titre value of blank sample

v. = Volume after dilution (100ml)

W. = Weight of dried sample (mg)

C. = Aliquot of the sample used (10ml)

14. = Nitrogen constant in mg.

0.01 = Normality of Hydrochloric acid

3.6.3 Hydrolysis of the sample

A gram of defatted material was weighed in a glass ampoule. 7 ml of 6NHCl was added and oxygen was evacuated by pumping nitrogen into the ampoule to prevent possible oxidation of specific amino acids during hydrolysis, such as methionine and cystine. After that, the glass ampoule was sealed with a Bunsen burner flame and baked at 105°C/5°C for 22 hours. The ampoule was allowed to cool before the tip was split off and the content was filtered to remove the humidity. It's worth mentioning that the hydrolysis of 6NHCl kills tryptophan. The filtrate was then evaporated to dryness using a rotary evaporator. The

residue was dissolved in 5mL of pH 2.0 acetate buffer and kept at 4°C in plastic specimen vials.

3.6.4 Loading of the hydrolysate into analyzer

Around 60µL of the sample was placed into the amino acid analyzer by dispensing into the analyzer's cartridge. The session of analysis lasted 45 minutes.

3.6.5 Calculation of amino acid values from chromatogram peaks

The net height of each peak generated by the TSM chart recorder (each representing an amino acid) was measured. By multiplying the height by half-height breadth, the area of each peak was determined. The norleucine equivalent (NE) for each amino acid in the reference combination was calculated using a formula.

$$NE = \frac{\text{Area of Norleucine peak}}{\text{Area of each amino acid}} \quad (3.6)$$

A constant S was calculated for each amino acid in the standard mixture:

Where $S_{std} = NE_{std}$ x Molecular weight x μMAA_{std}

Finally, the amount of each amino acid present in the sample was calculated in g/16gN or g/100g protein using the following formula:

Concentration (g/100g protein) = NH x W at NH/2 x Sstd x C

$$\text{Where } C = \frac{\text{Dilution x 16}}{\text{Sample Wt(g) x N(\%) x 10 x Vol. loaded}} \div \text{NH x W (nleu)} \quad (3.7)$$

Where: NH = Net Height

W = Width @ half height

Nleu = Norleucine

3.7 Feed Formulation

Four treatment diets were formulated and pelletized using ratios of the respective milled flours of Finger millet, Guinea corn and *Moringa oleifera* seeds as shown in the table 3.1 below.

Table 3.1: Composition of Formulated Ratios of Finger Millet, Guinea Corn and *Moringa oleifera* Seeds

Samples	A	B	C	D	E (Positive control)	F (Negative control)	G (Standard control)
Finger millet (*Eleucine coracana*)	50%	45%	40%	30%	100 % Rat Chow	100 % Rat Chow	100 % Rat Chow
Guinea corn (*Sorghum bicolor*)	30%	35%	40%	50%			
Moringa Oleifera seeds	20%	20%	20%	20%			
Total Received Diet	100 %	100 %	100 %	100 %	100 %	100 %	100 %

3.8 Experimental Design

A total of twenty one (21) adult albino rats between 100 and 120 g were purchased from Olatunji animal farm in ogbomosho. Under conventional laboratory settings, they were kept in plastic cages and provided commercial feed (rat pellet) and water *ad-libitum*. They were allowed to acclimatize for a week (7 days). Treatment groups; A, B, C, D, E (Positive control) and F (Negative control) were all induced with 150g per body kilogram weight of Alloxan monohydrate via intraperitoneal route or another week, all kept together but separated from group G (the standard control) which were uninduced. Glucose test was carried out on all the groups to check the blood glucose level prior treatment. The rats

induced with diabetes were divided into 4 treatment groups (A-D) as well as the positive (E) and negative control (F) of three (3) each and the three (3) un-induced (G) were kept separately under same conditions in their respective cages.

3.8.1 Dosage calculation and preparation of alloxan monohydrate stock solution

Alloxan monohydrate is a diabetogenic agent at a standard dose of 150mg/kg for rat via intraperitoneal route as reported in several experimental diabetes literatures (Erhirhie, et al., 2013; Oluwole, et al., 2012). It is in salt form.

The required dose of alloxan monohydrate to induce experimental diabetes intraperitoneally in a rat at a standard dose of 150 mg/kg can be calculated thus;

$$\text{Required dose of Alloxan for rats} = \frac{\text{weight of animals (g)}}{1000\text{g}} \times \text{standard dose (150mg)}$$

N/B: 2ml of normal saline was used as vehicle for induction of Alloxan monohydrate.

3.8.2 Feed administration and treatment

The respective formulated ratios of feeds for animals in were administered according to treatment as shown in table 3.1 above. Meanwhile rats in group E (positive control group) were treated with standard drug (Metformin HCl), rats in group F (negative control) where administered 2ml of normal saline while rats in group G (standard control) were given commercial rat pellets. Treatment lasted for a period of 21 days.

3.8.3 Dosage calculation and preparation of stock solution metformin HCl

Metformin HCl (with dosage formulations of 500 mg per tablet respectively) used in animal model for diabetic treatment with standard doses 100 mg/kg body weight was administered via oral intubation (Claudia, et al. 2003; Sohair & Salwa, 2011). The required dose was determined thus;

Required dose of Metformin HCl = $\frac{\text{weight of animals (g)}}{1000\text{g}} \times$ standard dose (100mg)

N/B: 2ml of normal saline was used as vehicle for administration of Metformin HCl.

3.9 Physiological Parameters

The weights of the animals were obtained before they started treatment, and then every two days afterwards. Each group's feed was weighed daily, and daily feed intake was estimated by weighing the remaining feed and subtracting the amount of feed (in grams) provided. Blood glucose test was done a week after diabetes was induced, and then blood glucose levels were checked twice a week afterwards.

3.10 Collection of Blood Sample

To ascertain the rats' final weights, they were weighed at the end of the 21-day feeding experiment. Shittu *et al.,* (2014) described jugular puncture as the method for obtaining a blood sample. Blood samples were collected in EDTA bottles for hematological analysis and plain bottles for biochemical analysis. Using an Eppendorf centrifuge 5702 spinning at 503 x g for 10 minutes, serum was extracted from whole blood collected for biochemical parameters.

3.11 Hematological Analysis

The following hematological parameters were measured using an auto hematological analyzer (Mindray BC – 5300 model): As reported by Akinnawo *et al.,* (2002) hemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell (RBC), platelet count, and total white blood cell differentials (TWBC).

3.11.1 Determination of fasting blood glucose

Fasting blood glucose was measured in mg/dL using Acuchek glucometer and Acuchek glucose strips with lot number 06656757 through the drop of blood sample obtained by puncture of the tail vein after overnight (12-15hours) fast of rats.

3.12 Biochemical Analysis

The presence of liver function enzymes was determined in the serum collected from albino rat blood samples.

3.12.1 Determination of serum aspartate transaminase (AST) activities

Serum AST was determined using Agappe diagnostic kit with Lot number P-PCT-1113.

Principle

L-Aspartate reacts with α-ketoglutarate in the presence of aspartate amino transferase (AST) to form oxaloacetate + L–Glutamate. The oxaloacetate react with NADH and H^+ in the presence of malate dehydrogenase (MDH) to form L–malate and NAD^+

$$\text{L–Aspartate} + \alpha\text{–ketoglutarate} \xrightarrow{\text{AST}} \text{Oxaloacetate} + \text{L–Glutamate}$$

$$\text{Oxaloacetate} + \text{NADH} + H^{+} \xrightarrow{\text{ADH}} \text{L–malate} + NAD^{+}$$

Procedure

1000µl of SGOT working reagent was added to 100µl of sample to be analysed. This was mixed and incubated at 37^{o}C for 1 minute. The change in absorbance per minute (OD/min) measured within 3 minutes.

SGOT (AST) activity was calculated from the expression below:

SGOT (AST) (U/L)= (ΔOD/mins) X 1745.

Where: ΔOD= change in absorbance or optical density

1745= the provided factor for estimation of assay (Normal procedure & High linearity procedure) in semi auto analyser.

3.12.2 Determination of serum alkaline phosphatase activities (ALP)

Alkaline phosphates (ALP) was determined using Agappe diagnostic kits with Lot number P-PCT-1211

Principle

Para-nitrophenyl phosphate reacts with water in the presence of alkaline phosphatase to produce para-nitrophenol + inorganic phosphate.

P–nitrophenyl phosphate + H_2O $\xrightarrow{ALP}$ P – nitrophenol + inorganic phosphate

Procedure

1000µl of ALP working reagent was added to 20µl of sample to be analysed. This was mixed and incubated at 37^{o}C for one minute. The change in absorbance per minute (ΔOD/min) was measured within 3 minutes.

The alkaline phosphatase activity was calculated using the formula below;

Alkaline phosphatase activity (u/l) = (ΔOD/min) X 2750

Where; 2750 = the provided factor for estimation of ALP using Semi Auto Analyser

ΔOD= change in absorbance or optical density.

3.12.3 Determination of serum alanine transaminase (ALT) activities

Serum Alanine Transaminase (ALT) activity was determined using Agappe diagnostic kits P-PCT-1325.

Principle

Alanine Transaminase catalyses the reaction involving the transamination of alanine and α–oxoglutarate to form pyruvate and glutamate. The activity of ALT is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4, -dinitrophenylhydrazine.

α – oxoglutarate + alanine $\xrightarrow{\text{ALT}}$ L-glutamate + Pyruvate

Procedure

1000µl of ALT working reagent was added to 100µl of sample to be assayed. The resultant solution was mixed and allowed to incubate at 37^{o}C for 1 minute. The change in absorbance per minute (ΔOD/min) was measured over a period of 3minutes. The ALT Activity was calculated using the formula, ALT activity (U/L) = (ΔOD/min) x 1745

3.13 Statistical Analysis

All of the data was triple-checked. The computer program Statistical Package for Social Sciences (SPSS) version 2.2.0 was used to conduct statistical analysis, which included one-way analysis of variance (ANOVA) and Duncan multiple range test (DMRT). A 95 percent confidence interval was used to examine the data. At $p \geq 0.05$, significant differences between means were identified, and data were presented as mean SEM.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

The result in Table 4.1 represents the proximate composition of *Moringa oleifera* seed, *Sorghum bicolor* and *Eleusine coracana*. The result shows moisture was significantly ($p \leq 0.05$) higher in *Sorghum bicolor* compared to *Eleusine coracana and Moringa oleifera,* while fat content was significantly higher ($p \leq 0.05$) in *Moringa oleifera* seed compared to *Sorghum bicolor* and *Eleusine coracana* while there was no difference between the two at $p \leq 0.05$. Also ash, fibre and protein content were significantly higher ($p \leq 0.05$) in *Moringa oleifera* compared to *Sorghum bicolor* and *Eleusine coracana* whereas *Sorghum bicolor* had significantly higher ($p \leq 0.05$) fibre and protein, and lower ash content compared to *Eleusine coracana*. The carbohydrate content was significantly highest ($p \leq 0.05$) in *Eleusine coracana* compared to *Sorghum bicolor* and *Moringa oleifera* seed respectively.

Table 4.1: Proximate Composition of *Moringa oleifera* Seed, *Sorghum bicolor* and *Eleusine coracana*

Parameters (%)	*Moringa oleifera* seed	*Sorghum bicolor*	*Eleusine coracana*
Moisture	7.265 ± 0.005^{a}	10.735 ± 0.005^{c}	7.515 ± 0.005^{b}
Fat	21.815 ± 0.005^{b}	4.265 ± 0.005^{a}	4.265 ± 0.005^{a}
Ash	6.325 ± 0.005^{c}	2.095 ± 0.005^{a}	3.135 ± 0.005^{b}
Fibre	11.825 ± 0.005^{c}	5.615 ± 0.005^{b}	3.385 ± 0.005^{a}
Protein	47.135 ± 0.005^{c}	16.175 ± 0.005^{b}	13.545 ± 0.005^{a}
CHO	4.965 ± 0.005^{a}	60.145 ± 0.005^{b}	67.725 ± 0.005^{c}

Values are reported as mean ± standard error of means. Values on the same row with different alphabetic superscript are significantly different at $p \leq 0.05$.

The result in Table 4.2 show the proximate composition of the formulated feeds at different ratio of *Moringa oleifera* seed, *Sorghum bicolor* and *Eleusine coracana*. The result shows that moisture content, fibre content and protein content were significantly highest ($p \leq 0.05$) in the 30 % *E. coracana,* 50 % *S. bicolor* and 20 % *M. oleifera* seed respectively while they were significantly lowest in 50 % *E. coracana,* 30 % *S. bicolor* and 20 % *M. oleifera* seed. Fat content though higher in 45 % *E. coracana,* 35 % *S. bicolor* and 20 % *M. oleifera* seed was not significant when compared to 50 % *E. coracana,* 30 % *S. bicolor* and 20 % *M. oleifera* seed and 30 % *E. coracana,* 50 % *S. bicolor* and 20 % *M. oleifera* seed, but it was significantly lower in 40 %*E. coracana,* 40 % *S. bicolor* and 20 % *M. oleifera* seed. Carbohydrate and ash content were significantly highest in 50 % *E. coracana,* 30 % *Sorghum bicolor* and 20 % *M. oleifera* seed compared to the other three ratios and significantly lowest in 30 % *E. coracana,* 50 % *S. bicolor* and 20 % *M. oleifera* seed.

Table 4.2: Proximate Composition of Feed Formulated at Different Ratio of *Eleusine coracana, Sorghum bicolor* and *Moringa oleifera* seed

Parameters (%)	**A**	**B**	**C**	**D**
Moisture	8.435 ± 0.005^{a}	8.595 ± 0.005^{b}	8.755 ± 0.005^{c}	9.085 ± 0.005^{d}
Fat	7.775 ± 0.005^{b}	7.785 ± 0.005^{b}	5.785 ± 0.005^{a}	7.770 ± 0.01^{b}
Ash	3.475 ± 0.005^{d}	3.425 ± 0.005^{c}	3.065 ± 0.005^{a}	3.245 ± 0.005^{b}
Fibre	5.655 ± 0.005^{a}	5.875 ± 0.005^{b}	5.995 ± 0.005^{c}	6.335 ± 0.005^{d}
Protein	20.965 ± 0.005^{a}	21.185 ± 0.005^{b}	21.325 ± 0.005^{c}	21.495 ± 005^{d}
CHO	61.935 ± 0.005^{d}	52.425 ± 0.005^{c}	52.165 ± 0.005^{b}	51.395 ± 0.005^{a}

Values are reported as mean ± standard error of means. Values on the same row with different alphabetic superscript are significantly different at $p \leq 0.05$.
A =50%Finger millet, 30% *Sorghum bicolor* and 20% *Moringa oleifera* Seeds,
B =45%Finger millet, 35% *Sorghum bicolor* and 20% *Moringa oleifera* Seeds,
C =40%Finger millet, 40% *Sorghum bicolor* and 20% *Moringa oleifera* Seeds,
D =30%Finger millet, 50% *Sorghum bicolor* and 20% *Moringa oleifera* Seeds

The result in Table 4.3 shows the amino acids composition of *Eleusine coracana, Sorghum bicolor* and *Moringa oleifera* seeds. The amino acid profile shows *Moringa oleifera* seed, *Sorghum bicolor* and *Eleusine coracana* contained 18 amino acids each. All the amino acids were significant at $p \leq 0.05$ among the three crops. Arginine and glycine were significantly highest ($p \leq 0.05$) in *Moringa oleifera* seed while they were significantly lowest ($p \leq 0.05$) in *S. bicolor*. Leucine, cysteine, alanine and typrosine were significantly highest ($p \leq 0.05$) in *S. bicolor* and lowest in *Moringa oleifera* seed. Lysine, isoleucine, phenylalanine, tryptophan, valine, methionine, proline, histidine, glutamic acid, threonine and serine were significantly highest ($p \leq 0.05$) in *Eleusine coracana* while aspartic acid was significantly the same ($p \leq 0.05$) in *Moringa oleifera* seed and *Eleusine coracana* grain.

Table 4.3: Amina acid profile of *E. coracana, S. bicolor* and *M. oleifera* seed

Amino acids(g/100g)	*Moringa oleifera* seed	*Sorghum bicolor*	*Eleusine coracana*
Leucine	5.645±0.005[a]	13.425±0.005[c]	9.215±0.005[b]
Lysine	3.335±0.005[b]	2.375±0.005[a]	4.605±0.005[c]
Isoleucine	3.985±0.005[b]	3.945±0.005[a]	4.185±0.005[c]
Phenylalanine	4.095±0.005[b]	3.795±0.005[a]	5.045±0.005[c]
Tryptophan	1.045±0.005[a]	1.255±0.005[b]	1.350±0.005[c]
Valine	3.095±0.005[a]	5.375±0.005[b]	5.720±0.005[c]
Methionine	0.955±0.005[a]	1.895±0.005[b]	2.350±0.005[c]
Proline	3.035±0.005[a]	9.135±0.005[b]	9.630±0.005[c]
Arginine	8.945±0.005[c]	4.815±0.005[a]	5.505±0.005[b]
Tyrosine	2.405±0.005[a]	4.130±0.005[c]	3.795±0.005[b]
Histidine	2.195±0.005[a]	2.195±0.005[a]	2.850±0.005[b]
Cysteine	1.995±0.005[b]	2.175±0.005[c]	0.355±0.005[b]
Alanine	3.405±0.005[a]	9.175±0.005[c]	8.035±0.005[b]
Glutamic acid	14.995±0.005[a]	20.510±0.005[b]	20.735±0.005[c]
Glycine	4.835±0.005[c]	3.245±0.005[a]	4.315±0.005[b]
Threonine	2.935±0.005[a]	3.155±0.005[b]	4.045±0.005[c]
Serine	3.795±0.005[a]	4.395±0.005[b]	4.495±0.005[c]
Aspartic acid	6.93±0.005[b]	7.435±0.005[a]	6.930±0.005[b]

Values are reported as mean ± standard error of means. Values on the same row with different alphabetic superscript are significantly different at $p \leq 0.05$.

The result in Table 4.4 shows the amino acids profile of Feed Formulated at different ratios of *Eleusine coracana, Sorghum bicolor* and *Moringa oleifera* seed. The result shows that lysine, isoleucine, phenylalanine, tryptophan, valine, methionine, proline, histidine, glycine and threonine were significantly highest ($p \leq 0.05$) in the 50 % *Eleusine coracana*, 30 % *Sorghum bicolor* and 20 % *Moringa oleifera* seed. Leucine, cysteinine, alanine and aspartic acid were significantly highest ($p \leq 0.05$) in the 30 % *Eleusine coracana*, 50 % *Sorghum bicolor* and 20 % *Moringa oleifera* seed. Except for tyrosine and glutamic acid, which were significantly highest ($p \leq 0.05$) in 40 % *Eleusine coracana*, 40 % *Sorghum bicolor* and 20 % *Moringa oleifera* seed or same in 35 % *Eleusine coracana*, 45 % *Sorghum bicolor* and 20 % *Moringa oleifera* seed, the remaining amino acids, though significantly different ($p \leq 0.05$) were in-between 50 % *Eleusine coracana*, 30 % *Sorghum bicolor* and 20 % *Moringa oleifera* seed and 30 % *Eleusine coracana*, 50 % *Sorghum bicolor* and 20 % *Moringa oleifera* seed.

Table 4.4: Amino Acid Composition of Feed Formulated at Different Ratios of *Eleusine coracana, Sorghum bicolor* and *Moringa oleifera* seed

Amino acids (g/100g)	A	B	C	D
Leucine	9.315±0.005^{a}	9.975±0.005^{b}	10.185±0.005^{c}	10.615±0.005^{d}
Lysine	3.685±0.005^{d}	3.565±0.005^{c}	3.455±0.005^{b}	3.235±0.005^{a}
Isoleucine	5.085±0.005^{d}	4.065±0.005^{c}	4.055±0.005^{b}	4.035±0.005^{a}
Phenylalanine	4.485±0.005^{d}	4.415±0.005^{c}	4.355±0.005^{b}	4.235±0.005^{a}
Tryptophan	1.265±0.005^{c}	1.255±0.005^{b}	1.245±0.005^{a}	1.245±0.005^{a}
Valine	5.095±0.005^{d}	5.075±0.005^{c}	5.055±0.005^{b}	5.025±0.005^{a}
Methionine	1.955±0.005^{d}	1.935±0.005^{c}	1.905±0.005^{b}	1.855±0.005^{a}
Proline	8.165±0.005^{d}	8.145±0.005^{c}	8.125±0.005^{b}	8.065±0.005^{a}
Arginine	5.995±0.005^{c}	5.995±0.005^{c}	5.915±0.005^{b}	5.845±0.005^{a}
Tyrosine	3.615±0.005^{a}	3.635±0.005^{c}	3.645±0.005^{d}	3.625±0.005^{b}
Histidine	2.545±0.005^{d}	2.335±0.005^{a}	2.475±0.005^{c}	2.405±0.005^{b}
Cysteine	0.865±0.005^{a}	1.315±0.005^{b}	1.405±0.005^{c}	1.550±0.005^{d}
Alanine	7.445±0.005^{a}	7.505±0.005^{b}	7.565±0.005^{c}	7.675±0.005^{d}
Glutamic acid	11.315±0.005^{a}	19.505±0.005^{c}	19.505±0.005^{c}	19.475±0.005^{b}
Glycine	4.105±0.005^{d}	4.045±0.005^{c}	3.950±0.005^{b}	3.850±0.005^{a}
Threonine	3.565±0.005^{d}	3.515±0.005^{c}	3.465±0.005^{b}	3.385±0.005^{a}
Serine	4.325±0.005^{c}	4.325±0.005^{c}	4.315±0.005^{b}	4.305±0.005^{a}
Aspartic acid	7.075±0.005^{b}	7.050±0.005^{a}	7.135±0.005^{c}	7.175±0.005^{d}

Values are reported as mean ± standard error of means. Values with on the same row with different alphabetic superscript are significantly different at $p \leq 0.05$.
A =50%Finger millet, 30% *Sorghum bicolor* and 20% *Moringa oleifera* Seeds,
B =45%Finger millet, 35% *Sorghum bicolor* and 20% *Moringa oleifera* Seeds,
C =40%Finger millet, 40% *Sorghum bicolor* and 20% *Moringa oleifera* Seeds,
D =30%Finger millet, 50% *Sorghum bicolor* and 20% *Moringa oleifera* Seeds

The result in Table 4.5 shows the vitamin constituent of *Eleusine coracana, Sorghum bicolor* and *Moringa oleifera* seed. The results show that *Eleusine coracana, Sorghum bicolor* and *Moringa oleifera* seed contained 13 vitamins. *Moringa oleifera* seed contains significantly higher ($p \leq 0.05$) concentration of vitamin constituents when compared to *Sorghum bicolor* and *Eleusine coracana.* Vitamins A, B1, B2, B3, B6, B9, B12, C, D, E and K were significantly highest ($p \leq 0.05$) in *Moringa oleifera* seed compared to *Sorghum bicolor* and *Eleusine coracana* while Vitamins B5 and B7 were significantly highest ($p \leq 0.05$) in *Sorghum bicolor* compared to *Moringa oleifera* seed and *Eleusine coracana.*

Table 4.5: Vitamin Constituent of *E. coracana, S. bicolor* and *M. oleifera* Seed

Vitamins	***Moringa oleifera* seed**	***Sorghum bicolor***	***Eleusine coracana***
A	16.0261 ± 0.061^{c}	0.0150 ± 0.005^{a}	0.1050 ± 0.005^{b}
B1	1.975 ± 0.005^{c}	0.1150 ± 0.005^{a}	0.2600 ± 0.005^{b}
B2	11.134 ± 0.004^{c}	0.0300 ± 0.010^{a}	0.1100 ± 0.010^{b}
B3	6.485 ± 0.005^{c}	2.2150 ± 0.005^{b}	0.7900 ± 0.010^{a}
B5	0.275 ± 0.005^{b}	0.735 ± 0.005^{c}	0.250 ± 0.005^{a}
B6	19.250 ± 0.050^{c}	0.190 ± 0.010^{b}	0.0450 ± 0.01^{a}
B7	2.085 ± 0.005^{b}	3.935 ± 0.005^{c}	0.770 ± 0.01^{a}
B9	1.775 ± 0.005^{c}	0.60 ± 0.010^{a}	0.545 ± 0.005^{b}
B12	3.465 ± 0.295^{b}	0.465 ± 0.005^{a}	0.205 ± 0.005^{a}
C	12.645 ± 0.005^{c}	1.525 ± 0.005^{b}	0.3050 ± 0.005^{a}
D	1.750 ± 0.050^{b}	0.035 ± 0.005^{a}	0.165 ± 0.005^{a}
E	1.630 ± 0.010^{c}	0.075 ± 0.005^{a}	0.165 ± 0.005^{b}
K	1.705 ± 0.005^{c}	0.065 ± 0.005^{a}	0.295 ± 0.005^{b}

Values are reported as mean ± standard error of means. Values on the same row with different alphabetic superscript are significantly different at $p \leq 0.05$.

The result in Table 4.6 shows the vitamin constituents of the Feed Formulated at *Eleusine coracana, Sorghum bicolor* and *Moringa oleifera* seed. The vitamin B1, C and D constituent were significantly highest ($p \leq 0.05$) in the 50 % *Eleusine coracana,* 30 % *Sorghum bicolor*and20 % *Moringa oleifera* seed as opposed vitamin B3, B5, B7 and B12 which were significantly lowest ($p \leq 0.05$) in the same compounded ratio. In the 30 % *Eleusine coracana,* 50 % *Sorghum bicolor*and20 % *Moringa oleifera* seed, vitamins B3, B5, B7 and K were significantly highest ($p \leq 0.05$) while B2, B9 and D were significantly lowest ($p \leq 0.05$) in those compounded ratios. There were no statistical significance ($p \leq 0.05$) among the difference blend ratios for vitamins A, B2, B6 and E.

Table 4.6: Vitamin Constituent of the Feed Formulated from Different Ratio of *E. coracana, S. bicolor* and *M. oleifera* Seed

Vitamin	A	B	C	D
A	3.255±0.005^{a}	3.250±0.005^{a}	3.245±0.005^{a}	3.235±0.005^{a}
B1	0.605±0.005^{d}	0.560±0.000^{c}	0.535±0.005^{b}	0.515±0.005^{a}
B2	2.255±0.005^{a}	2.255±0.005^{a}	2.255±0.005^{a}	2.245±0.005^{a}
B3	2.360±0.005^{a}	2.465±0.005^{b}	2.475±0.005^{b}	2.595±0.005^{c}
B5	0.295±0.005^{a}	0.365±0.005^{b}	0.365±0.005^{b}	0.465±0.005^{c}
B6	4.050±0.005^{a}	4.050±0.05^{a}	4.030±0.01^{a}	4.025±0.005^{a}
B7	1.985±0.005^{a}	2.145±0.005^{b}	2.305±0.005^{c}	2.655±0.005^{d}
B9	0.655±0.005^{c}	0.635±0.005^{b}	0.805±0.005^{d}	0.565±0.005^{a}
B12	0.895±0.005^{a}	0.915±0.005^{a}	0.915±0.005^{a}	0.995±0.005^{b}
C	4.585±0.005^{d}	3.205±0.005^{b}	1.255±0.005^{a}	3.385±0.005^{c}
D	0.455±0.005^{b}	0.445±0.005^{b}	0.435±0.005^{ab}	0.425±0.005^{a}
E	0.435±0.005^{a}	0.435±0.005^{a}	0.425±0.005^{a}	0.415±0.005^{a}
K	0.155±0.005^{b}	0.025±0.005^{a}	0.275±0.005^{c}	0.260±0.000^{c}

Values are reported as mean ± standard error of means. Values on the same row with different alphabetic superscript are significantly different at $p \leq 0.05$.
A =50%Finger millet, 30% *Sorghum bicolor* and 20% *Moringa oleifera* Seeds,
B =45%Finger millet, 35% *Sorghum bicolor* and 20% *Moringa oleifera* Seeds,
C =40%Finger millet, 40% *Sorghum bicolor* and 20% *Moringa oleifera* Seeds,
D =30%Finger millet, 50% *Sorghum bicolor* and 20% *Moringa oleifera* Seeds

The result in Table 4.7 shows the mineral content of *E. coracana, S. bicolor* and *M. oleifera* seed. The result showed there were eight (8) mineral in each of *E. coracana, S. bicolor* and *M. oleifera* seed. Magnesium, calcium, sodium, copper, iron and potassium were significantly highest ($p \leq 0.05$) in *Moringa oleifera* seed while phosphorus was significantly highest ($p \leq 0.05$) in *Eleusine coracana*. There was no significant difference ($p \leq 0.05$) in zinc level of *Sorghum bicolor* and *Eleusine coracana*, even though the zinc level in those two were significantly higher ($p \leq 0.05$) than that of *Moringa oleifera*.

Table 4.7: Mineral Content of *E. coracana, S. bicolor* and *M. oleifera* seed

Mineral	***Moringa oleifera* seed**	***Sorghum bicolor***	***Eleusine coracana***
Mg	16.605 ± 0.005^{c}	3.275 ± 0.005^{a}	4.937 ± 0.007^{b}
Ca	12.155 ± 0.005^{c}	11.765 ± 0.005^{b}	10.755 ± 0.005^{a}
Na	7.735 ± 0.005^{b}	7.385 ± 0.005^{a}	7.395 ± 0.005^{a}
Cu	0.975 ± 0.005^{c}	0.2050 ± 0.005^{a}	0.3950 ± 0.005^{b}
Zn	0.755 ± 0.005^{a}	0.785 ± 0.005^{b}	0.805 ± 0.005^{b}
Fe	5.380 ± 0.000^{c}	3.945 ± 0.005^{b}	1.325 ± 0.005^{a}
K	14.565 ± 0.005^{c}	12.675 ± 0.005^{a}	13.575 ± 0.005^{b}
P	19.650 ± 0.050^{a}	28.850 ± 0.050^{b}	40.100 ± 0.100^{c}

Values are reported as mean ± standard error of means. Values on the same row with different alphabetic superscript are significantly different at $p \leq 0.05$.

The result in Table 4.8 show the mineral composition of feed formulated at different ratio of *E. coracana, S. bicolor* and *M. oleifera* seed. The result show magnesium, and potassium were significantly highest ($p \leq 0.05$) in 50 % *E. coracana,* 30 % *S. bicolor* and 20 % *M. oleifera* seed while calcium, iron and potassium were significantly lowest ($p \leq 0.05$) in the same ratio. Calcium and iron were significantly highest ($p \leq 0.05$) in 30 % *E. coracana,* 50 % *S. bicolor* and 20 % *M. oleifera* seed while magnesium, copper and potassium were significantly lowest ($p \leq 0.05$) in the same ratio. Phosphorus was significantly highest ($p \leq 0.05$) in 45 % *E. coracana,* 35 % *S. bicolor* and 20 %*M. oleifera* seed while zinc was significantly lowest ($p \leq 0.05$) in that same ratio. There was no significant difference ($p \leq 0.05$) in sodium for all the four feed ratios.

Table 4.8: Mineral Content of Formulated Feed at Different Ratios of *E. coracana, S. bicolor* and *M. oleifera* Seed

Parameters	A	B	C	D
Mg	6.765 ± 0.005^{d}	6.695 ± 0.005^{c}	6.605 ± 0.005^{b}	6.435 ± 0.005^{a}
Ca	11.335 ± 0.005^{a}	11.385 ± 0.005^{b}	11.435 ± 0.005^{c}	11.545 ± 0.005^{d}
Na	7.465 ± 0.005^{a}	7.460 ± 0.010^{a}	7.455 ± 0.005^{a}	7.455 ± 0.005^{a}
Cu	0.455 ± 0.005^{b}	1.015 ± 0.005^{c}	0.445 ± 0.005^{b}	0.425 ± 0.005^{a}
Zn	0.795 ± 0.005^{b}	0.685 ± 0.005^{a}	0.785 ± 0.005^{b}	0.785 ± 0.005^{b}
Fe	2.935 ± 0.005^{a}	2.955 ± 0.005^{b}	3.185 ± 0.005^{c}	3.355 ± 0.005^{d}
K	13.445 ± 0.005^{d}	13.415 ± 0.005^{c}	13.365 ± 0.005^{b}	13.195 ± 0.005^{a}
P	13.615 ± 0.005^{a}	32.145 ± 0.005^{d}	31.575 ± 0.005^{c}	30.445 ± 0.005^{b}

Values are reported as mean ± standard error of means. Values on the same row with different alphabetic superscript are significantly different at $p \leq 0.05$.
A =50%Finger millet, 30% *Sorghum bicolor* and 20% *Moringa oleifera* Seeds,
B =45%Finger millet, 35% *Sorghum bicolor* and 20% *Moringa oleifera* Seeds,
C =40%Finger millet, 40% *Sorghum bicolor* and 20% *Moringa oleifera* Seeds,
D =30%Finger millet, 50% *Sorghum bicolor* and 20% *Moringa oleifera* Seeds

The result in Table 4.9 shows the blood glucose level of the albino rats formulated feeds (Group 1 to 4), those fed with chow with Metformin HCl treatment (Group 5) and without treatment (Group 6 and 7). The result shows there was significant difference ($p \leq 0.05$) in the blood glucose level of all the groups. The un-induced and untreated group had blood glucose level that was significantly lowest ($p \leq 0.05$) at the start of the treatment, followed by the groups treated with 30 % *E. coracana,* 50 % *S. bicolor* and 20 %*M. oleifera* seed, 50 % *E. coracana,* 30 %*S. bicolor* and 20 %*M. oleifera* seed, Metformin HCl, induced and untreated, 45 % *E. coracana,* 35 % *S. bicolor*and20 % *M. oleifera* seed and 40 % *E. coracana,* 40 %*S. bicolor* and 20 %*M. oleifera* seed in that order. At the end of the 21 days treatment, the un-induced and untreated group still had the significantly lowest ($p \leq 0.05$) blood glucose level, followed by the group feed with 50 % *E. coracana,* 30 %*S. bicolor* and 20 %*M. oleifera* seed, Metformin HCl, 30 % *E. coracana,* 50 % *S. bicolor* and 20 %*M. oleifera* seed, 45 % *E. coracana,* 35 % *S. bicolor*and20 % *M. oleifera* seed, 40 % *E. coracana,* 40 %*S. bicolor* and 20 %*M. oleifera* seed and lastly the group induced and not treated.

When compared along the column, the blood glucose level of all the groups fell significantly ($p \leq 0.05$) and continuously over the period of the administration of the formulated feeds and Metformin HCl treatment except for the un-induced and untreated groups. The percentage change in the blood glucose level is shown in Figure 4.1.

Table 4.9: Blood Glucose Level of the Albino Rats (Group) Managed/treated with the Formulated Fed, Metformin HCl Control and Other Controls

Parameters (mg/dl)	A	B	C	D	E	F	G
Day 1	$115.000\pm1.000^{bc}{}_{f}$	$124.500\pm1.500^{d}{}_{f}$	$134.000\pm1.000^{e}{}_{f}$	$112.000\pm2.000^{b}{}_{f}$	$117.000\pm1.000^{c}{}_{f}$	$124.000\pm1.000^{d}{}_{f}$	$81.500\pm1.500^{a}{}_{ab}$
Day 5	$112.500\pm2.500^{c}{}_{e}$	$120.000\pm1.000^{d}{}_{e}$	$129.000\pm0.500^{e}{}_{e}$	$106.000\pm1.000^{b}{}_{e}$	$112.000\pm2.000^{c}{}_{e}$	$121.000\pm1.500^{d}{}_{e}$	$80.000\pm1.000^{a}{}_{a}$
Day 9	$108.500\pm1.500^{c}{}_{d}$	$114.500\pm1.500^{d}{}_{d}$	$124.500\pm1.500^{e}{}_{d}$	$102.500\pm2.500^{b}{}_{d}$	$104.000\pm1.000^{bc}{}_{d}$	$125.500\pm1.500^{e}{}_{d}$	$81.000\pm1.000^{a}{}_{ab}$
Day 13	$103.500\pm1.500^{b}{}_{c}$	$110.500\pm1.500^{c}{}_{c}$	$123.000\pm2.000^{d}{}_{c}$	$106.000\pm3.000^{bc}{}_{c}$	$100.500\pm2.500^{b}{}_{c}$	$120.500\pm1.500^{d}{}_{c}$	$82.500\pm1.500^{a}{}_{ab}$
Day 17	$106.500\pm2.500^{c}{}_{b}$	$107.000\pm3.000^{c}{}_{b}$	$116.000\pm1.000^{d}{}_{b}$	$99.500\pm0.500^{bc}{}_{b}$	$97.500\pm3.500^{b}{}_{b}$	$123.500\pm2.500^{d}{}_{b}$	$82.500\pm0.500^{a}{}_{ab}$
Day 21	$90.000\pm1.000^{b}{}_{a}$	$98.000\pm1.000^{d}{}_{a}$	$106.500\pm1.500^{e}{}_{a}$	$94.000\pm1.000^{c}{}_{a}$	$90.500\pm1.500^{b}{}_{a}$	$119.000\pm1.000^{f}{}_{a}$	$83.500\pm1.500^{a}{}_{b}$

Values are reported as mean ± standard error of means. Values on the same row with different alphabetic superscript are significantly different($p\leq0.05$) while values along the column with different alphabetic subscripts are significantly difference ($p\leq0.05$).
A= induced and treated with 50% Finger millet, 30% *Sorghum bicolor* and 20% *Moringa oleifera* seeds
B= induced and treated with 45% Finger millet, 35% *Sorghum bicolor* and 20% *Moringa oleifera* seeds
C= induced and treated with 40% Finger millet, 40% *Sorghum bicolor* and 20% *Moringa oleifera* seeds
D= induced and treated with 30% Finger millet, 50% *Sorghum bicolor* and 20% *Moringa oleifera* seeds
E= induced and treated with Metformin HCl
F= induced and untreated
G= uninduced and untreated

The result in Figure 4.1 shows the percentage change in the blood glucose level of the Albino Rats managed/treated with the formulated feeds from different ratios of *E. coracana, S. bicolor* and *M. oleifera* seed, Metformin HCl treated control group, the induced and non-treated group and the un-induced and non-treated group. There were statistically significant reduction in the blood glucose level of all the group except the un-induced and un-treated negative control group which gained blood glucose level over the period of the treatment. Rats in Group E which were treated with Metformin HCl Standard drug had the highest reduction (23.08 %) in their blood glucose level. The groups treated/managed with the formulated feeds (Groups A to D) from different ratios of *E. coracana, S. bicolor* and *M. oleifera* seed had 21.74 %, 20.97 %, 20.90 % and 16.07 % reduction respectively. The group that were induced but were not treated or managed had a 4.03 % reduction in their blood glucose level, which was significantly lower ($p \leq 0.05$) when compared to the groups treated/managed with formulated diets and Metformin HCl Standard drugs. Group G (the un-induced and non-treated group) had a significant ($p \leq 0.05$) gained of blood glucose level. The Percentage reduction is calculated by subtracting the final blood glucose concentration from the initial, dividing it by the initial blood glucose concentration and multiplied by 100%.

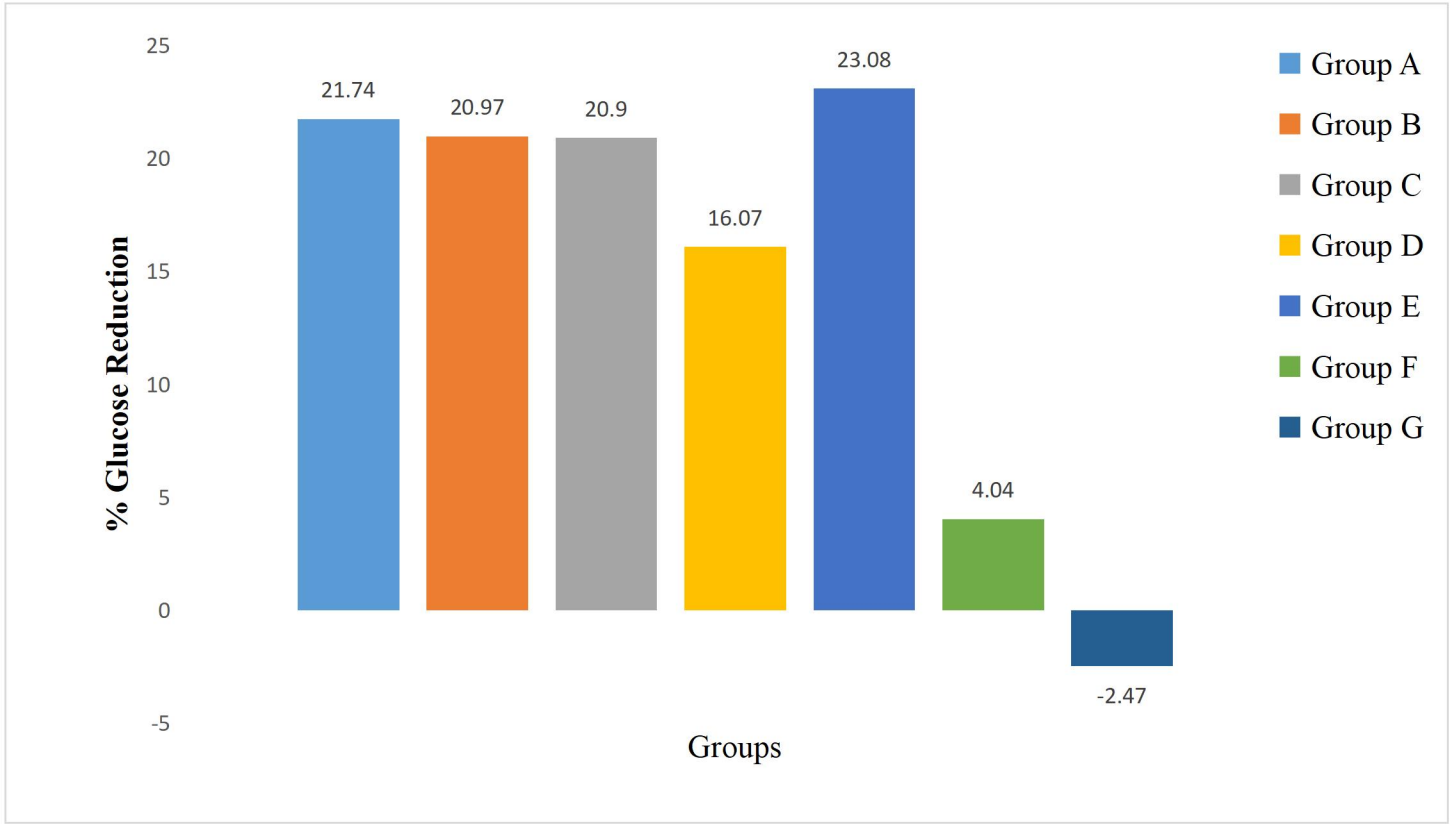

Figure 4.1: Percentage Changes in Blood Sugar in Various Groups of Rats.

A= induced and treated with 50% Finger millet, 30% *Sorghum bicolor* and 20% *Moringa oleifera* seeds
B= induced and treated with 45% Finger millet, 35% *Sorghum bicolor* and 20% *Moringa oleifera* seeds
C= induced and treated with 40% Finger millet, 40% *Sorghum bicolor* and 20% *Moringa oleifera* seeds
D= induced and treated with 30% Finger millet, 50% *Sorghum bicolor* and 20% *Moringa oleifera* seeds
E= induced and treated with Metformin HCl
F= induced and untreated
G= uninduced and untreated

The result in Table 4.10 shows the hematological indexes of Albino rats fed/managed with different ratios of formulated feed from *E. coracana, S. bicolor* and *M. oleifera* seed, Metformin HCl treated control group, the induced and non-treated group and the un-induced and non-treated group. Although most hematological parameters were within the normal ranges, packed cell volume (PCV) was significantly highest ($p \leq 0.05$) in the un-induced and non-treated group, followed by the induced but non-treated group. The least PCV level was obtained for the group managed with 30 % *E. coracana,* 50 % *S. bicolor a*nd 20 % *M. oleifera* seed followed by the group managed with 40 % *E. coracana,* 40 % *S. bicolor* and 20 % *M. oleifera* seed, 45 % *E. coracana,* 35 % *S. bicolor* and 20 % *M. oleifera* seed and 50 % *E. coracana,* 30 % *S. bicolor* and 20 % *M. oleifera* seed and Metformin treated group respectively. The same pattern was repeated for heamoglobin estimate (HB).

Red blood cell (RBC) count were significantly highest ($p \leq 0.05$) in the standard control and negative control groups while it was significantly lowest ($p \leq 0.05$) in the group managed with 30 % *E. coracana,* 50 % *S. bicolor*and20 % *M. oleifera* seed. Similar trend was repeated for white blood cells (WBC) counts. Platelets count was significantly highest ($p \leq 0.05$) in the group managed with 30 % *E. coracana,* 50 5 *S. bicolor*and20 % *M. oleifera* seed while it was significantly lowest ($p \leq 0.05$) in the un-induced and non-treated group. Neutrophile was significantly highest ($p \leq 0.05$) in the group managed with 50 % *E. coracana,* 30 % *S. bicolor* and 20 % *M. oleifera* seed and least ($p \leq 0.05$) in the group managed with 40 % *E. coracana,* 40 % *S. bicolor* and 20 % *M. oleifera* seed. Lymphocyte was significantly highest ($p \leq 0.05$) in the group managed with 40 % *E. coracana,* 40 % *S. bicolor* and 20 % *M. oleifera* seed. There were no significant difference ($p \leq 0.05$) in monocyte and eosiniphile for all groups.

Table 4.10: Hematological Parameters of Rats in Various Treatment Groups

Parameters	A	B	C	D	E	F	G
PCV	47.050±0.50^{d}	44.950±0.06^{c}	43.500±0.50^{b}	40.050±0.50^{a}	44.990±0.10^{c}	55.000±0.01^{e}	55.980±0.02^{f}
HB	15.650±0.05^{d}	15.050±0.05^{c}	14.700±0.01^{b}	13.290±0.01^{a}	14.990±0.02^{c}	18.200±0.10^{e}	18.450±0.20^{f}
RBC	7.750±0.05^{c}	7.450±0.05^{b}	7.200±0.10^{b}	6.650±0.05^{a}	7.450±0.05^{b}	9.150±0.05^{d}	9.150±0.20^{d}
WBC	5.150±0.05^{b}	5.400±0.10^{b}	5.300±00^{b}	4.550±0.20^{a}	5.250±0.20^{b}	6.150±0.05^{c}	6.200±0.10^{c}
Platelets	225.350±0.30^{b}	226.150±0.050^{bc}	227.500±0.10^{d}	226.400±0.10^{c}	225.650±0.20^{bc}	226.050±0.05^{bc}	224.500±0.30^{a}
Neutrophile	33.500±0.50^{d}	31.050±0.050^{b}	29.980±0.02^{a}	31.500±0.50^{bc}	33.100±0.10^{cd}	32.000±0.10^{bcd}	32.000±00^{bcd}
Lymphocyte	61.500±0.50^{ab}	62.500±0.50^{bc}	64.000±0.50^{d}	60.500±0.50^{a}	61.500±0.50^{ab}	62.500±0.50^{bc}	63.500±0.50^{cd}
Monocyte	2.000±00^{a}	2.500±0.50^{a}	2.000±00^{a}	2.000±00^{a}	2.000±00^{a}	2.000±00^{a}	2.000±00^{a}
Eosiniphile	1.000±00^{a}	1.000±00^{a}	1.000±00^{a}	1.000±00^{a}	1.000±00^{a}	1.000±00^{a}	1.000±00^{a}

Values are reported as mean ± standard error of means. Values on the same row with different alphabetic superscript are significantly different ($p \leq 0.05$).
A= induced and treated with 50% Finger millet, 30% *Sorghum bicolor* and 20% *Moringa oleifera* seeds
B= induced and treated with 45% Finger millet, 35% *Sorghum bicolor* and 20% *Moringa oleifera* seeds
C= induced and treated with 40% Finger millet, 40% *Sorghum bicolor* and 20% *Moringa oleifera* seeds
D= induced and treated with 30% Finger millet, 50% *Sorghum bicolor* and 20% *Moringa oleifera* seeds
E= induced and treated with Metformin HCl
F= induced and untreated
G= uninduced and untreated

The result in Figure 4.2 shows the activities of the liver function enzymes in the blood serum of the different groups of the albino rats treated/managed with both drug and formulated feeds. The result shows the aspartate transaminase (AST) activities was significantly highest ($p \leq 0.05$) in the group fed with 50 % *E. coracana,* 30 % *S. bicolor* and 20 % *M. oleifera* seed and least in the group administered Metformin HCl standard control drug. The alanine transaminase (ALT) was significantly highest ($p \leq 0.05$) in the group induced without treated (Group F) compared to the other groups while it was least in the group fed or managed with 45 % *E. coracana,* 35 % *S. bicolor* and 20 % *M. oleifera* seed. The alkaline phosphatase (ALP) activities was significantly highest ($p \leq 0.05$) in the group induced with diabetes mellitus without treated and the group induced with diabetes mellitus and managed with 50 % *E. coracana,* 30 % *S. bicolor* and 20 % *M. oleifera* seed. In contrasts, it was significantly least ($p \leq 0.05$) in group induced with diabetes mellitus and treated with Metformin as well as the un-induced and the un-treated group.

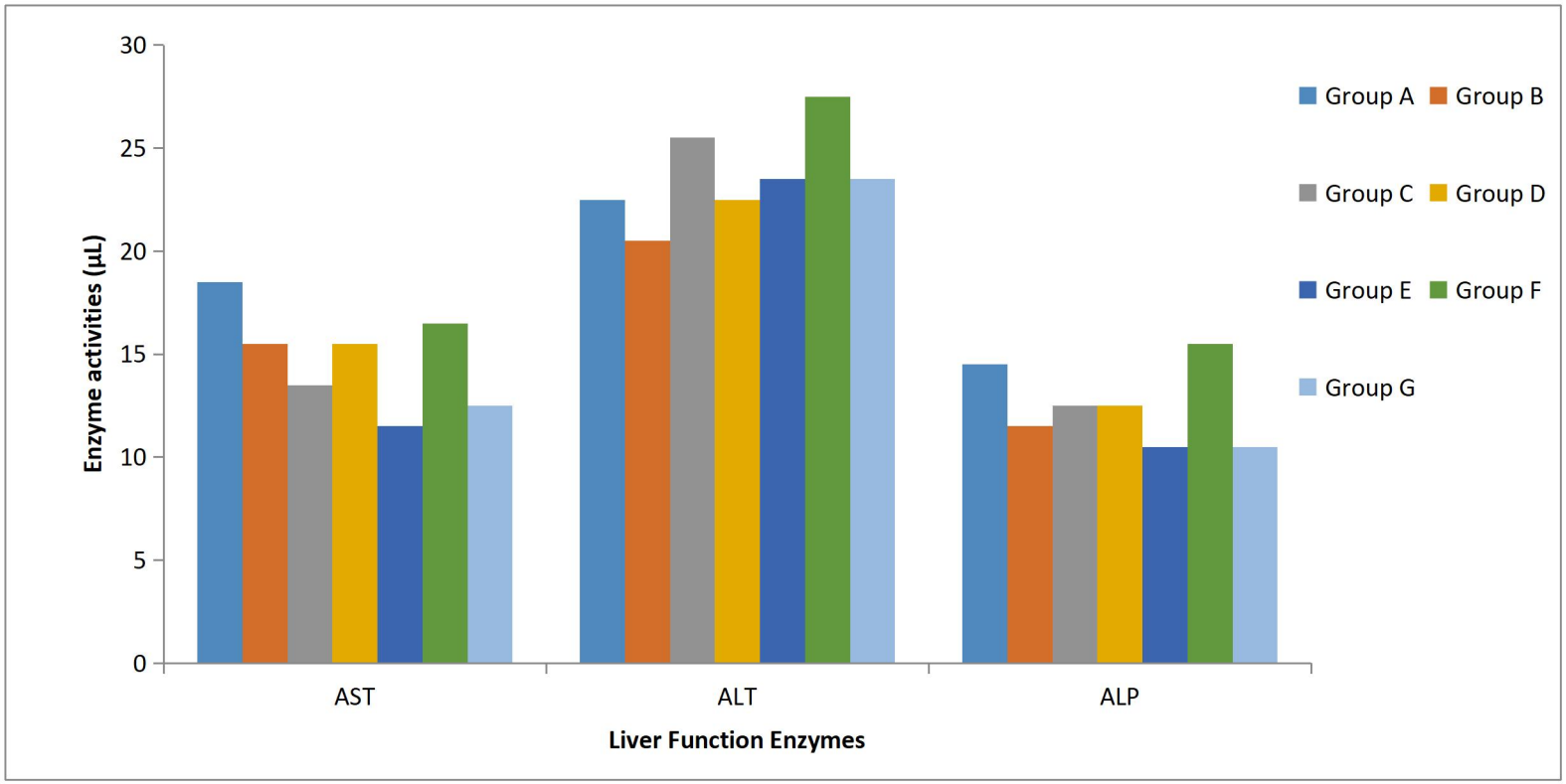

Figure 4.2: Activities of Biochemical Parameters of Rats in Various Treatment Groups

A= induced and treated with 50% Finger millet, 30% *Sorghum bicolor* and 20% *Moringa oleifera* seeds
B= induced and treated with 45% Finger millet, 35% *Sorghum bicolor* and 20% *Moringa oleifera* seeds
C= induced and treated with 40% Finger millet, 40% *Sorghum bicolor* and 20% *Moringa oleifera* seeds
D= induced and treated with 30% Finger millet, 50% *Sorghum bicolor* and 20% *Moringa oleifera* seeds
E= induced and treated with Metformin HCl
F= induced and untreated
G= uninduced and untreated

4.2 Discussion

The nutritional *Moringa oleifera* seeds, *Eleusine coracana* and *Sorghum bicolor* were assessed in this study through the comparative evaluation of the proximate, Amino acids, Vitamin and mineral composition of the individual samples as well as the formulated feeds as part of exploration into its hypoglycemic mechanisms.

4.2.1 Proximate composition

The findings of this study shows that the significantly higher fat (21.815 %), ash (6.325 %), fibre (11.825 %) and protein (47.135 %) content and significantly low carbohydrate (4.965 %) content of *Moringa oleifera* seed revealed that *Moringa oleifera* seed has potential to lower blood glucose level compared to the carbohydrate rich *Sorghum bicolor* (60.145 %) and *Eleusine coracana* (67.725 %). This study agrees with the reports of (Madibuike *et al*., 2015) and that of Mbah *et al.,* (2012) that suggest *M. oleifera* seeds had high protein, lipids, fibre and Ash content.

Protein is the major functional and structural component of all the cells of the body, exerting an insulinotropic effect and thus promotes insulin secretion, which indeed leads to enhanced glucose clearance from the blood. Also, the functional benefits of dietary fibre when present in the human diet is its ability to reduce the rate of absorption of glucose after consumption of high glycemic carbohydrate-containing foods, leading to a blunted blood glucose response curve and less demand for insulin. When considering the physiology of starch and sugar digestion and glucose absorption, it is possible to elucidate several plausible mechanisms by which both soluble and insoluble fibres might contribute to Glycemic control: reduction in gastric emptying, modification of release of digestion- and fermentation-related hormones, inhibition of amylase activity and delayed starch hydrolysis,

reduction in diffusion of amylolytic products to the small intestinal microvilli, and/or the development of an absorptive barrier layer through interactions with the mucosa. The ash content provides a measure of the total amount of minerals within a food (Nielsen, 2002).

The relatively lower moisture content (7.265 %) of *Moringa oleifera* seeds implies its higher shelf life compared to *Eleusine coracana* and *Sorghum bicolor*. As revealed by the proximate composition of the formulated feed from different ratio of the three starting materials, the overall carbohydrate *Eleusine coracana* of the feeds was lowered while fat, protein and ash content tends toward a more balanced diets. Sandoz *et al.,* (2016) made similar observations, while emphasizing the importance of food augmentation.

4.2.2 Amino acids

The significantly higher amino acids, for example lysine (4.605), isoleucine (4.185), phenylalanine (5.045), proline (9.630) and glutamic acid (20.735) in finger millet (*Eleusine coracana*) and *Sorghum bicolor* (leucine (13.425), tyrosine (4.130), cysteine (2.175), alanine (9.175), proline (9.630) and glutamic acid (20.735) can make up for the significantly lower amino acid constituents in *Moringa oleifera* such leucine (5.645), methionine (0.955), tyrosine (2.405), proline (3.035). Although most of the amino acids in the different ratio of the formulated feed were significant, there is bridge in the gap, as the difference in the amino acid constituents of the formulated feeds were closer in value and within a more balanced range. This shows that compounding different food materials can result in a more balanced diet or nutrition. Compounding of feed has been reported by Fanzo (2014) to bring about deficiency elimination, diet balancing as well as medical benefits.

Also, supplementation of amino acid had gained acceptance as an important treatment therapy for diabetes mellitus as well as it associated complications (Shimomura and Kitaura, 2018). Sulfur containing amino acids such as methionine which was high in *Eleusine coracana* (2.350) and cysteine which was significantly higher in *Sorghum bicolor* (2.195) were known players in the treatment of pathological disorders oxidative stress (Yoon, 2016). Meijer *et al.,* (2015) report that cysteine, homocysteine and N-acetyl cysteine moiety has regulatory effect on insulin secretion as well as glucose level in the plasma. In addition, S-adinosyl methionine or methionine was shown through studies to increase the mitochondrial DNA density in the muscles of the skeleton (Athrerton *et al.,* 2016), bring about improvement insulin sensitivity (Zhang *et al.,* 2019) as well as prevent weight gain.

4.2.3 Mineral and vitamins

The study found that *Moringa oleifera* had significantly higher vitamins such as vitamin A (16.261), vitamin B1 (1.975), vitamin B2 (11.134) and vitamin C (12.645) content which can augment for low vitamin content of *Eleusine coracana* and *Sorghum bicolor* (0.105, 0.015) for vitamin A, (0.110, 0.030) for vitamin B2 and (0.190, 0.045) for vitamin B6 respectively. This assertion was confirmed by the relatively balanced vitamin constituents in the formulated feed from the different ratio of the three food materials. For instance, unlike the significantly different values obtained for the individual raw material in Table 4.5, Table 4.6 shows there was no significant difference in the vitamin A, B2, B6 and E constituents. While the wide gaps observed for B1, B3, B5, B7, B9, B12, C, D and K vitamins were closed by the blending at different ratios. Similar observation was reported by Bashaw *et al.,* (2020) who worked on Nutrition Therapy in Eosinophilic Esophagitis—

Outcomes and Deficiencies. Most of these vitamins are linked to diabetes type 2 management and prevention (Valdes-Ramos *et al.,* 2015)

Minerals were found to be significantly highest in *Moringa oleifera* and lower in *Sorghum bicolor* and *Eleusine coracana.* These high minerals in *Moringa oleifera* can augment for the low minerals in *Sorghum bicolor* and *Eleusine coracana.* This will bring about mineral balancing in the formulated diets, hence better nutritional performance due to the fortifying potential of the *Moringa oleifera*. The formulated feed in Table 4.8 revealed that the mineral deficiency in *Eleusine coracana* and *Sorghum bicolor* were augmented by the mineral rich *Moringa oleifera.* Blank *et al.,* (2016) postulated that mineral deficiency augmentation as one of the benefits of feed formulation from a mineral-rich and a mineral deficient food sources. Also, mineral and vitamins play a significant role in glucose metabolism and the utility food supplementation are relevant to prevention and management of diabetes, particularly, type 2 diabetes mellitus (Christie-David *et al.,* 2015). Evidence exist for relationships between vitamin B complexes, antioxidants (Khan *et al.,* 2015) (vitamins A, C, E and carotenoids), vitamin D (Nakashima *et al.,* 2016), vitamin K, sodium, calcium, potassium and magnesium- and glucose metabolism, (Kibiti and Afolayan, 2015). Minerals serve as Co-enzymes which help to boost insulin sensitivity and the vitamins addresses the various diabetic complications (such as retinopathy, neuropathy, cardiovascular diseases, atherosclerosis, kidney damage, free radicals among others). The availability of the minerals and vitamins present in these formulated diets compensates for its deficiency in diabetic patient (Khawandanah & Tewfik, 2016; Yahaya *et al.,* 2021).

4.2.4 Hematological parameters

The result of the animal study revealed that the glucose level changed significantly over the 21 days of study period. While there were significant reductions in the blood glucose level of the group managed with Metformin HCl standard drug as well as the groups managed with the different ratios of *Eleusine coracana* and *Sorghum bicolor* at fixed ratio of *Moringa oleifera.* There was a gradual reduction in the blood glucose level of the groups managed with formulated feeds (Group A to D), Metformin HCl standard drug (Group E) and the negative control group (Group F). This high potential for blood glucose level reduction demonstrated by the formulated feeds could be as a result of high protein, ash, fat and fibre content in the formulated feeds (Table 4.9) (Adams *et al.,* 2018). This high potential for blood glucose level reduction was found to increase with increase in the ratio of *Eleusine coracana* to that of *Sorghum bicolor* at fixed ratio of *Moringa oleifera.* This could be as a result of high fibre and low glycemic index of finger millet (*Eleusine coracana*) (Chappalwar *et al.,* 2013). Hence, the feeds with higher ratio of *Eleusine coracana* stood a better chance in the management of diabetes mellitus at fixed ratio of *Moringa oleifera.*

At the end of the 21 days of the animal study, the Metformin HCl a standard drug used for management of diabetes mellitus and used as positive control in this study recorded a 23.08% reduction in the blood glucose level of the animal group it was used to manage. This was against 21.74 % reduction recorded for the group of animals managed with 50% *E. coracana,* 30% *S. bicolor* and 20% *M. oleifera* seed and 20.79% recorded for the group managed with 45% *E. coracana,* 35% *S. bicolor* and 20% *M. oleifera* seed (Figure 4.1). Based on the evidences from the result, it can be suggested that the formulated feed from

different ratios of *Eleusine coracana* and *Sorghum bicolor* at fixed ratio of *Moringa oleifera* were equally effective in the management of diabetes mellitus in Alloxan induced albino rats because of the balance in the nutrient constituents. This finding agreed with the finding of Adams *et al.,* (2018)

Another observation from the study was the fact that the blood glucose level of Alloxan induced albino rats dropped naturally over the period of the experiment by 4.03 % (Group F, induced but not treated or managed), but the reduction can be made four to five faster with the formulated management feeds (Group A to D, induced and managed with the formulated management feeds) as well as Metformin HCl treated group (Group E). Also, without any form of induction or management, the albino rats gained a 2.47 % in their blood glucose level of the standard control group (Group G). Hence, feeding the albino rats with just rat chow over time can lead to increased blood glucose level.

4.2.5 Liver function parameters

The measurement of activities of various enzymes in the tissues and body fluids plays a significant role in disease investigation and diagnosis (Malomo, 2000), assault on the organs/tissues and to a reasonable extent the toxicity of the drug (Yakubu *et al.,* 2003). Tissue enzymes can also indicate tissue cellular damage caused by chemical compounds long before structural damage that can be picked by conventional histological techniques (Yakubu *et al.,* 2003).

Aminotransferases, which include alanine aminotransferase (ALT) and aspartate aminotransferase (AST), are involved in the transfer of an amino group from a 2-amino acid to a 2-oxoacid with the aid of pyridoxal phosphate as a cofactor for optimal activity. A

rise in any of these is an indicator of damage to cystolic and/or mitochondrial membranes (Crook, 2006).

AST is present in high concentrations in hepatic, renal, cardiac, and skeletal muscle cells and erythrocytes; hence damage to any of these tissues may increase plasma AST levels (Saka *et al*, 2011; Ige *et al*, 2011). Even though the AST, ALT and ALP were all within the normal range, the significantly higher ($P < 0.05$) AST activity observed in the serum of rats in group A may not necessarily be as a result of liver damage. Ekpo, (2011) fed rats with insects supplemented diets low in protein and reported elevated levels of AST. Therefore, increased serum activity of ALT may be ascribed to low protein quality of the various supplemented diets, poor feeding (unpalatiability) or length of feeding (Ekpo *et al*, 2011).

Alkaline phosphatase (ALP) is present in high concentrations in the bone, mucosa of the small intestine, kidney, liver and plasma. Damage in any of these organs may cause a release of ALP into the bloodstream. ALP is also a marker for the plasma membrane and endoplasmic reticulum used to assess the integrity of plasma membrane and endoplasmic reticulum (Akanya *et al.,* 2014). An increase in serum ALP activity is also associated with bone growth (Ramaiah, 2007). The significant increase in serum ALT and ALP activity observed in the group F (the negative control) may be as a result of liver damage as a result of diabetic impact with no treatment administered.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The following conclusions can be drawn from the results and the findings of this study. First, that *Moringa oleifera* seed was richer in protein, ash, fat, fibre, minerals and vitamins as compared to *Eleusine coracana* and *Sorghum bicolor* which are richer in carbohydrate. Hence, combining at different ratios produced hybrid feed that is nutritionally more balanced than the individual food materials.

The formulated feeds demonstrated a high potential for blood glucose level reduction. This high potential for blood glucose level reduction was found to increase with increase in the ratio of *Eleusine coracana* to that of *Sorghum bicolor* at fixed ratio of *Moringa oleifera.* Hence, the feeds with higher ratio of *Eleusine coracana* stood a better chance in the management of diabetes mellitus at fixed ratio of *Moringa oleifera.*

At the end of the 21 days of the animal study, the Metformin a standard drug used for management of diabetes mellitus and used as positive control in this study recorded a 23.08% reduction in the blood glucose level of the animal group it was used to manage. This was against 21.74% reduction recorded for the group of animals managed with 50 % *E. coracana,* 30 %*S. bicolor* and 20% *M. oleifera* seed and 20.79% recorded for the group managed with 45% *E. coracana,* 35% *S. bicolor* and 20% *M. oleifera* seed. Based on the evidences from the result, it can be concluded that the formulated feed from different ratios of *Eleusine coracana* and *Sorghum bicolor* at fixed ratio of *Moringa oleifera* were equally effective in the management of diabetes mellitus in Alloxan induced albino rats.

Another conclusion that can be drawn from the study was the fact that the blood glucose level of Alloxan induced albino rats dropped naturally over the period of the experiment by 4.03 % (Group F, induced but not treated or managed), but the reduction can be made four to five faster with the formulated management feeds (Group A to D, induced and managed with the formulated management feeds)as well as Metformin treated group (Group E). Also, without any form of induction or management, the albino rats gained a 2.47% in their blood glucose level of the standard control group (Group G). Hence, feeding the albino rats with just rat chow over time can led to increased blood glucose level.

Finally, the diabetes mellitus formulated management feeds have no effect on the hematological parameters as the hematological parameters were within the normal ranges, even though there were significant differences among the various treatment groups. Also, the blood serum enzymes (aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP)) activities were within normal ranges. This means the formulated feeds had no damaging effect on the liver or kidney where these enzymes are stored. The formulated feeds are there for effective for the management of diabetes mellitus and safe for use since it had no negative on the biochemical as well as hematological parameters of the albino Wistar rats.

5.2 Recommendations

From the results and the findings of this study, the following recommendations are made for further search for solutions to the problem *Diabetes mellitus.*

1. This study considers effects of the formulated feed against Metformin, induced but non-treated or managed group and un-induced and non-treated or managed

group.Further study is hereby recommended to evaluate the effects of the individual food crops against the combined ratios in the management of diabetes.

2. While organ toxicity or damage are not expected, it is recommended that acute and chronic toxicity or histopathology assessment be carried out to determine the safety of the formulated feeds on the organs.

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