EFFECTS OF *Sesamum indicum* COMPOUNDED DIET ON ALLOXAN-INDUCED DIABETIC RATS.

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CERTIFICATION

This is to certify that I, Ekeke Kelechi Light (20184142358), completed the research project titled "**Effects of *Sesamum indicum* Compounded Diet on Alloxan-Induced Diabetic Rats**" in partial fulfillment of the requirements for the award of the degree of M.Sc. in Biochemistry at the department of Biochemistry of the Federal University of Technology, Owerri.

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DEDICATION

I humbly dedicate my study to God Almighty in appreciation of his kindness and grace shown to me throughout the course of this investigation.

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TABLE OF CONTENTS

Title page - - - - - - - - - i

Certification - - - - - - - - - ii

Dedication - - - - - - - - - iii

Acknowledgement - - - - - - - - - iv

Abstract - - - - - - - - - v

Table of Contents - - - - - - - - - vi

List of Tables - - - - - - - - - vii

List of Figures - - - - - - - - - ix

List of Plates - - - - - - - - - x

CHAPTER ONE: INTRODUCTION

1.1 Background of Study - - - - - - - 1

1.2 Statement of Problem - - - - - - - 2

1.3 Aim and Objectives - - - - - - - 2

1.3.1 Aim - - - - - - - - - 2

1.3.2 Objectives - - - - - - - - 3

1.4 Significant of the Study - - - - - - - 3

1.5 Justification of the Study - - - - - - - 3

CHAPTER TWO: LITERATURE REVIEW

2.1*Sesamum indicum* - - - - - - - - 4

2.1.1Scientific Classification of *Sesamum indicum* - - - - 5

2.1.2 Plant Habitat and Characteristics of *Sesamum indicum* - - - 6

2.1.3 Nutritional Profile of *Sesamum indicum* - - - - 7

2.1.4 Medicinal Properties of *Sesamum indicum* and Health Issues - - 8

2.2. Diabetes - - - - - - - - - 12

2.2.1 Diabetes Mellitus - - - - - - - 12

2.2.1.1 History of Diabetes Mellitus - - - - - - 13

2.2.1.2 Types of Diabetes Mellitus - -- - - - - 14

2.2.1.2.1 Type 1 Diabetes Mellitus - - - - - - 14

2.2.1.2.2 Type 2 Diabetes Mellitus - - - - - - 14

2.2.1.2.3 Gestational Diabetes Mellitus - - - - - 15

2.2.1.3 Diagnosis, Treatment and Management of Diabetes Mellitus - 15

2.2.2 Hyperglycemia and Hypoglycemia - - - - - 17

2.3 Alloxan - - - - - - - - - 18

2.3.1 Chemical Structure of Alloxan - - - - - - 19

2.3.2 Biological Effects of Alloxan - - - - - - 20

2.3.3 Mechanism of Action of Alloxan - - - - - 20

2.4 Liver - - - - - - - - - - 21

2.4.1 The Structure of the Liver - - - - - - 22

2.4.2 Functions of the Liver - - - - - - - 22

2.4.3 Diseases of the Liver - - - - - - - 23

2.4.4 Mechanism of Liver Damage - - - - - - 23

2.4.5 Liver Function Test (LFT) - - - - - - 24

2.4.5.1 Relevance of Liver Function Test - - - - - 24

2.4.5.2 Aspartate Transaminase (AST) - - - - - 25

2.4.5.2.1 Clinical Significance of Aspartate Transaminase (AST) - - 26

2.4.5.3 Alanine Transaminase (ALT) - - - - - - 26

2.4.5.3.1 Clinical Significance of Alanine Transaminase - - - 27

2.4.5.4 Alkaline Phosphatase (ALP) - - - - - - 28

2.4.5.4.1Clinical Significant of ALP - - - - - - 29

2.4.5.5 Albumin - - - - - - - - 29

2.4.5.6 Bilirubin - - - - - - - - 30

2.4.5.7 Total Protein - - - - - - - - 31

2.4.5.7.1 Clinical Significant of Total Protein - - - - 31

2.5 Lipid Profile Test - - - - - - - - 32

2.5.1 Triglyceride - - - - - - - - 32

2.5. 2 High Density Lipoprotein (HDL) - - - - - 32

2.5.3 Cholesterol - - - - - - - - 33

2.5.4 Low Density Lipoprotein (LDL) - - - - - - 33

2.5.5 Very Low-density Lipoprotein (VLDL) - - - - - 33

2.6 Kidney - - - - - - - - - 34

2.6.1 Function of the Kidney - - - - - - - 34

2.6.2 Kidney Diseases - - - - - - - 35

2.6.3 Kidney Function Test - - - - - - - 35

2.6.3.1 Serum Urea - - - - - - - - 35

2.6.3.2 Serum Creatinine - - - - - - - 36

2.7 Feed Formulation - - - - - - - - 36

2.7.1 Important Consideration in Feed Formulation - - - - 38

2.7.2 Composition of Constituent - - - - - - 39

2.7.2.1 Level of Fiber - - - - - - - - 39

2.7.2.2 Fat Supplementation Level - - - - - - 39

2.7.2.3 Protein Level - - - - - - - - 40

2.7.3 Feed Formulation Techniques - - - - - - 40

CHAPTER THREE: MATERIALS AND METHODS

3.1 Materials, Apparatus and Equipment - - - - - 42

3.2 Reagents - - - - - - - - - 42

3.3 Methodology - - - - - - - - 42

3.3.1 Procurement of Animals - - - -- - - 42

3.3.2 Experimental Design - - - - - - - 43

3.4 Sample Collection and Preparation - - - - - 44

3.5 Proximate Analysis Methods - - - - - - 44

3.5.1 Moisture Content - - - - - - - 44

3.5.2 Ash Content - - - - - - - - 44

3.5.3 Crude Fiber Content - - - - - - - 45

3.5.4 Crude Fat Content - - - - - - - 45

3.5.5 Crude Protein content - - - - - - - 46

3.5.6 Carbohydrate Determination - - - - - - 46

3.6 Phytochemical Analysis using GC-MS - - - - - 46

3.7 Feed Formulation - - - - - - - - 47

3.8 Biochemical Analysis - - - - - - - 48

3.8.1 Methods of Liver Function Tests - - - - - 48

3.8.1.1 Aspartate Transferase Activity - - - - - 48

3.8.1.2 Alanine Transferase activity - - - - - - 48

3.8.1.3 Albumin Determination - - - - - - 49

3.8.1.4 Alkaline Phosphatase Determination - - - - - 49

3.8.1.5 Bilirubin Determination - - - - - - - 49

3.8.1.6 Total Protein Determination - - - - - - 49

3.8.2 Methods of Kidney Function Test - - - - - 50

3.8.2.1 Urea - - - - - - - - - 50

3.8.2.2 Creatinine - - - - - - - - 50

3.8.2.3 Potassium - - - - - - - - 51

3.8.2.4 Sodium - - - - - - - - 51

3.8.2.5 Bicarbonate - - - - - - - - 51

3.8.3 Methods of Lipid Profile - - - - - - 52

3.8.3.1 Cholesterol Test - - - - - - - 52

3.8.3.2 Triglyceride Test - - - - - - - 52

3.8.3.3 HDL Test - - - - - - - - 53

3.8.3.4 Total Bilirubin Test - - - - - - - 53

3.8.3.5 LDL Test - - - - - - - - 54

3.9 Histology of Organ - - - - - - - 54

3.10 Alloxan Preparation - - - - - - - 55

3.11 Statistical Analysis - - - - - - 55

CHAPTER FOUR: RESULTS

Results - - - - - - - - - 56-85

CHAPTER FIVE: DISCUSSION AND RECOMENDATION

5.1 Discussion - - - - - - - - 86

5.2 Conclusion - - - - - - - - 89

5.3 Contribution to Knowledge - - - - - - 89

5.3 Recommendation - - - - - - - - 89

References - - - - - - - - - 90-98

Appendix - - - - - - - - - 99-101

LIST OF TABLES

Table	page
3.1: Animal Grouping and Treatment	43
4.1: Proximate Composition of *Sesamum indicum* Seed	57
4.2: Phytocompounds Identified in *Sesamum indicum* by GC/MS	59
4.3: % Composition of different Compounded Feed	62
4.4: Relative Body Weight of Albino Rat on day 7 and 14	66
4.5: Relative Weight (%) of Liver, kidney and pancreas of Albino Rat after Sacrifice	68
4.6: Effect of compounded diet on Liver Function Parameters of Albino Rat	71
4.7: Effect of compounded diet on Kidney Profile Parameters of Albino Rat	74
4.8: Effect of compounded diet on Lipid Profile of Albino Rat	76

LIST OF FIGURES

Figure	page
2.1: *Sesamum indicum* Seed	6
2.2: Structure of Alloxan	19
4.1: Effect of Treatment on Blood Glucose Level of Albino Rat at 3rd 7th 10th & 14th Day	64

LIST OF PLATES

Plate	page
A: Photomicrograph of Pancreas for Group 1-6 Mag X 400 H & E	78
B: Photomicrograph of Pancreas for Group 1 – 3 and 7-9 Mag X 400 H & E	79
C: Photomicrograph of Kidney for Group 1-6 Mag X 400 H & E	81
D: Photomicrograph of Kidney for Group 1-3 and 7-9 Mag X 400 H & E	82
E: Photomicrograph of Liver for Group 1-6 Mag X 400 H & E	84
F: Photomicrograph of Liver for Group 1-3 and 7-9 Mag X 400 H & E	85

ABSTRACT

The purpose of this study was to assess the effects of *Sesamum indicum* compounded diet on alloxan induced diabetic rats. For the study, which lasted 21 days, 36 male albino rats were divided into nine groups of four rats each (Grp 1 Normal control, Grp 2 Diabetic control, Grp 3 standard drug-Glibenclamide treated, Grp 4, 5, and 6 diabetic rats fed with 15%, 30%, and 60% compounded diet respectively, and Grp 7, 8 and 9 rats fed with 15%, 30%, and 60% compounded diet respectively for 14 days prior to diabetes induction). A single intraperitoneal dosage of freshly produced alloxan (140 mg/kg body weight) was used to induce diabetes. Acu-check Glucometer was used to measure blood sugar levels. Utilizing conventional spectrophotometric techniques, values for lipid, kidney, and liver function were assessed. When compared to the diabetic control at the conclusion of the study, the blood glucose levels of groups 4, 5, and 6 fed on 15%, 30%, and 60% compounded diets, respectively, were reduced below 200. After two weeks of feeding on the compounded diet, group 7, 8, and 9 were induced. It was found that their blood glucose levels were still < 200. The trend in Lipid profile (CHOL, TRIG, and LDL) parameters and blood glucose were similar, with a significant difference of $P<0.05$, although the HDL, which was low in untreated diabetics, increased in the groups fed the compound diet. Comparing groups fed the compounded diet to the diabetic control group, the level of Kidney parameters showed a decrease at a significant difference of $P<0.05$. The parameters of Liver function showed the same pattern. From the relative organ weight result, the groups fed with the compounded diet showed a weight decrease in the Liver and Kidney while the Pancreas increased compared to the diabetic group that has the opposite. There is no significant difference between the groups fed with the diet before induction of diabetes and the group induced before feeding with the diet. The result of this research proves that the compounded diet has both Ameliorative and Protective properties against diabetes mellitus.

Key Words: *Sesamum indicum*, Alloxan, Diabetes, Ameliorative, Protective

CHAPTER ONE

INTRODUCTION

1.1 Background of study

The utilization of plant-based products is one of the growing interests in the treatment of diabetes. It has been established that several plant products have medicinal potential. There have been numerous medications developed to treat diabetes, but the most of them have unfavorable side effects (Gupta *et al.*, 2006). For the majority of diabetics, out of reach. A dietary supplement that makes use of foods or plant items that have been shown to have a hypoglycemic impact and are also readily available locally can help with this problem (Onyechi & Ibeanu, 2010). According to Raskin and Ripoll (2004), 25% of medicines are made from plant products.

Dietary supplements are substances that include nutrients and are intended to enhance the diet of particular animals. This is composed of, but is not limited to, vitamins, minerals, enzymes, and other substances in either a standalone or mixed form (Ogboye *et al.,* 2018). The sesame plant is one of the plants that has historically made a significant contribution to food and medicine (Olaleye *et al.,* 2019). Sesame contains a bioactive component called sesamin that can shield the liver from oxidative damage (Anilakuma *et al.,* 2010).

The list of the most difficult pathological conditions in the twenty-first century now includes diabetes. According to the 2010 diabetes statistics, 285 million individuals worldwide are projected to be carriers of the disease, with type 2 diabetes accounting for 90% of cases. According to Wild et al. (2004), this led in 1.4 million deaths worldwide, making it the eighth greatest cause of death, and it is predicted to increase by 2030. Nearly one-third of diabetes cases are now undiagnosed, which is extremely concerning (Michael & Fowler, 2003).

Diabetes mellitus is a chronic medical illness that develops when the pancreas is unable to produce enough of the hormone insulin, which controls blood sugar, or when the body's own insulin cannot be used properly. Increased blood glucose is mostly caused by inadequate insulin synthesis, which leads to impaired function or damage to the body's systems like the heart and blood vessels.

Some of the indications and symptoms of diabetes include excessive thirst, frequent urination, weight loss or increase, exhaustion, and flu-like symptoms. Early on, these symptoms may be negligibly faint or even absent (WHO, 2009).

About 90% of diabetic people have type 2 diabetes, which is characterized by insulin resistance, while only 5% of diabetic patients have type 1 diabetes (complete insulin shortage).

1.2 Statement of Problem

It is essential to provide an alternative with little to no side effects for the treatment of diabetes due to the increasing prevalence of diabetes in the world and some noticeable negative effects of the majority of medications used to treat it. Dietary supplements are now a possibility, and research into diabetes treatment is becoming more and more interested in them.

This investigation was necessary since sesame seed has been shown in earlier studies to have therapeutic potential and can be used as a dietary supplement.

1.3 Aim and Objectives

1.3.1 Aim

This study is aimed at evaluating the effects of sesamum indicum (beniseed) compounded diet on alloxan induced diabetic albino rat.

1.3.2 Objectives

i. To compound diet using *sesamum indicum* as dietary supplement for the diabetic rat with appropriate nutritional requirement.

ii. To ascertain the proximate composition of *sesamum indicum*

iii. To ascertain the Phytochemical constituent of *sesamum indicum*

iv. To evaluate the effect of the *sesamum indicum* supplemented diet on the diabetic rat by assessing the;

a) Lipid profile of the diabetic rat
b) Liver enzyme biomarkers (ALP ALT and AST)
c) Kidney function (serum urea, Na, K and creatinine)
d) Histology of the liver, pancreas and kidney
e) Organ weight (Liver, Kidney and Pancreas)

1.4 Significance of the Study

This study will advance and add to the numerous investigations towards finding a long-term treatment for diabetes. It may also offer information on the effects of a compounded sesamum indicum diet on rats that have been given an alloxan-induced diabetes.

1.5 Justification of Study

Sesamum indicum is a therapeutic plant product that has been shown in the literature to treat a number of ailments, including diabetes. There is, however, no published research on the assessment of the effect of sesamum indicum supplemented diet on alloxan induced diabetic rats at the time of this study. Therefore, the research's findings will offer some useful experimental data on the subject at hand.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Sesamum indicum*

Beniseed, also known as Sesamum indicum, is a flowering plant in the family Sesamum. It grows naturally in tropical areas of the world and is cultivated for its edible seeds, which are produced in pods. It is one of the oldest oil seed harvests ever known, dating back more than 3000 years, according to records (Ram *et al.,* 1990). It has several different species, some of which are native to sub-Saharan Africa and are wild. India is where the best cultivar was discovered (Ogasawara *et al.,* 1988). It is known to thrive in environments or locations where other crops may fail because of its remarkable tolerance to drought-like conditions (Ram *et al.,* 1990).

According to the FAO (2012), Burma was the world's greatest producer of sesame seeds in 2013, with harvesting totalling over 4.76 million metric tons. The greatest importer of sesame seeds was Japan, while India was the largest exporter of the seed (FAO, 2012).

The seeds are oval in shape, somewhat flattened, and thinner at the eye (hilum) than the other end. The seed has a milligram weight of 20 to 40. The testa, or seed coat, is typically either ribbed or smooth (Oplinger *et al.,* 1990).

Sesamum indicum is well-known to produce some of the seeds with the highest oil content. It is a typical element in cuisines all around the world and has a deep nutty flavor (Oplinger *et al.,* 1990). It is a key source of vegetable oil for cooking in Nigeria and is used as a soup ingredient (Bedigan, 2006). Sesamum indicum, which is used in animal nutrition, is advantageous in the preparation of foods such halaweh, java beans, and bennimix in Sierra Leone (Namiki, 1995; Abou-Gharbia *et al.,* 2000; Kanu *et al.,* 2007).

Although it is usually used as a component of soup and is a significant source of edible/cooking oil, there are other alternate uses for it that are advantageous to both people and animals. The

seeds are sprinkled on top of hamburger buns and added to breads like bagels. While whole seeds are present in many salads and baked snacks, they can also be baked into crackers, frequently in stick form (Nweke *et al.,* 2011).

Sesame seeds can be used raw or roasted in a variety of cuisine recipes (Alegbejo *et al.,* 2003). Roasted seeds are used in soups or can be made into candy by combining them with caramelized sugar. Margarine and other complex cooking fats are made with sesame oil (Bedigian, 2007). Additionally, it serves as a synergist for insecticides based on pyrethrin as well as a transporter for medications and fragrances (Bedigian, 2007).

Poor grades are used in the production of soaps, paints, lubricants and lamp oil. Sesame cake is an excellent livestock feed and a raw material for several foodstuff (Bedigian, 2003). The oil is used to treat cough and ear ache. Sesame seeds are valued for their laxative effect (Alegbejo *et al.,* 2003; Bedigian, 2003; Bedigian, 2007).

2.1.1 Scientific Classification of *Sesamum indicum*

Kingdom	*Plantae*
Class	*Tracheophytes*
Sub class	*Angiosperms*
Order	*Lamiales*
Family	*Pedaliaceae*
Genus	*Sesamum*
Species	*S. indicum*
Binomial name	*Sesamum indicum*

Fig 2.1 *Sesamum indicum* Seeds

2.1.2 Plant Habitat and Characteristics of *Sesamum indicum*

Sesamum indicum, a member of the Pedaliaceae family of plants, is an annual shrub with branches or no branches and white bell-shaped blooms with hints of blue, red, or yellow (Martin & Leonard, 1964). It is grown for the purpose of producing seeds that are high in oil content. It is available in a variety of hues, from milky white to charcoal black.

Sesame appears to be appreciated more highly in the West and Middle East than in the Far East, where the darker types are favored. Tropical, subtropical, and southern temperate regions of the earth, particularly in Africa, China, South America, and India, are home to sesame. It is extremely important to the economy and is mainly cultivated by small farmers in underdeveloped nations.

Tropical climes, sandy, well-drained soil, hot weather, and moderate rainfall are ideal for the plant's growth. It can take up to four months for the seeds to fully ripen after being sown in the spring. It is an annual tropical herbaceous plant that reaches a height of around 1-2 m. The plant's leaves range in shape from oblong to lanceolate and have hairs on both sides. Its odor

is reputed to be disagreeable. The foxglove-like, purple to pale flowers are followed by 3 cm capsules or fruits that contain many of seeds (McCormick, 2001).

Each plant produces between 15 and 20 fruits, each of which has between 70 and 100 seeds. It takes between 80 and 180 days for it to reach maturity, at which point the stems are cut and hung upside-down to allow the ripe seeds to fall out and be collected on mats. With an annual yield of over four billion pounds worldwide, mechanical harvesting is also used.

2.1.3 Nutritional profile of *Sesamum indicum*

In hot climes, the very stable sesamum indicum oil hardly ever becomes rancid. According to Toma and Tabekhia (1979), it contains a high amount of unsaturated fatty acids with a composition of 14% saturated, 39% mono-unsaturated, and 46% poly-unsaturated fatty acids. Sesame seeds contain dietary fiber as well as 3.2% glucose, 2.6% fructose, and 0.2% sucrose as their main sources of carbohydrates. Additionally, they have positive physiological effects that have been demonstrated in both humans and experimental animals, such as antioxidant activity, blood pressure lowering potential, and serum cholesterol lowering potential (Sirato-Yasumoto *et al.*, 2001).

About 95% of the primary protein component (globulin) in sesame is made up of 13S globulin, which is simple, salt soluble, very vulnerable to heat denaturation, and resembles soybean 11S globulin in subunit structure but is more hydrophobic. The last characteristic shows the necessity to alter the functioning of sesame proteins before they can be used in the processing of dairy products. This trait restricts the use of sesame proteins in specific food formulation, particularly in fluids and beverages. Sesame is well known for being low in lysine and high in sulfur-containing amino acids. According to Kapadia et al. (2002), it also includes 5% phytate and 2.5 oxalic acid.

Oxalic acid can be found in the hulls; however, decortication can remove the majority of it. Decorticated sesame seeds are made up of 45–63% oil, 19–31% proteins, 14%–15% carbs, and 3%–3% ash. Sesame meal lacks anti-tryptic chemicals in contrast to several oilseeds.

Sesame oil is extremely high in polyunsaturated fat, which is used to make margarine and cooking oils. Sesamin and sesamolin, two distinct chemicals, make up its composition. Both substances, which are lignans, have been demonstrated to lower cholesterol in people (Ogawa *et al.,* 1995; Hirata *et al.,* 1996), to lower blood pressure, and to raise vitamin E levels in animals (Yamashita *et al.,* 2002; Kamal-Eldin *et al.,* 1995).

Sesame seeds are a great source of calcium and copper. Phosphorus, iron, magnesium, manganese, zinc, and vitamin B1 are also abundant in it. Chlorosesamone (2-chloro-5, 8-dihydroxy-3-3methyl-2-butenyl)-1, 4-naphthoquinone), a chlorinated red naphthoquinone pigment with antifungal properties, has been discovered in sesame root (Hasan *et al.,* 2000).

Anthrasesamones A, B, and C, three anthraquinones, were identified from the sesame root in a different study (Furumoto *et al.,* 2003). In higher plants, anthrasesamone C, an uncommon chlorinated anthraquinone, is present. According to Phillipa et al. (2005), sesame seeds have a greater total phytosterol concentration than Brazil and English walnuts (113 mg and 95 mg/100 g, respectively). 74.0% of the daily value (DV) for copper, 31.6% of the DV for magnesium, and 35.1% of the DV for calcium are all found in just a quarter cup of sesame seeds. This diverse mineral composition translates into a wide range of therapeutic qualities.

2.1.4 Medicinal Properties of *Sesamum indicum* and Health Issues

Numerous medical benefits of sesame seeds have been discovered. Sesame oil has been used to treat wounds for hundreds of years because it is emollient, demulcent, and moderately laxative. According to Anilakumar et al. (2010), the oil has natural antibacterial activities against Staphylococcus and Streptococcus, two common skin pathogens, as well as anti-fungal,

anti-viral, and anti-inflammatory actions (on athlete's foot fungus). Malignant melanoma *in vitro* and the growth of human colon cancer cells have both been shown to be inhibited by sesame oil (Smith & Salerno, 1992). According to research by Sirato-Yasumoto et al. (2001), the oil also demonstrates antioxidant action and has been shown to raise good cholesterol (HDL) and lower bad cholesterol (LDL).

According to Anilakuma et al. (2010), the bioactive component sesamin, which is generated from sesame, has been shown to shield the liver from oxidative damage. By lowering the liver's lipogenic enzymes, it also promotes fat oxidation and lowers lipogenesis (Ide *et al.,* 2003). Sesame seeds and other foods high in zinc should be a regular part of an older man's healthy diet in order to help maintain bone mineral density and prevent osteoporosis (Hyun *et al.,* 2004).

Natural supplies of phytosterols, fiber that protects the heart, minerals, and good fats are abundant in sesame seeds. English walnuts and Brazilian walnuts have the lowest total phytosterol contents (113 mg/100g and 95mg/100g, respectively), while sesame seeds have the greatest (400–413 mg/100g). According to Anilakumar et al. (2010), phytosterols are thought to lower cholesterol levels in the blood, improve immunological function, and lower the risk of several malignancies.

In Iran and other East Asian nations, sesame seed and oil are among the medicinal plants that have long been valued as traditional health foods (Roghani *et al.,* 2013). In Iran, particularly in the provinces of Yazd and Khuzestan, a respectable number of sesame seeds are grown and used. More than 90% of the grain produced in Iran is utilized to produce oil and sesame butter, also known as "Ardeh" there.

One of the natural sesame seed products, ardeh can be made by grinding the whole sesame seed and contains no additives, either chemical or nonchemical. Both sesame oil and sesame butter

contain sizable amounts of mono- and polyunsaturated fatty acids, as well as vitamin E. According to Sankar et al. (2011), sesame also contains sesamin, sesamol, episesamin, and sesamolin. About 50% of sesame seeds' calories come from oil, while 20% come from protein.

According to Khaneshi et al. (2013), oleic acid (poly unsaturated) makes up 43% of the sesame oil, followed by linoleic acid (poly unsaturated) at 35%, palmitic acid (saturated) at 11%, and stearic acid (monosaturated) at 7%.

Sesame oil is emollient, demulcent, and moderately laxative. Additionally used as a poultice are its seeds and young leaves. There are numerous medical and pharmaceutical uses for the oil. Sesamin has been discovered to defend the liver from oxidative harm. The oil has been used for treating wounds for a long time. For common skin pathogens like Staphylococcus and Streptococcus as well as common skin fungi like athlete's foot fungus, it is naturally anti-bacterial. It is also anti-inflammatory and antiviral. The oil has been utilized in recent studies by Ayurvedic doctors in Holland to treat a number of chronic conditions, including hepatitis, diabetes, and migraines. The acetic acid-induced writhing model in mice was used to assess the ethanolic extract of Sesamum indicum's analgesic efficacy (Nahar, 2009).

According to a study, using sesame oil as mouthwash reduced the number of bacteria that causes gingivitis by 85%. Sesame oil has successfully treated chronic sinusitis when used as nose drops and inhaled back into the sinuses. It eliminates Streptococcus and other common cold bacteria when gargled down the throat. It benefits those with psoriasis and dry skin conditions. It serves as a helpful natural UV shield.

After being exposed to the sun or wind, sesame oil is applied to soothe burns. In order to control dry scalp dandruff and eliminate the bacteria that cause it, it feeds and nourishes the scalp. It has been used to eliminate lice infestations in children's hair with success. It guards against the damaging effects of chlorine in swimming pool water on the skin. Sesame oil can help

neutralize the flow of oxygen radicals that radiation therapies inevitably produce when administered before to and following radiation treatments (Cooney *et al.,* 2001).

Sesame seed oil is used on newborn skin to prevent dermatitis brought on by the acidity of bodily wastes, especially in the diaper-covered areas. It offers defense against typical skin infections in the nose and ears. The oil swabbed in the nose provides protection against airborne viruses and bacteria for pupils who are around other students who have colds and the sniffles (Morris, 2002).

Peroxisome Proliferator Activator Receptor Alpha (PPAR alpha) is a receptor in the body that is activated by the phytochemical sesamin, which is contained in the seed. PPAR alpha plays a role in the regulation of lipid metabolism, specifically the transcription of genes involved in the â-oxidation of fatty acids and lipogenesis. It is abundantly expressed in muscle, liver, kidneys, and the heart. Fatty acid oxidation enzyme gene expression is increased when PPAR alpha is activated, but lipogenic enzyme gene expression is decreased. Sesamin, in other words, speeds up the body's ability to burn fat while reducing the amount of fat that is stored there (Penalvo *et al.,* 2006).

Additionally, this substance can stop the rise in serum triacylglycerol levels that occurs in rats after consuming ethanol (Akimoto, 1993). The liver and skeletal muscle cells' mitochondria and peroxisomes are where fat is mostly metabolized. By boosting the expression levels of the enzymes involved in the â-oxidation of fatty acids, sesamin's activation of PPAR alpha promotes fat oxidation in these organelles (Sirato-Yasumoto *et al.,* 2001).

Sesamin has been demonstrated to reduce the lipogenic gene expression of acetyl-CoA carboxylase, fatty acid synthase, and sterol regulatory element binding protein-1 (SREBP-1) in the liver, which results in less fat esterification and consequently decreased fat production (Ide *et al.,* 2003). When fatty acid oxidation rises to a level where the liver cannot digest all the

fatty acids for energy, ketogenesis takes place. Ketone bodies are created in the liver from extra acetyl-CoA (produced through the catabolism of fat, glucose, and amino acids) and released into the bloodstream for utilization by other tissues, including the brain.

Because the brain primarily uses glucose as fuel on low-carb diets, ketogenesis is a crucial function. The brain will turn to ketone bodies for energy when its glucose levels are low. Ketone body synthesis has been demonstrated to be increased by sesamin (Fukuda, 1998).

2.2 Diabetes

Diabetes, which is most commonly used to refer to diabetes mellitus, is loosely translated as having too much sugar in your urine (glycosuria). Diabetes also refers to a number of rare diseases. The most typical of them is diabetes insipidus (without taste), which causes polyuria, or the production of a lot of urine. According to Shiferaw and Ayalew (2018), there are two forms of diabetes: diabetes mellitus and diabetes insipidus.

2.2.1 Diabetes Mellitus

Diabetes mellitus is a metabolic disorder that develops either when the pancreas produces insufficient amounts of the hormone insulin (which controls blood sugar) or when the body has trouble using the insulin that is generated. Chronic hyperglycemia, which impairs function or seriously harms body organs such the liver, kidneys, heart, nerves, and blood vessels, is mostly caused by defects in insulin secretion (WHO, 2009). According to Kantarova et al. (2006), it is characterized by glucose intolerance and fasting hyperglycemia.

Diabetes mellitus is characterized by excessive thirst, frequent urination, weight loss or gain, lethargy, and flu-like symptoms. Early diabetes symptoms can be extremely subtle and frequently go unnoticed. One of the common metabolic diseases, diabetes mellitus involves both micro and macro vascular consequences that significantly increase morbidity and death. It is regarded as one of the top five killers worldwide (Bhupesh *et al.*, 2008).

Records indicate that a sizable fraction of the population worldwide has diabetes mellitus. According to epidemiological data, 2.8% of people worldwide had diabetes in 2000, and that number may rise to 4.4% by 2030. It affected persons of all ages and from all ethnicities (Xing *et al.,* 2022).

According to statistical study, there will be 57 million diabetics in India in 2025, up from 15 million in 1995 (Shikarwar & Patil, 2010). Additionally, many flaws in the patho-physiology of diabetes contribute to morbidity and mortality (Ivorra *et al.,* 1989).

In order to cure or manage diabetes with its various pharmacologic activities, research is currently focused on food, traditional medicinal plants, and herbs (Chandramohan *et al.*, 2008). Although numerous phytoconstituents with anti-diabetic action have been extracted and analyzed from numerous medicinal plants, scientists have continued to study medicinal plants in an effort to find anti-diabetic medications or leads for the medical community.

2.2.1.1 History of Diabetes Mellitus.

Aretaeus of Cappadocia created the word diabetes from the Greek verb (diabainein). He created it by combining the verb "bainein," which means to walk or stand, with the prefix "dia," which means across or apart. The word diabetes, derived from the verb diabeinein, which meant "to stride, walk, or stand with legs asunder," meant "one that straddles," or more specifically, "a compass, siphon." The sense "siphon" led to the use of diabetes as the name for a disease involving the release of excessive amounts of urine (Polonsky, 2012).

The term "diabetes" was first used in English in a 1425-era medical treatise. The Latin word mellitus, which translates to "honey" in English, was introduced by Thomas Willis in 1675 to describe the sweet taste of the urine. Ancient Greeks, Chinese, Egyptians, Indians, and Persians had all noted this sweet taste in pee. Matthew Dobson established in 1776 that the sweetness

of the urine and blood of diabetics was due to an overabundance of a particular type of sugar (Sanders, 2012).

2.2.1.2 Types of Diabetes Mellitus.

2.2.1.2.1 Type 1 Diabetes Mellitus: Other names for this kind of diabetes include juvenile onset diabetes (JOD) and insulin dependent diabetes mellitus (IDDM). In this case, the pancreas either fails to generate insulin or only produces it in very little amounts. Symptoms typically strike people under the age of 20 all of a sudden. The majority of instances happen between puberty (10–12 years for girls and 12–14 years for guys).

An autoimmune condition known as IDDM occurs when the body's natural defenses mistakenly target healthy tissues. It is thought that the islet cells are mistakenly classified as foreign objects by the body's auto immunological defense system, leading to their destruction. These islet cells are the -cells, or insulin-producing cells, found in the pancreas.

Viruses and a mix of genetic and environmental factors may cause IDDM. It may also develop from the pancreas being surgically removed. Since this type of diabetes mellitus can soon become lethal, it requires prompt treatment with diet and insulin injections. If untreated, IDDM not only contributes to the accumulation of glucose in the blood but may also have an impact on how the body burns fat.

As a result of the body's inability to convert glucose into energy, body fat is broken down as a substitute energy source, which causes an increase in the number of ketone bodies to be produced in the blood and interfere with cellular respiration, which can cause comas (Maruf, 2017).

2.2.1.2.2 Type 2 Diabetes Mellitus: Non-insulin dependent diabetes mellitus and adult-onset diabetes are other names for this kind of diabetes mellitus. It typically affects those over 40 and advances gradually. The body's delicate balance between insulin goes haywire in this situation

even if the pancreas is still producing insulin and it is not promoting the glucose uptake in muscles and tissues needed for energy. As a result, glucose accumulates in the blood and urine (Mathers & Loncar, 2016).

Non-insulin dependent diabetes mellitus is typically a hereditary condition, despite the fact that the exact source of this dysfunction is unknown. The increased incidence of this condition may be influenced by other risk factors like obesity and particular lifestyle choices. Type 2 diabetes symptoms in industrialized nations include recurrent infections or skin sores that heal slowly or not at all, as well as generalized fatigue and limb numbness. Tablets that lower blood glucose levels are frequently effective at controlling non-insulin dependent diabetes mellitus (Mathers & Loncar, 2016).

2.2.1.2.3 Gestational Diabetes: In numerous ways, gestational diabetes mellitus (GDM), which involves a combination of relatively insufficient insulin secretion and responsiveness, resembles type 2 diabetes. It can get better or go away after delivery and affects 2% to 5% of all pregnancies. Although it is completely manageable, gestational diabetes must be closely monitored by a doctor the entire time.

2.2.1.3 Diagnosis, Treatment and Management of Diabetes Mellitus.

Diabetes mellitus currently has no known treatment. The goal of treatment is to maintain euglycemia, or blood sugar levels that are as close to normal as possible without endangering the patient. After a person has fasted for roughly eight hours, the level of glucose in the blood is measured to determine if they have diabetes.

A glucose tolerance test, which compares blood glucose levels before and after a certain amount of sugar is consumed, is typically used by doctors to diagnose diabetes. Controlling blood glucose levels is the mainstay of treatment for diabetes once it has been identified in order to avoid complications. This can be achieved with consistent exercise, a properly

monitored diet, and medication, depending on the type of diabetes. To give the body the hormone it does not make, people with Type 1 diabetes need to take insulin (two to four times per day).

It can't be taken orally since it gets destroyed in the digestive system. Therefore, historically, insulin-dependent diabetics have injected the medication using a hypodermic needle or a pump the size of a beeper attached to a needle implanted under the skin.

An insulin formulation that can be breathed and is subsequently absorbed by blood in the lungs was given the go-ahead by the US Food and Drug Administration in 2006. An individual's level of physical activity, diet, and the presence of other medical conditions can all have an impact on how much insulin they require. People with Type 1 diabetes typically use a meter multiple times per day to check the glucose level in a drop of blood they acquire by pricking a fingertip. To keep the blood sugar at a normal level, they can then modify the insulin dosage, the amount of activity, or the amount of food consumed.

People with Type 1 diabetes must carefully manage their diets by spacing out meals and snacks throughout the day in order to prevent the supply of insulin from being insufficient to support cell absorption of glucose. Additionally, they must consume foods rich in complex sugars, which digest slowly and result in a slower rise in blood sugar levels.

For convenience and speed of therapy, patients typically self-administer insulin by injection or using automatic medication injectors that contain an insulin cartridge that may be carried in the pocket (Thanabalasingham & Owen, 2011). Although most people with Type 1 diabetes work to reduce their blood glucose levels, too low levels can also have negative health effects. For instance, taking too much insulin by a person with Type 1 diabetes can result in low blood sugar levels. Hypoglycemia may occur from this.

When hypoglycemia occurs, a person can treat their symptoms by taking glucose pills or eating high-sugar foods like fruit juices or hard candies. In addition to insulin, oral medicines may be used to treat type 2 diabetes. Treatment for Type 2 diabetes patients starts with diet control, exercise, and weight loss, albeit these measures may not be sufficient in the long run.

In order to create a diet plan that controls blood sugar levels so that they do not rise quickly after a meal, people with Type 2 diabetes frequently consult nutritionists. A recommended meal often has moderate protein (10% to 20% of total calories), low fat (30% or less of total calories), and a variety of carbs such beans, veggies, and grains. Self-oral drugs are unsuccessful; a person with Type 2 diabetes may require insulin doses or an oral medication and insulin combination. According to Santaguida et al. (2015), about 50% of people with Type 2 diabetes need oral drugs, 40% need insulin or a mix of insulin and oral meds, and 10% rely only on diet and exercise.

2.2.2 Hyperglycemia and Hypoglycemia.

Hyperglycemia is a disorder in which the blood sugar level excessively rises (up to four times the normal level) as a result of the body producing too little insulin or too much excess pituitary hormone. Although this condition is not fatal on its own, it is a sign of a serious illness (diabetes mellitus).

Patients with diabetes do not pass away from hyperglycemia, but if they do not receive insulin injections, they may pass away from other conditions like the build-up of toxins brought on by altered fat metabolism; a diabetic's body uses fat as a substitute for the sugar it cannot use.

A condition known as hypoglycemia or insulin shock occurs when an excessive amount of insulin is administered into the body, lowering the level of sugar to a risky low. Weakness, shakiness, uneasiness, anxiety, and fainting are some of the typical symptoms of hypoglycemia. Patients may also exhibit noticeable personality changes and exhibit signs of intoxication.

In people with diabetes mellitus, hyperinsulinism, an excess of insulin, or an excess of insulin produced by the body are the causes of hypoglycemia. Because glucose is converted to glycogen in the liver, muscles, and fat in the adipose tissues when hyperinsulinism develops, insulin is crucial for controlling carbohydrate metabolism (Cryer, 2012).

2.3 Alloxan

Friedrich Wohler and Justus von Liebig made the discovery of alloxan. It was first created in 1818 by Luigi Valentino Brugnatelli and is one of the earliest known organic compounds (Gaspare, 1818). Wohler and Liebig gave it its name in 1838 (Wohler and Liebig 1838). Combining the words "allantoin" with "Oxalsäure" (oxalic acid), "Alloxan" is the outcome. Alloxan, which is an organic molecule, a urea derivative, a carcinogen, and a cytotoxic glucose analog, is also known chemically as 5, 5-dihydroxyl pyrimidine-2, 4, 6-trione. The substance has a relative molecular mass of 142.06 and the chemical formula $C_4H_2N_2O_4$.

Among the well-known diabetogenic agents, which also include di-thizone, monosodium glutamate, gold thio-glucose, high fructose load, high glucose load, and anti-insulin serum, alloxan and streptozotocin (STZ) are the most frequently used in diabetes studies to evaluate the anti-diabetic potential of both pure compounds and plant extracts.

In the beginning, rabbits were used to demonstrate the alloxan model of diabetes (Dunn *et al.*, 1943). The purple dye murexide, which Carl Wilhelm Scheele discovered in 1776 as the result of a difficult in-situ multistep reaction between alloxantin and gaseous ammonia, was made using alloxan. Murexide is produced when the unisolated intermediate uramil condenses with the alloxan that is released during the reaction.

2.3.1 Chemical Structure of Alloxan

The 5-carbonyl group in alloxan's chemical structure is highly reactive with thiol groups, suggesting a structure-function relationship in alloxan toxicity or diabetogenicity (Lenzen *et*

al., 1992). Glucokinase contains two thiol groups (-SH) in its binding site, making it particularly vulnerable to oxidation by alloxan**.**

Alloxan's attachment to glucokinase causes the creation of a disulfide bond, which inactivates the enzyme. The quick occurrence of this process within the first minute of the enzyme's exposure to alloxan accounts for the selective suppression of glucose-stimulated insulin secretion that is typically observed within a few minutes of alloxan injection (Dunn *et al.,* 1943).

Although glucokinase is the most vulnerable thiol enzyme to alloxan attack in the beta cells, alloxan can also suppress the activity of numerous other functionally significant thiol enzymes (phosphofructokinase, aconitase, hexokinase, and calmodulin-dependent protein kinase). The same mechanism may likely be responsible for the inhibitory action of alloxan on insulin biosynthesis (Niki *et al.,* 1976), as it prevents glucose oxidation and, consequently, the production of ATP. Lack of ATP inhibits the signal-generating metabolic flux necessary for glucose-stimulated insulin secretion (Lenzen, 1988).

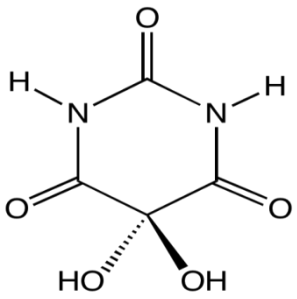


Fig 2.2: Structure of Alloxan

2.3.2 Biological Effects of Alloxan

Alloxan, a hydrophilic and chemically unstable molecule with a structure similar to glucose, is what causes the pancreatic beta cell to selectively take it up and accumulate it (Gorus *et al.,* 1982). Due to their comparable shapes, the beta cell's plasma membrane's glucose transporter (GLUT2) can move them into the cytosol (Elsner *et al.,* 2002). Additionally, the thiol group

reactivity of alloxan has been linked to its ability to selectively limit glucose-induced insulin production by inhibiting glucokinase.

This inhibitory feature is recognized as the principal pathophysiological effect of alloxan, which derives from the thiol group reactivity of alloxan. The thiol groups of glucokinase (glucose phosphorylating enzyme) are particularly vulnerable to oxidation by alloxan (Lenzen & Panten, 1988).

It is obvious that the inhibition of glucose-induced insulin secretion by alloxan is the result of the thiol reactivity of the glucokinase because alloxan inhibits many cellular functions at higher concentrations, including the ability to oxidize thiol groups of many functionally important enzymes like hexokinase, phosphofructokinase, calmodulin-dependent protein kinase, aconita.

The formation of cytotoxic ROS is the result of a cyclic reaction between alloxan and its reduction product, dialuric acid, which by auto-oxidation generates superoxide radicals, hydroxyl radicals, and H_2O_2, which may be attributed as the mechanism underlying the cytotoxic action of alloxan to insulin-producing cells (Lenzen & Panten, 1988).

2.3.3 Mechanism of Action of Alloxan.

Uncertainty surrounds the precise mechanism underlying alloxan's selective -cytotoxicity. Alloxan presumably has a harmful effect on -cells by preferentially interacting with specific plasma membrane elements, according to Coopertein Watkins and Lazarow in 1964. D-mannitol and insulin are two extracellular fluid markers that can diffuse into the surrounding incubation medium as a result of the increased permeability**.**

It is possible that alloxan interacts with the membranes of the cytoplasmic organelle in -cells, but the fact that only cell components of intermediate cells are affected by alloxan suggests that there is no comparable damage to their plasma membrane as occurs in the cell. These

mimics the remarkably selective identification and autophagy of these organelles in intermediate cells.

Alloxan is known to cause nephro-toxic changes as well as diabetic renal changes, but no ultra-structural investigation has been carried out to distinguish between the toxic and diabetic effects of alloxan on tubules and glomeruli (Lenzen, 2018). When given to mice, the poisonous glucose analogue alloxan selectively kills the insulin-producing cells in the pancreas and causes alloxan diabetes, a form of diabetes mellitus with features resembling type 1 diabetes in humans.

Alloxan preferentially accumulates in -cells through absorption via the GLUT2 glucose transporter, which makes it harmful only to insulin-producing pancreatic cells. Reactive oxygen species (ROS) are produced by alloxan in the presence of intracellular thiols in a cyclic reaction with its reduction product, dialuric acid. Free radicals produced in this redox reaction are what start Alloxan's harmful effect on -cells (Szkudelski, 2011).

2.4 The Liver

According to Smithus (2014), the liver is the largest glandular organ in the body and weighs between 1-36 kg (31b, or 1/50 of the body weight) (Guyton & Hall, 2000). It functions as both the primary chemical factory and a significant food storage facility. The liver is separated into four lobes, each of which is different in size, shape, and color (Lieber, 2000).

2.4.1 The Structure of The Liver

It is located below the diaphragm on the right side of the abdominal cavity (Smithus, 2014). The hepatic artery and the portal vein are the two major blood arteries that supply the liver (Wright, 1993). The hepatic artery transports oxygen-rich blood from the heart's aorta. Food that has been digested leaves the small intestine through the portal vein (Wright, 1993).

The liver contains several subdivided blood arteries that end in tiny capillaries. A lobule is reached by each capillary. Hepatocytes, the primary metabolic cells of the liver, make up each of the thousands of lobules that make up the liver tissue (Cabot *et al.*, 2003).

The common hepatic duct, which opens alongside the pancreatic duct in the hollow side of the duodenum, is made up of two ducts that start in the liver. Bile, which is created by the liver cells and is stored in the gall bladder, which is located inside the liver (Slu, 2014).

The liver's right lobe is bigger than its left lobe. Each lobe is further divided into numerous smaller lobes that are each roughly the size of a pinhead, contain numerous liver cells, and are connected by bile ducts. A network of lymph, bile, and blood capillaries runs throughout the entire liver structure. Bile is secreted by the liver cell and accumulates in bile capillaries before joining together to form bile ducts (Slu, 2014).

2.4.2 Functions of the Liver

The liver serves a variety of purposes (Hbooks, 2014). The following processes are used to carry out these functions:

i. Organic metabolism
ii. Lipid metabolism
iii. Digestive function
iv. Carbohydrate metabolism
v. Protein metabolism
vi. Endocrine function
vii. Clotting function,
viii. Excretory and degradative function,

2.4.3 Disease of the Liver

The liver serves a variety of essential purposes. When the liver quits functioning, death occurs. Doctors may be able to transplant a healthy liver from a deceased donor into some patients

with advanced liver disease (Gentilini *et al.,* 1990). There are numerous ways that liver disease might present itself. The following list of liver disease symptoms is extremely significant:

i. Jaundice (a yellowish discoloration of the skin and whites of the eyes)
ii. Cholestasis (reduction or stoppage of bile flow),
iii. Cirrhosis liver enlargement,
iv. portal hypertension (abnormally high blood pressure in the veins that brings blood flow from the intestine to the liver),
v. ascites (accumulation of fluid in the abdominal cavity),
vi. Hepatic encephalopathy (a liver disorder in which toxins build up in the blood leading to brain dysfunction and liver failure) (Mumoli *et al.*, 2006).

2.4.4 Mechanism of Liver Damage

The liver is vulnerable to damage from drugs and other chemicals because of its metabolism and close connection to the gastro intestinal tracts. Portal veins, which carry medicines and xenobiotics in concentrated form, are responsible for bringing 75% of the blood to the liver straight from the spleen and gastro intestinal tract.

There are numerous methods that either cause hepatic injury or exacerbate the damaged mitochondria. Its dysfunction causes an excessive number of oxidants to be released, which damages the hepatic cells. The buildup of bile acids inside the liver as a result of injury to hepatocytes and bile duct cells encourages additional liver damage (Patel *et al.,* 1998).

Kupffer cells, stellate cells that store fat, and leukocytes are examples of non-parenchyma cells that play a part. Chemicals cause a wide range of clinical and pathological liver injuries, depending on the pattern of injury.

ALT, ALP, and AST are three biochemical markers that are frequently used to detect liver disease. Therefore, a rise in either ALT levels greater than three times the upper limit of normal or ALP levels greater than twice normal level when combined with elevated ALT or ALP is regarded as liver damage. (2006) Mumoli et al.; Benichou (1990).

2.4.5 Liver Function Test (LFT)

In order to provide information regarding the condition of a patient's liver, the liver function test (LFT) set of clinical biochemical laboratory blood assays was developed (Lee, 2009). Prothrombin time, albumin, AST, ALP, and other markers are assessed. When a patient has some degree of intact liver function, liver transaminases (AST and ALT) are helpful indicators of liver injury (Johnston, 1999).

Although the majority of liver diseases first manifest only moderate symptoms, it is crucial that they are caught early. The results of various tests are related to cellular integrity (transaminase), functionality (albumin), and diseases of the biliary system (gamma-glutamyl transferase and ALP).

2.4.5.1 Relevance of Liver Function Test

The assessment and treatment of patients with hepatic dysfunction benefit from a variety of biochemical testing. These tests can be used to:

- Identify the presence of liver illness;
- Differentiate between various liver disorders;
- Calculate the degree of known liver damage;
- Monitor the effectiveness of treatment.

In order to make sure that particular medications (anticonvulsants are a good example) are not harming the person's liver, some or all of these measurements are also performed on those

individuals taking certain medications (often twice a year for routine instances) (Nic *et al.,* 2014).

2.4.5.2 Aspartate Transaminase (AST)

An essential enzyme in the metabolism of amino acids is aspartate transaminase, also known as serum glutamic oxaloacetic transaminase (SGOT). It is a pyridoxal phosphate (PLP) dependent transaminase enzyme that catalyzes the reversible transfer of an alpha amino group from aspartate to glutamate.

AST is a marker for liver health that is found in the liver, heart, skeletal muscles, kidney, brain, and RBC. It is frequently evaluated clinically. The interconversion of aspartate and alpha ketoglutarate to oxaloacetate and glutamate is catalyzed by aspartate transaminase.

Aspartate (ASP) + α- ketoglutarate $\xleftrightarrow{\text{AST}}$ oxaloacetate + glutamate (GLU)

In order to transfer the amino group from aspartate or glutamate to the appropriate keto-acid, AST, a prototype transaminase, needs PLP as a cofactor. The cofactor switches back and forth between PLP and the pyridoxamine phosphate (PMP) form during the process (Kirsh *et al.,* 1984).

Following the conversion of alpha ketoglutarate to glutamate, which then proceeds through oxidative deamination to generate ammonium ions excreted as urea, this enzyme is essential in the breakdown of amino acids. Oxaloacetate, a crucial step in the citric acid cycle, can be used to produce aspartate in the opposite process (Berg *et al.,* 2006).

2.4.5.2.1 Clinical Significance of Aspartate Transaminase (AST)

AST like alanine transaminase (ALT) is associated with liver parenchyma cells. The difference is that alanine transaminase (ALT) is found predominantly in the liver, with clinically negligible quantities found in the kidneys, heart, and muscles, while AST is found in the liver, heart (cardiac muscles), skeletal muscles, kidneys, brain and RBC.

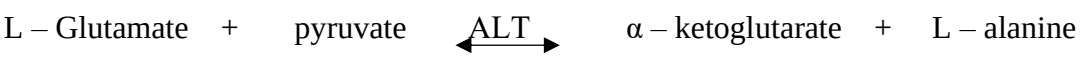
Thus, compared to AST, ALT is a more accurate biomarker of hepatic inflammation. Myocardial infarction, acute pancreatitis, acute hemolytic anemia, severe burns, acute renal disease, muscle skeletal disease, and trauma are a few conditions that might cause an increase in AST. In 1954, AST was established as a biochemical marker for the detection of acute myocardial infarction; however, cardiac troponins have since supplanted AST as the preferred biomarker for this diagnosis (Gaze, 2007).

AST is commonly measured clinically as a part of diagnostic liver function tests, to determine liver health. Laboratory tests should always be interpreted using the reference range from the laboratory that performed the test.

2.4.5.3 Alanine Transaminase (ALT)

Alanine transaminase is a transaminase enzyme also known as serum glutamic pyruvic transaminase (SGPT) or alanine amino transferase (ALAT). It is found in plasma and in various bodily tissues but is most commonly associated with the liver. It catalyzes the two parts of alanine cycle (Nyblom, 2006).

It catalyzes the transfer of an amino group from L-alanine to alpha ketoglutarate, with the products of this reversible transamination reaction being pyruvate and L-glutamate.



2.4.5.3.1 Clinical Significance of Alanine Transaminase (ALT)

It is frequently assessed clinically to assess liver health as part of a diagnostic examination of hepatocellular damage. utilized for diagnostic purposes. According to Ghouri et al. (2010), it is generally always expressed in international units/liter. The typical reference range for experimental investigations is 10–40 U/L, despite occasional variations in particular reference range values for patients (Wang *et al.*, 2012).

A noticeable diurnal variation can be seen in alanine transaminase. The reference ranges from the laboratory that produced the result should always be used to interpret test results. Significantly, increased ALT levels frequently point to underlying health issues such viral hepatitis, diabetes, congestive heart failure, liver damage, bile duct obstruction, or myopathy. Because of this, ALT is frequently used to check for liver issues.

An inadequate intake of choline in the diet might also result in elevated ALT. Elevated ALT levels, however, may not always indicate the presence of health issues. ALT levels fluctuate throughout the course of the day as is typical, and they can rise in reaction to vigorous activity (Paul & Giborney, 2000).

Measuring additional enzymes can help narrow down the potential underlying reason when excessive levels are discovered in the blood. Alkaline phosphate measurements, for instance, can be used to distinguish between biliary duct issues and excessive ALT levels caused by liver cell destruction.

Creatine kinase enzyme measurements can also be used to rule out ALT levels associated with myopathy. Numerous medications, including antibiotics and anti-inflammatory medicines, can raise ALT levels. For many years, the American Red Cross deferred blood donations from donors who had increased ALT levels as part of the battery of tests to guarantee the security of its blood supply.

Because there was no particular test available for hepatitis C at the time, the goal was to identify donors who might be infected (Paul *et al.,* 2000).

2.4.5.4 Alkaline Phosphatase (ALP)

Dephosphorylation, also known as alkaline phosphatase dephosphorylation, is the process of removing the phosphate group from a variety of compounds, including nucleotides, proteins, and alkaloids. Alkaline phosphatase works well in an alkaline environment, as its name

suggests. According to Tamas et al. (2002), it is occasionally used synonymously with basic phosphatase.

Alkaline phosphatase is typically used in the laboratory to avoid self-ligation by eliminating phosphate monoester (Maxam & Gilbert, 1980).

Since DNA typically comprises phosphate groups on the 5' end, alkaline phosphatase has emerged as a useful tool in molecular biology labs. By removing these phosphates, the DNA is kept linear until the subsequent stage of the process for which it is being prepared. Ligation is the mechanism by which the 5' end of the DNA attaches to the 3' end. In order to measure the presence of labelled DNA through subsequent phases in the procedure or experiment, the phosphate group must be removed to enable radio labeling (replacement with radioactive phosphate groups) (Maxam *et al.*, 1980).

Alkaline phosphatase from shrimp is the most practical for this function because, once it has completed its task, it is the simplest to deactivate. Alkaline phosphatase is also utilized in enzyme immunoassays as a label. In the dairy business, it is frequently used as a sign that cow's milk has been pasteurized.

The color of a Para-Nitro phenyl phosphate substrate in a buffered solution can be used to detect this molecule, which is denatured by the high temperature encountered during pasteurization (Ashaffenburg & Mullen, 1949). Cow milk would normally become yellowish within a few minutes, whereas properly pasteurized milk should show no change. However, there are certain exceptions, such as when some bacteria create heat-stable alkaline phosphatase.

2.4.5.4.1 Clinical Significant of ALP

Adults typically have ALP levels between 20 to 140 IU/L; however, children and pregnant women have much greater amounts. Always use the reference range provided by the lab that

conducted the test when interpreting results from blood testing. The bile ducts can get blocked, which might result in high ALP levels.

ALP is a consequence of osteoblast activity, which occurs when there is active bone growth, like in Paget's disease of the bones. Additionally, there is an increase in those who have celiac disease but are untreated (Preussner & Harold, 1998). Less frequently than excessive levels, lower levels of ALP are seen. The cause of the increased alkaline phosphatase is unknown.

Reduced levels of ALP may be caused by hypophosphatasia, starvation, magnesium shortage, hypothyroidism, pernicious anemia, and Wilson disease, among other disorders or diseases. Oral contraceptives are another group of medications that have been shown to lower alkaline phosphatase (Schiele *et al.,* 1998).

2.4.5.5 Albumin

Serum albumin is the most prevalent member of the family of globular proteins known as albumins (derived from the Latin albumen (egg) white; dried egg white). All proteins that are water soluble, somewhat soluble in concentrated salt solution, and susceptible to heat denaturation are members of the albumin family. The blood plasma frequently contains albumins, which differ from other blood proteins in that they are not glycosylated.

Serum albumin, alpha fetoprotein, vitamin D binding protein, and afamin are a few blood transport proteins that have evolutionary connections (Haefliger *et al.,* 1989).

The primary protein in human blood plasma is albumin (Farrugia, 2010). Water, cations (including Ca2+, Na+, and K+), fatty acids, hormones, bilirubin, thyroxin (T4), and drugs (including barbiturates) are among the substances it binds. Its primary job is to control the blood's colloidal osmotic pressure. A fetal plasma protein called alpha fetoprotein (also known as alpha fetoglobulin) binds different cations, fatty acids, and bilirubin. Vitamin D binding

protein binds to fatty acids as well as vitamin D and its metabolites. Alpha albumin's biological function has not yet been established (Farrugia, 2010).

2.4.5.5.1 Clinical Significant of Albumin

There is no proof that albumin lowers mortality in individuals with low blood volume when compared to less expensive options such as normal saline or that albumin lowers mortality in patients with burns and low albumin levels. As a result, the Cochrane group advises against using it outside of clinical studies. Albumin is occasionally employed as a surfactant in acoustic droplet vaporization (ADV). Through the use of occlusion therapy, ADV has been suggested as a cancer treatment (Lo, 2007).

2.4.5.6 Bilirubin

This is the primary bile pigment in humans, and when it is increased, it produces jaundice, a yellow coloring of the skin and eyes. The RBC's heme component is mostly broken down to produce bilirubin. The liver absorbs it during blood flow, passes it through the bile ducts, and then secretes it into the bile. Less than 1.2 mg/dl of bilirubin circulates in the blood of healthy persons.

The breakdown of RBC, for example, increases the synthesis of bilirubin and reduces its clearance from the blood stream. Jaundice is typically visible at levels higher than 3 mg/dl. The bilirubin is very non-specific and may be increased in a variety of liver or biliary tract diseases. However, as blood bilirubin represents the liver's capacity to absorb, process, and produce bilirubin into the bile, it is usually regarded as a genuine test of LFT (Preussner & Harold, 1998).

2.4.5.7 Total Protein

Including clotting factors, this is a quantitative assessment of the concentration of all serum proteins. Both albumin and immunoglobulin, primarily IgA, IgM, and IgG, are important

proteins. Numerous more proteins are counted in the measurement, but none of them alone makes up more than 50% of the whole. Both urine and cerebrospinal fluid can be tested for total protein (Jaypee, 2012).

2.4.5.7.1 Clinical Significant of Total Protein

Since total protein minus albumin equals globulin, whose main component is immunoglobulin, total protein is measured in serum to provide an indication of the total immunoglobulin concentration. The LFT occasionally includes total protein. A decrease in albumin may counteract the rise in total protein caused by several chronic liver diseases by decreasing immunoglobulin.

Total protein may indicate that the chronic liver disease has an auto immune component. Therefore, IgM, IgG, and IgA levels are typically elevated in primary biliary cirrhosis, auto immune chronic hepatitis, and alcoholic liver disease, respectively. Patients with humoral immune deficiencies may have low serum levels, but as IgG is the main component, a lack of either IgA or IgM may not have a substantial impact on total protein (Jaypee, 2012). Every immunoglobulin class should be measured if immunodeficiency is suspected.

2.5 Lipid Profile Test

A collection of medical tests known as "lipid profile tests" are used as a preliminary screening method for lipid problems. Triglycerides, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), and occasionally very low-density lipoprotein (VLDL) are all included in the test (Garrett, 2002).

2.5.1 Triglycerides

Esters of glycerol and three fatty acids constitute what are known as triglycerides. Triglycerides serve as both energy sources and carriers for dietary fat in the metabolism (Elias, 1983). It aids in the bidirectional transference of adipose fat and blood from the liver as a blood lipid.

Glycerol and three different fatty acid molecules are combined to create triglycerides. High triglyceride levels in the blood have been associated with atherosclerosis in humans, which increases the risk of heart disease and stroke. A significant negative association between triglyceride level and HDL cholesterol can help explain some of the danger (Robert *et al.*, 2010).

2.5.2 High Density Lipoprotein (HDL)

One of the main lipoprotein subgroups that facilitates the movement of lipids like cholesterol and triglycerides through the water-based blood stream is HDL. HDL carries roughly 30% of blood cholesterol in healthy people. Because HDL particles can remove cholesterol from inside arteries and carry it back to the liver for excretion or neutralization, HDL is frequently referred to as "good cholesterol" (Donovan *et al.*, 2001).

High concentrations of HDL (above 60 mg/dl), according to epidemiological research, are protective against cardiovascular disease. Low HDL cholesterol levels are a well-established independent risk factor for cardiovascular disease (Donovan *et al.*, 2001).

2.5.3 Cholesterol

The liver or intestines manufacture cholesterol, a waxy steroid of fat (Emma, 2009). It is a crucial part of membranes and an amphipathic lipid. It serves as the starting point for the synthesis of all other steroids in the body, as well as important hormones such adrenocorticol and the sex hormone, D vitamins, and bile acids (Robert *et al.*, 2010). The liver or intestines manufacture cholesterol, a waxy steroid of fat (Emma, 2009). It is a crucial part of membranes and an amphipathic lipid. It serves as the starting point for the synthesis of all other steroids in the body, as well as important hormones such adrenocorticol and the sex hormone, D vitamins, and bile acids (Robert *et al.*, 2010).

2.5.4 Low Density Lipoprotein (LDL)

One of the five main lipoprotein groups is low density lipoprotein. It is sometimes referred to as bad cholesterol due to its ability to carry fat molecules into artery walls, draw macrophages, and promote atherosclerosis (Cardio, 2008).

Each native LDL particle promotes emulsification, which allows the fatty acids it is carrying to circulate throughout the body in the water outside of cells by encircling and packing them. There is only one apolipoprotein B-100 molecule per particle. (APO B-100, a protein with a mass of 514KDA and 4563 amino acid residues. According to Jones et al. (2001), each LDL molecule has a highly hydrophobic core made up of hundreds to thousands of esterified and non-esterified cholesterol molecules as well as polyunsaturated fatty acids known as linoleate.

2.5.5 Very Low-Density Lipoprotein (VLDL)

These lipoproteins, which are also included in VLDL along with hepatic cholesterol, Apo-B-100, C-11, and E, are made in the liver from glycerol and fatty acids. When VLDL is released, Apo- B-100 is the main lipoprotein present. Plasma HDL is used to produce apo-E and C-11. When VLDL enters peripheral tissues, their half-life in serum is only 1 to 3 hours; Apo C-11 activates LPL, releasing fatty acids that are absorbed by adipose tissue and muscles. The remainder now falls under the category of IDL (Intermediate Density Lipoprotein), and it contains more cholesterol and less TAG.

2.6 The Kidney

On either side of the vertebral column, in the back of the abdominal cavity, is where the kidney is located. It is made up of a pyramid-shaped interior medulla that is pale and has a reddish-brown cortex on the outside. About 2 million nephrons, or similarly designed functional units, make up the pyramids. Each of these nephrons is made up of a small tube with a Bowman's capsule-shaped epithelial sac at the top end.

The glomerulus, which forms sacs and is located in the cortex of the kidney, contains clusters of capillaries that are born from afferent arterioles. Convoluted tubules at its proximal and distal ends surround this glomerulus. The loop of Henle is formed by a narrow piece of the proximal convoluted tubule that enters the medulla. The distal convoluted tubule winds around in the vicinity of the parent glomerulus as it travels from the thick section of the loop of Henle to the collecting ducts. The collecting tubules, which descend into the medullary area and drain urine into the renal pelvis, are where all distal convoluted tubules come to a stop (Vander, 1991).

2.6.1 Functions of the Kidney

Fluid in the body varies within specific bounds in terms of volume, pH, and composition. This consistency is primarily preserved by the kidney. The following are some of the different kidney's functions:

- Regulation of water content of the body
- Regulation of the electrolyte content of the body
- Maintenance of the normal acid-base equilibrium of the body etc

2.6.2 Kidney Disease

Increased urine osmolality can cause calcium oxalate salts to develop and deposit in the renal tubules, which can result in kidney stones. Another form of kidney illness known as ketone body production results from an abundant but incomplete oxidation of fatty acids. The overproduction of ketone bodies caused by starvation and untreated diabetes mellitus has various related health issues. The health of the glomeruli and tubular cells, a regular blood supply, proper hormone secretion, and normal hormonal feedback control are all necessary for the kidney to operate normally (Valtin & Schafer, 1995).

2.6.3 Kidney Function Tests

The renal function test is another name for this. They either identify the degree of functional damage in kidneys that are known to be diseased or they detect any potential renal damage in patients who are suffering from kidney disorders. Blood analysis for urea and creatinine is the key component of a renal function test.

The significance of a quick examination and measurement of specific electrolytes in urine, however, can also be crucial. When diagnosing acid-base disorders in particular, blood analysis for the electrolyte profile of Na^+, K^+, Cl^-, and HCO_3^- may also be used to monitor renal function (Varley *et al.*, 1976).

2.6.3.1 Serum Urea

The deamination reaction utilized in the production of urea, a by-product of protein metabolism, is a step in the metabolism of amino acids. It develops in the liver and is eliminated by urination. One molecule of ATP, CO_2, and NH_2 are combined to form carbamol phosphate, which is then converted into urea by the Mg_2^+ ion-dependent carbamol phosphate synthase. The urea cycle then results in the regeneration of orthnine. The kidney then removes the urea that has been created in this way from the bloodstream. Therefore, the kidney's capacity to remove urea from circulation has a large impact on the amount of urea in circulation in the blood (rather than only protein intake and metabolism) (Varley *et al.*, 1976).

2.6.3.2 Serum Creatinine

Anhydride of creatine is creatinine. It is created when creatine is dehydrated and is the by-product of creatine metabolism in muscles. The kidney, liver, and pancreas use amino acids to make creatine. After then, the blood carries it to other organs where it gets phosphorylated. At a rate of roughly 2% per day, creatinine is produced from creatine and creatine phosphate. The urine contains creatinine that has been produced. To assess renal disease, the blood's creatinine

concentration is measured. The degree of renal impairment is tracked by a rise in serum creatinine and/or urea concentration (Varley *et al.,* 1976).

2.7 Feed formulation

Feed formulation is the process of calculating the quantity of feed that must be combined to provide a single, consistent diet for animals that meets all of their nutritional demands. In light of its function in guaranteeing appropriate nutrition, it is one of the main operations of the feed industry.

It is a crucial step in the production of animal feed, ensuring that the ingredients are used economically for the best growth of rats. Because it requires a thorough understanding of poultry and the ingredients, it is crucial that the formulation be accurate to prevent any negative effects on a significant amount of animal feed. Scientists studying animal production believe that altering the components of animal diets is the most efficient strategy to control animal growth, reproduction, and survival rates.

The lack of materials for animal feed manufacturing in Nigeria is a significant issue, particularly for the livestock business. The cost of feed accounts for more than half of the price of producing farm animals for meat (Oyenuga, 1969). Feed scientists and nutritionists are constantly looking for low-cost alternative sources of feed component that can increase livestock production to their desired level in an effort to keep up with the rising demand for livestock for human consumption feed ingredients. The lack of clear nutritional information on the less well-known substitutions is a significant drawback to using non-traditional feed ingredient replacements. Millet, corn (a source of carbohydrates), groundnuts, and crayfish (a source of protein) are potential non-conventional feed ingredient sources.

The entire amount of food provided to the animals each day is referred to as their ration. Contrarily, ration formulation is the process by which various feed components are blended in

an essential ratio to give the animal the right amount of nutrients needed at a specific time. Despite the rising cost of raw materials, in order to keep a respectable profit margin and labor, there must be a design to reduce the cost of production and still maintain high level of performance in the rats.

Animals of different species or classes require varied amounts of energy (carbohydrate and fats), proteins, minerals, and vitamins to support their various physiological processes, such as body upkeep, reproduction, egg and milk production, and so forth. The process of creating poultry feed is really difficult. It entails choosing a blend of feed components that sufficiently meets the indicated nutritional needs as well as the needs of other animals.

The main goal of both commercial and on-farm feed millers is "least cost" formulation, which is defined as producing a technically satisfactory feed as inexpensively as possible. In order to determine the least-expensive ration, the formulation of rations for poultry (Kibaara, 2005) places a strong emphasis on the use of linear programming. Least-cost strategy is referred to as reasonable cost. Since the calculation's outcome can be unworkable and unsuitable for feeding the animals, ration formulation involves more than just doing mathematical calculations to satisfy the needs of the animals. As a result, the feed formulation must be assessed before being fed to the animals.

2.7.1 Important Considerations in Feed Formulation:

Making a successful feed formulation requires taking into account a number of crucial aspects, including the following:

Acceptability to the animals: The ration must be pleasant to the animals in order to encourage intake. Since animals must swallow the feed in order for it to be effective, any food that an animal rejects are useless.

Digestibility: In order for the animals to benefit from the nutrients in the feed, they must be digested and released into the gastrointestinal tract. For instance, fowl are unable to tolerate diets heavy in fibre.

Cost: Different combinations of feed ingredients can be used to meet the animal's needs. However, there can only be one least-cost formulation when the prices of these components are taken into account. The animal's needs should be addressed, and the targeted goals should be accomplished, using the least expensive ration.

Anti-nutritional Factors and Toxins: These things prevent animals from digesting particular nutrients by rendering them inaccessible to them. for instance, the antitrypsin component in soybean meal. Some of the elements in feed might also contain poisonous substances that, when administered in large doses, could harm the animal. These ingredients should either be used in smaller quantities or not at all (Talat, 2004).

2.7.2 Composition of Constituents

2.7.2.1 Level of Fiber

Due to their unusual digestive system, animals can develop well when fed a high-fibre diet. When no fat is given, diets containing 180–210 acid detergent fibre (ADF) g kg–1 (corresponding to 9.7–10.3 MJ digestible energy (DE) kg–1) achieve the highest growth rates. Animals used for fattening are unable to maintain their DE intake over this fibre level. In comparison to diets with the recommended values, high fibre diets (350 g ADF kg1 DM) reduce average daily growth and feed conversion rate by 30% and 50%, respectively. Young animals may be more susceptible to this impairment (De Blas *et al.*, 1995; Feugier *et al.*, 2006).

In order to reduce the frequency of diarrhoea, high-fibrous diets are commonly created. Numerous studies have demonstrated that an increase in dietary ADF content from 190–210 to 240–260 g kg1 actually increases sanitary risk (mortality plus morbidity; Feugier *et al.*, 2006)

and fattening mortality as well as impairment in mucosal structure (Alvarez *et al.*, 2007). In contrast, to increase DE intake and weight gain and to minimize total and caeca mean retention time, dietary fiber content must be at a minimum.

2.7.2.2 Fat Supplementation level

Numerous authors have investigated the effects of including 30 g kg1 of various types of fat in fiber diets for fattening rabbits, including tallow, lard, deodorized oleins, and sunflower oil (Partridge *et al.*, 1986; Santoma *et al.*, 1987; Fernandez & Fraga, 1992). The addition of fat improved feed efficiency (by 7%) and energy digestibility (by an average of 5%), but not growth rate, as feed intake fell by 6%. There was no interaction between the type and quantity of supplementary fat. Due to the effects of fat quality on carcass quality and pellet stability, the value of adding fat to fattening feeds should be determined on an energy-cost basis.

The addition of 35 g fat kg1 to diets raised DE consumption by an average of 14.5%, which led to an increase in milk output and an 8.5% rise in litter weight at weaning. Puppy mortality dropped in litters with more than nine pups, but the type of diet had no effect on the breeding does' body weight, fertility, or prolificacy (Fraga *et al.*, 1987).

Additionally, studies have demonstrated that n-3 polyunsaturated fatty acid-rich meals from fish oil or linseed increase the reproductive effectiveness of breeding rat does and reduce breastfeeding mortality. Another investigation has demonstrated a positive effect of dietary linseed supplementation on the semen quality of bucks (Castellini *et al.*, 2003).

2.7.2.3 Protein Level and Source

Animal diets contain a wide range of energies. The best way to express total protein needs is as a ratio of digestible protein to digestible energy. Fraga et al. (1983) used 12 meals comprising from 7.9 to 11.7 g DP MJ1 DE to examine the impact of a change in this ratio on the

performance of fattening pigs. For diets with a DP: DE ratio of 10 g DP MJ1 DE, the maximum DE intake and average daily gain were attained.

Therefore, when dietary DE increases from 9.5 to 11.5 MJ kg1, the ideal DP level should be raised from 95 to 115 g kg1. Dietary DP: DE ratios below and beyond this optimum have a negative impact on feed efficiency and fattening performance. If the DP:DE ratio is maintained at 9.5–10 g MJ1 and the amino acid supply is sufficient, it has been found that dietary crude protein concentrations of around 140 g kg1 do not hinder growth performance (Carabano *et al.,* 2009).

2.7.3 Feed Formulation Techniques

There are various ways to formulate rations, but they all aim to provide the necessary balance of nutrients at the lowest feasible cost. Several of these include:

Pearson Square Calculation: The following are some advantages of this approach of feed formulation: It is helpful in balancing for the protein requirements because it is straightforward, easy to understand, and reasonably simple.

The following are a few of this method's drawbacks: There are just two feed ingredients used. Other nutrient requirements, such as vitamins and minerals, are given less attention (Talat, 2004).

Simultaneous Equation approach: This technique uses a straightforward algebraic equation as an alternative to the Pearson Square approach. The benefit of this method over the Pearson Square method is that both protein and energy can be balanced. When balancing increasingly complex diets, it is often helpful to take into account more than two feed ingredients simultaneously.

Trial-and Error Approach: As the name suggests, the formulation is adjusted until the animal's nutrient needs are met. The trial-and-error approach can be used manually on paper or on a computer with the use of spreadsheet programs like Excel, Lotus123, and Quattro pro.

Using this technique, it is possible to create a ration that fully satisfies the animals' nutritional needs. This method's drawback is that it is time-consuming and laborious before producing a largely good result.

The two-by-two matrix approach uses two separate feed ingredients to address two nutrient needs. The questions are solved by solving a series of equations using a 2 x 2 matrix that has been set up.

The most popular technique for determining least cost feed formulation is linear programming (LP). This approach was initially created in 1947 by G.B. Dantzig to address some planning issues with the U.S. Air Force, but it is now widely employed in a variety of industries. This technique uses a set of mathematical equations to identify the least expensive ingredient combination.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials, apparatus and equipment

Wistar albino rats (male), Conified cages, Blender (Hondah 206), Electronic weighing balance, Glucometer (Acu check), Glucometer strip (Acu check), Hand glove, Funnel, What man filter paper, measuring cylinder (100 ml), Beaker (250 ml), 1ml Sterile syringe, Spatula, Cotton wool, Permanent marker, Dissecting tools, Surgical blade, amber bottles, *Sesamum indicum*, Groundnut cake, Bone meal, Fish meal, Premix, Wheat offal, Elephant grass, Normal salt, Confined cage, Feeding troughs, Lithium heparin bottle (for blood sample collection), Refrigerator (Pr-134.GK 2000), Spectrophotometer (NV201 2002), Sterile bottles, Centrifuge.

3.2 Reagents

All reagents used were of pure analytical grades commercially available. Alloxan, Normal Saline, Formalin (90%), Ethanol (98%), Distilled water, Aspartate amino transferase reagent test kit (RANDOX), Alanine amino transferase reagent test kit (RANDOX), Alkaline phosphatase reagent test kit (Quimica clinica aplicada QCA), Albumin reagent test kit (RANDOX), Bilirubin reagent test kit (RANDOX), Total protein reagent test kit (RANDOX), Cholesterol reagent test kit (RANDOX), Triglyceride reagent test kit (RANDOX), High density Lipoprotein (HDL) reagent test kit (RANDOX), Very low-Density lipoprotein (VLDL) reagent test kit (RANDOX)

3.3 Methodology

3.3.1 Procurement of animal

36 male rats weighing between 120 and 180g each were purchased from the animal farm of the Animal Science department at the University of Nigeria Nsukka in Enugu state. Their weights were measured and divided into nine (9) sets, each set containing four (4) rats. They have their own space in a Conified cage. They were subjected to acclimatization for one week while

receiving the standard control compounded food (devoid of Sesamum indicum) and unlimited access to water.

3.3.2 Experimental Design

As indicated below, there were 4 animals in each group and the experimental animals were maintained in 9 contained cages that were carefully constructed to prevent the mixing of food and rat feces.

Table 3.1 Animal Grouping and Treatment

Groups	No of Rats	Feeding
1 (control)	4	Compounded diet without *Sesamum indicum*
2 (diabetic but Untreated)	4	Compounded diet without *Sesamum indicum*
3 (standard drug treated)	4	Compounded diet without *Sesamum indicum* and administered Glibenclamide
4 (diabetic treated)	4	Compounded diet with 15% *Sesamum indicum*
5 (diabetic treated)	4	Compounded diet with 30% *Sesamum indicum*
6 (diabetic treated)	4	Compounded diet with 45% *Sesamum indicum*
7 (non-diabetic)	4	Compounded diet with 15% *Sesamum indicum* and induced with diabetics after 14 days
8 (non-diabetic)	4	Compounded diet with 30% *Sesamum indicum* and induced with diabetics after 14 days
9 (non-diabetic)	4	Compounded diet with 45% *Sesamum indicum* and induced with diabetics after 14 days

The albino rats' weight and glycemia levels were examined throughout the experiment on the first, third, seventh, tenth, and fourteenth days using an Acu Check Glucometer and an electronic weighing scale, respectively. At the end of 14 days, the animals from groups 1 through 5 were sacrificed; their blood samples and organs (liver, kidney, and pancreas) were collected in sterile bottles and lithium heparin bottles, respectively. The animals from groups

6 through 9 were sacrificed 72 hours (3 days) following the induction of diabetes. They were all brought to the lab for histology testing and a clinical evaluation.

3.4 Sample Collection and Preparation

Off-white Sesamum indicum growing in Nigeria was acquired from the Ogbete market in Enugu State in the southeast of the country, and it was identified at the Federal University of Technology Owerri's department of plant science and biotechnology. They were cleaned with water, allowed to air dry for a week, milled, and stored in a container with clear labels for proximate and phytochemical analysis as well as feed formulation.

3.5 Proximate Analysis Methods

To determine the percentage proportion of moisture, ash, crude fiber, crude fat, protein, and carbohydrate on the Sesamum indicum, proximate analysis was carried out using the AOAC 2010 technique.

3.5.1 Moisture Content

The moisture content of the seed was calculated using the AOAC 2010 technique. An oven was used to dry the washed Petri dish. The Petri dish was filled with 2g of the sample, and the combined weight of the Petri dish and sample was recorded before oven drying (W1). The Petri dish and sample were placed in the oven and baked for three hours at 100°C before the weight (W2) was recorded. Drying continues until the weight remains constant. The formula was used to determine the percentage moisture content;

$$\% \text{ Moisture} = \frac{W_1 - W_2}{\text{Weight of Sample}} \times 100$$

3.5.2 Ash Content

Ash content was calculated using the AOAC 2010 technique. Platinum crucible that was empty was cleaned, dried, and weighted (W1). The sample was weighed at 2g into the platinum

crucible, and the weight was recorded as W2, before the sample was heated to 550°C for three hours. After cooling the sample in a desiccator, the weight (W3) was recorded. The formula was used to get the percent Ash content;

$$\% \text{ Ash content} = \frac{W_3 - W_1}{W_2 - W_1} \times 100$$

3.5.3 Crude Fiber

Using petroleum ether to remove the fat from 2g of the sample, 200ml of a solution containing 1.25g of H_2SO_4 per 100ml of solution was added, and it was heated at reflux for 30 minutes. On a fluted funnel, the solution was filtered through many layers of cheese cloth. After that, it was rinsed in hot water to remove any remaining acidity. The final residue was filtered through a thin but tight pad of washed and burned asbestos in a Gooch crucible after being placed to a beaker and boiling for 30 minutes with 200ml of a solution containing 1.25g of carbonate free NaOH per 100ml. It was weighed, burnt, cooled, and then dried in an electric oven before being weighed once more.

$$\% \text{ Crude fiber} = \frac{\text{Weight of fiber}}{\text{Weight of sample}} \times 100$$

3.5.4 Crude Fat

At 105–110°C, 250ml of clean boiling flasks were dried. To cool, it was moved into a desiccator. The boiling flask was weighed after being filled with around 300 ml of petroleum ether (b.pt 40-60) and labeled with the appropriate weights. Cotton wool was used to make a light plug in the extraction thimble. The petroleum ether was collected in the top container of the setup and emptied into a container for reuse after the Soxhlet apparatus had been built and allowed to reflux for roughly 6 hours. The flask was taken out of the oven when it was nearly clear of petroleum ether, dried at 105-110^{oC} for an hour, transferred into a desiccator, allowed to cool, and then weighed.

3.5.5 Crude Protein

In order to avoid the sample from hitting the side walls of each flask, 0.5g of the sample was carefully weighed into a 30ml Kjedahl flask before the flask was sealed and shaken. The Kjedahl catalyst mixture was then added in 0.5g increments. In a digesting rack over fire, the mixture was cooked continuously until a clear solution materialized. After cooling for 30 minutes, the clear solution was given a chance to stand before being added to with roughly 100 ml of distilled water to prevent caking. After this, 50 ml of the clear solution was then transferred to the Kjedahl distillation apparatus. As soon as 5ml of 40% NaOH was added to the digested sample in the apparatus, distillation began immediately and continued until 50 drops got into the receiver flask. Thereafter, it was titrated to pink color using 0.01N hydrochloric acid. A 100ml receiver flask containing 5ml of 2% boric acid and an indicator mixture consisting of 5 drops of Bromocresol blue and 1 drop of methylene blue was placed under a condenser.

$$\% \text{ Nitrogen} = \text{Titer value} \times 0.01 \times 14 \times 4$$

3.5.6 Carbohydrate Determination

$$\% \text{ Carbohydrate} = 100 - (\% \text{ Protein} + \% \text{ Moisture} + \% \text{ Ash} + \% \text{ Fat} + \% \text{ Fiber})$$

3.6 Phytochemical Analysis Using GC-MS

The milled sample was also subjected to GC-MS phytochemical investigation. The approach is the AOAC 2007 approach. In order to absorb moisture, 20g of the homogenized material was weighed and combined with 60g of anhydrous sodium sulphate in an agate mortar. The homogenate was added to a 500 ml beaker along with 300 ml of n-hexane, and extraction was carried out using the 300 ml of n-hexane over the course of 48 hours at EPA 354°C. The resultant crude extract was dried using a rotary vacuum evaporator at 40°C, and the residue was then transferred with n-hexane into a 5ml Florisil column for cleanup.

The Florisil was heated overnight in an oven at 130°C, transferred to a beaker with a capacity of 250 ml, and put in a desiccator. On an 8ml column with glass wool filled, 0.5g of anhydrous NaSo4 was added to 0.1g of activated Florisil (magnesium silicate), 60 - 100nm mesh. To condition the packed column, 5ml of n-hexane was added. While gently pressing the top of the column until the Florisil settled thoroughly in the column, the stopcock was opened to let n-hexane to flow out till it just touches the top of sodium sulphate into a receiving vessel. The extract was transferred from an evaporating flask to the column using a disposable Pasteur pipette. The evaporating flasks were then rinsed twice with 1ml portions of n-hexane and added to the column. The eluate was then collected into an evaporating flask and rotary dried before being dissolved in 1ml n-hexane for gas chromatographic analysis.

A 100 ml volumetric flask was used to dilute the 1 ml n-hexane extract to the desired concentration after it had been dissolved in 50 ml of chloroform. The majority of the chloroform was evaporated at ambient temperature before 1 ml of the inter-esterification reagent (20% vol benzene and 55% vol methanol) was added to seal it. This mixture was then heated at 40°C in a water bath for 30 minutes. The organic sample was extracted with hexane and water after inter-esterification so that the final ratio of the reagent, hexane, and water is 1;1;1. The mixture was stirred briskly. Centrifugation was used to separate the stable emulsion that had formed after two minutes of hand shaking. A tiny test tube was used to transfer about half of the upper hexane phase for injection. The GC was given time to warm up while the oven was preheated to 180°C. The temperature condition was set while the instrument warmed up, and when it was ready, the "Not Ready" light went out and the sample was run.

3.7 Feed Formulation

The method described by Onyeike et al. (2015) was used to implement the sesamum indicum compounded diet. Groundnut cake, wheat offal, maize flour, premix, bone meal, sesame seed, elephant grass, fish meal, and regular salt were used to manufacture the diet. They were all ground, weighed in accordance with the necessary ratio, and properly combined.

3.8 Biochemical Analysis

Blood samples from albino rats that had been sacrificed were subjected to biochemical examination to ascertain how the treatment affected the liver enzymes, lipid profile, and kidney function parameters.

3.8.1 Methods of Liver Function Tests

RANDOX test kits were used to conduct liver function testing. The parameters for the liver function were determined using the Daniel and Marshall, 1999 technique.

3.8.1.1 Aspartate Amino Transferase (AST) Activity

The aspartate amino transferase activity was measured using the technique described by Daniel and Marshall in 1999. After collecting the blood samples in a lithium heparin bottle, the plasma and serum were separated using a spinner. Sample and Blank were the labels on two test tubes. 100 ml of sample and 500 ml of reagent 1 (R1) were pipetted into test tubes labeled "sample" and "blank," respectively. After adding 100 l of distilled water, the mixture was incubated for 30 minutes. After 20 minutes of incubation, 500 l of reagent 2 (R2) and 5000 l of sodium hydroxide (NaOH) were added. The absorbance was measured using a UV/visible spectrophotometer at 546 nm, and the standard table was used to determine the AST activity in the serum/plasma.

3.8.1.2 Alanine Amino Transferase (ALT)

Blood samples were drawn and put into a vial containing lithium heparin; the samples were then spined to separate the serum from the plasma. 500 l of reagent 1 (R1) was pipetted into each of two test tubes after they had been labeled. The sample test tube received 100 l of the sample, while the blank test tube received 100 l of distilled water. Following a 30-minute incubation period, 500 l of reagent 2 (R2) and 500 l of sodium hydroxide (NaOH) were added to the test tubes. The UV spectrophotometer was used to measure the absorbance, and the standard tables were used to determine the activity of ALT in the serum.

3.8.1.3 Albumin Determination

Calculating albumin required using 60% of the total protein.

3.8.1.4 Alkaline Phosphatase determination

Two test tubes with the labels "standard" and "test" were used to separate the blood samples after they had been collected and spun for 5 minutes. Both test tubes received 1000µl of alkaline phosphatase water by pipette. In each test tubes, a drop of alkaline phosphatase substrate was introduced. Five minutes were given for it to stand. Alkaline phosphatase color developer 5000µl was pipetted into both tubes. A UV Spectrophotometer was used to measure the absorbance at a wavelength of 540–550 nm. the conc. was calculated using

$$\frac{absorbance of sample \times 30}{absorbance of standard}$$

3.8.1.5 Bilirubin Determination

A lithium heparin bottle was used to collect the blood sample, which was then spun to separate the plasma from the whole blood. Two test tubes with the labels "test" and "blank" were used. 200µl of reagent 1 (R1) and 50µl of reagent 2 (R2) were pipetted into each test tube. Reagent 3 (R3) in the amount of 1000µl was pipetted into each test tube. 200µl of the plasma and 200µl of distilled water were pipetted into test tubes marked "test" and "blank" and incubated for 10 minutes at 20-250C, respectively. 1000µl of reagent 4 (R4) was pipetted into each test tube, and the absorbance was measured with a UV/visible spectrophotometer at a wavelength between 560-600 nm. After setting the wave length and zeroing the machine, the concentration was determined using the formula 185 X sample absorbance.

3.8.1.6 Total Protein Determination

Two test tubes with the labels "Standard" and "Test" were used to separate the blood samples after they had been collected and spun for 5 minutes. A total of 1000µl of R1 was pipetted into each test tube, followed by 20µl each of the standard and test, which were then incubated at

250 C for 30 minutes. A UV spectrophotometer was used to measure the absorbance at 546 nm, and the total protein concentration was determined by multiplying the A sample by 190.

3.8.2 Methods for Kidney Function Test

3.8.2.1 Urea

The sample's urea content was determined using the Valtin and Schafer, 1995 technique. The material was gathered, placed into a bottle of lithium heparin, separated, and spined. 100µl of reagent 1 (R1) was pipetted into two test tubes labeled "sample" and "standard," and the test tubes were then both sealed. In each test tube, 10µl of the standard reagent and sample were pipetted in. After 10 minutes of incubation, 2500µl of reagent 2 (R2) was added to both. In both test tubes, 2500µl of reagent 3 (R3) was introduced. After 15 minutes of incubation in warm water at 370°C, the absorbance was measured with a calorimeter using a wavelength range of 530–570 nm. The concentration of urea was calculated using

$$\frac{\text{A sample} \times \text{standard conc.}}{\text{A standard}}$$

3.8.2.2 Creatinine

Creatinine was determined using the method described by Valtin and Schafer in 1995. The sample was taken and poured into a container containing lithium heparin. To remove the whole cell from the plasma, it was spined. R1a and R1b were pipetted into the container in equal amounts. The working reagent was pipetted into a cuvette and 1ml (1000µl) was used to zero the calorimeter at 490nm. The sample was put to the cuvette in 100µl and thoroughly mixed. After 30 seconds, the first absorbance (Ab1) was read, and exactly 2 minutes later, the second absorbance (Ab2). The process was repeated for the standard, and the DA standard and DA sample were obtained by calculating the difference between Ab2 and Ab1 for the standard and sample. The absorbance of creatinine was calculated using;

$$\frac{DAsample \times Std\ conc}{DA\ standard}$$

3.8.2.3 Potassium

Valtin & Schafer, 1995 was used in determination of potassium concentration of the sample. The sample was collected the and poured into lithium heparin or plain bottle. It was spined to achieve separation of the plasma/serum and the blood cells, 1ml(1000µl) of the potassium reagent was pipetted into tubes labelled sample and standard. 10µl of sample and standard were added into appropriate tubes and mixed properly. It was incubated at room temperature for 3 minutes; the spectrophotometer was zeroed and the absorbance read at 500nm. The concentration of the potassium was calculated with;

$$\frac{Absorbance\ of\ sample\ \times conc.of\ std}{Absorbance\ ot\ standard}$$

3.8.2.4 Sodium

The technique applied was Valtin & Schafer's procedure from 1995, in which the sample was gathered and poured into a lithium bottle. It was spined, and 50µl of water was added to the sample and standard test tubes after 1 ml of filtrate regent was pipetted into the blank sample and standard test. For 30 minutes, all of the tubes were shaked ferociously and continuously. With 1 ml of acid reagent pipetted into test tubes for the blank sample and standard, it was centrifuged for 10 minutes. Each tube received 50 l of color reagent, 50µl of supernatant from the centrifuge tubes, 50µl of the blank sample and standard, and the spec was zeroed at 550 nm before the absorbance was measured**.** Calculation was done with;

$$\frac{Absorbance\ of\ blank - Absorbance\ of\ sample \times conc.of\ std}{Absorbance\ \ of\ blank - Absorbance\ of\ standard}$$

3.8.2.5 Bicarbonate

The sample's bicarbonate concentration was calculated using Valtin & Schafer, 1995. 'Blank', 'standard', 'control', and 'sample' test tubes each contained 10 ml of carbon dioxide reagent, which was pipetted into them. The test tubes were incubated at 37^{c} for three minutes. 5 ml of water, the standard, and the sample were pipetted into the cuvettes labeled blank, standard, and

sample, respectively, with the spectrophotometer set at 340 nm. They were all gently combined by inversion, then incubated for five minutes. The absorbance of all cuvette was read at 340nm and calculation done by;

$$\text{Conc.} = \frac{abs.blank - abs.sample}{absblank - abs.standard} \times conc.of\ standard$$

3.8.3 Methods for Lipid Profile Test

Venness and Diehl method 1989 was used in determining the parameters in Lipid Profile of this study.

3.8.3.1 Cholesterol Test

The Venness and Diehl 1989 method is used to calculate cholesterol levels. Two test tubes with the label "sample" and "standard" and 1000µl of the cholesterol reagent were pipetted into each test tube after the sample was collected, poured into a lithium heparin bottle, and spined to separate the blood. Also pipetted into the sample and standard test tubes, respectively, were 10µl of the sample and standard reagent. After 10 minutes of incubation, the absorbance at 540 nm wavelength was measured**.** The concentration was calculated using;

$$\text{Concentration} = \frac{\text{absorbance of sample} \times \text{concentration of standard reagent}}{\text{Absorbance of standard}}$$

3.8.3.2 Triglyceride Test

To separate the blood, the sample was gathered, placed into a lithium heparin vial, and spined. A total of 1000 microliters of the cholesterol reagent were pipetted into two test tubes labeled sample and standard. Additionally, 10 microliters each of the sample and standard reagents were pipetted into the test tubes before being incubated for 10 minutes. At 540 nm, the absorbance was measured and calculated with;

$$\text{Conc of cholesterol} = \frac{\text{absorbance of sample} \times \text{concentration of standard reagent}}{\text{Absorbance of standard}}$$

3.8.3.3 HDL TEST

A sample of blood was taken, and it was then put into a lithium heparin bottle with a spine to separate the blood. 500µl of the HDL reagent was pipetted into two test tubes labeled sample and standard, respectively. Both test tubes received 200µl of the cholesterol standard reagent and 200µl of the sample reagent, respectively, and were then incubated for 10 mins. Another 10 minutes were spent spinning; this divided the contents into two levels, upper and lower. The deposit or residue is at the lower level, and the filtrate or supernatant is at the top level. A second set of test tubes were designated as the cholesterol standard, and 1000µl of the cholesterol reagent and 100µl of the earlier-designated standard's supernatant were pipetted into the two newly-designated test tubes. The HDL test and standard test tubes for cholesterol were performed similarly and let to stand for 10 minutes. The absorbance was read and the conc of cholesterol calculated.

$$VLDL = Trig /2.2$$

$$VLDL = Total\ Chol + (HDL + LDL)$$

$$TRIG/2.2 = Total\ Chol + HDL + LDL$$

3.8.3.4 Total Bilirubin Test

A lithium heparin bottle was filled with the collected blood sample, and the bottle was spun to separate the blood. Reagent 1 (R1) in quantities of 200µl was poured into two test tubes with the labels "Test" and "Blank," respectively. A drop of 50µl of reagent 2 (R2) and 1000µl of reagent 3 (R3) were poured into each test tube. 200µl of the plasma and 200µl of distilled water were pipetted into test tubes labeled "test" and "blank," respectively, and incubated at 20 to 25 oC for 10 minutes. 1000µl of reagent 4 (R4) was added to each test tube, and the absorbance was measured with a calorimeter using a 560–600 nm wavelength range. After choosing the wavelength, the device is zeroed with the blank. The formula 185 X sample absorbance is used to determine the concentration.

3.8.3.5 LDL

LDL = Trig/2.2 + Total Chol +HDL

3.9 Histology of Organs

For the histopathology of the organs, Slaoul & Fiette's 2011 approach was employed. The organs were processed by running them through progressively stronger grades of alcohol, including 30%, 50%, 70%, 90%, and absolute alcohol, after being fixed in a sizable amount of 10% neutral buffered formalin that covered the whole organ. After dehydration, the alcohol was removed, and the organ was submerged in Xylene for three hours. Then, in the embedding oven, it was transferred from the cleaning agent to a bath of molten paraffin wax. By dispersion into the surrounding wax, the cleaning agent was now gone from the organ.

The hot paraffin wax was poured into the mold. The organ was moved from the paraffin bath to the mould using a pair of blunt-nosed forceps that had been electrically heated. Once more warmed, the forceps were used to position the organs so that they were lying in the appropriate plane. To ensure that any wax that may have solidified during the transfer from the mold is melted, the forceps were moved around the organs. The organ-facing side of the mold was touched up with the matching label from the paraffin bah. Blown air was used to cover the surface until a thin wax film had hardened. After carefully transferring the mold to a container of cold water, the wax was left to solidify for 30 minutes.

The block was maintained for trimming when it was taken out of the mold. At this point, the extra wax was carefully removed and fastened to a holder. A rotary microtome was used to slice the organs into sections. The object's vertical rise and fall in relation to the knife edge had an impact on this. Before being affixed to the slides, the greased parts were gently heated to flatten them. For secure adhesion, a portion adhesive was applied. The portion was dewaxed, hydrated, briefly heated to 10 degrees over its melting point on a hot plate, and then submerged

in Xylene for 30 minutes prior to staining. To get rid of Xylene and make sure it didn't get transferred to the lower grade alcohol, it was moved to pure alcohol for 30 minutes. The slide was then exposed to 90% alcohol for 30 seconds, followed by another 30 seconds in 70% alcohol. After a thorough cleaning in distilled water, it spent 30 minutes being submerged in Harris Haematoxylin. After being properly cleaned with running water, the slide was differentiated in 1% alcohol until only the cell nuclei retained the stain. After being submerged in flowing water for 5 minutes, it was counter stained for 1 minute with eosin, dehydrated in escalating alcohol concentrations, cleaned in Xylene, and mounted using dibutylpthalate, polystyrene, and Xylene. Photomicrographs were obtained while the thin sections were being inspected under a high-resolution microscope using photography equipment.

3.10 Alloxan Preparation

140mg/kg was used for the preparation of the alloxan and was prepared based on the body weight of the individual albino rats. The calculation was done thus;

140mg – 1000g
14mg – 100g
0.14mg – 10g
0.014mg – 1g

The milligram of alloxan administered to each rat = 0.014mg x body weight of the rat

3.11 Statistical Analysis

The data obtained were analyzed using ANOVA and values for $P< 0.05$ were considered statistically significant.

CHAPTER FOUR

RESULT

4.1: Proximate Composition (%) of *Sesamum indicum* Seed

Table 4.1 showed the presentation of the results of proximate analysis of *Sesamum indicum* seed. The proximate composition presented are; Carbohydrate, Protein, Lipid, Ash, Moisture and Fiber composition. They are presented in mean $\pm$ standard error of mean deviation.

From the result, it showed that Lipid has the highest value of 46.33% followed by Protein with a value of 32.2% and Carbohydrate value of 6.51%. The Moisture, Fiber and Ash content were observed to have 5.07%, 4.72% and 5.15% values respectively.

Table 4.1: Proximate Composition (%) of *Sesamum indicum* Seed

S/No	Component	Mean Value
1	Moisture	5.07 ± 0.10
2	Crude Protein	32.2 ± 0.15
3	Crude Fiber	4.72 ± 0.04
4	Crude Lipid	46.33 ± 0.21
5	Ash	5.15 ± 0.12
6	Carbohydrate	6.51 ± 0.02

Data are presented as Triplicate means ± standard deviation

4.2: Phytocompounds Identified in *Sesamum indicum* Seed by GC/MS

Table 4.2 showed the result of various phytocompounds identified in *Sesamum indicum* using GC/MS. The result showed that about 20 phytocompounds were identified showing their Retention time, Area and Ref. Butyl 9 Octadecenoate and n-Propyl 9-Octadecenoate were the first compounds to be identified at the retention time of 5.250 and area of 1.58% and Ref of 195600 and 182557 respectively.

Table 4.2: Phytocompounds Identified in *Sesamum indicum* Seed by GC/MS

Pk#	RT	Area %	Compounds	Ref#	CAS	Qual
1	5.250	1.58	Butyl 9 octadecenoate n-Propyl 9-octadecenoate	195600 182557	1000336-74-7 1000336-71-6	46 38
2	5.455	0.22	2-Thiopheneacetic acid,3-tridecyl ester 1-Docosene	182322 167463	1000280-66-7 001599-67-3	35 35
3	6.885	1.18	9-Octadecenal, (Z) – Decyl 4-nitrophenyl ether 1-Decyloxy-2nitrobenzene	126829 139051 139050	002423-10-1 031657-37-1 098311-79-6	80 60 50
4	7.137	0.19	Hexadecanoic acid, 4-nitrophenyl, ester 1H-Indene, 2-butyl-5-hexyloctahydro-	225497 125028	001492-30-4 055044-33-2	27 25
5	7.225	0.14	Methyl 12-oxo-9-dodecenoate 9-Octadecenoic acid (Z)-, tetradecyl ester	89368 262981	022418-58-2 022393-85-7	30 25
6	7.425	1.33	Benzene, 1, 3-dichloro- Benzene, 1, 2-dichloro-	22509 22501	000541-73-1 000095-50-1	95 95
7	7.788	0.46	Octadecane,1(ethenyloxy) -1-Octadecanesulphonyl chloride ,Oxalic acid, cyclobutyl tetradecyl ester	155863 206960 197215	000930-02-9 1000342-70-4 1000309-70-9	43 38 38
8	8.362	0.78	Butyl 9-octadecenoate Cyclooctane, methyl- 1-Fluorononane	195600 11771 22849	1000336-74-7 001502-38-1 000463-18-3	52 49 49
9	8.521	1.49	2-Ethyl-1-dodecanol Undecane, 1-bromo- Octane, 2, 5, 6-trimethyl-	78269 96187 29376	019780-33-7 000693-67-4 062016-14-2	59 50 49
10	8.612	2.07	1-Hexadecanesulfonic acid,3,5-Dichloro-2,6-dimethyl-4-pyridylester Docosylpentafluoro propionate Isobutylnonyl carbonate	263024 261805 105955	040220-85-7 1000351-80-9 959311-27-4	55 52 49

11	8.876	3.69	Hexadecane,3-methyl- Docosane, 2, 21-dimethyl-	102605 195685	006418-43-5 077536-31-3	86 72
12	8.980	1.49	Nonane Undecane, 3-methyl- Undecane	12938 39984 29356	000111-84-2 001002-43-3 001120-21-4	80 64 64
13	9.059	1.08	Decane 2, 6, 10-trimethyl- Decane Tetradecane,	19648 19649 102610	000124-18-5 000124-18-5 014905-56-7	86 86 86
14	9.138	1.73	2,6-Dimethyldecane Octane, 2-methyl-Undecane, 3, 7-dimethyl-	39979 12946 51418	013150-81-7 003221-61-2 017301-29-0	64 64 59
15	9.217	0.46	Tricosane, 2-methyl- Carbonic acid, decyl prop-1-en-2-yl ester Tricosane, 2-methyl-	195680 104084 195681	001928-30-9 1000382-90-5 001928-30-9	87 86 81
16	9.430	7.79	Decane, 3, 8-dimethyl- Hydroxylamine,O-decyl- Undecane, 3, 7-dimethyl-	40006 42310 51418	017312-55-9 029812-79-1 017301-29-0	72 72 64
17	9.558	1.46	Decane, 3,8-dimethyl-Undecane 4, 6-dimethyl- Decane, 3, 6-dimethyl-	40006 51429 40000	017312-55-9 017312-82-2 017312-53-7	76 64 59
18	9.622	2.98	Undecane Hexane, 3, 3-dimethyl- Undecane	29357 7774 29354	001120-21-4 000563-16-6 001120-21-4	78 72 64
19	9.737	3.24	Dodecane Octane, 3, 5-dimethyl- Heptane, 2, 4-dimethyl-	39972 19687 12960	000112-40-3 015869-93-9 002213-23-2	68 64 64
20	9.816	4.74	Heptadecane, 2, 6, 10, 14- Tetramethyl Tridecane Carbonic acid, dodecyl vinyl ester	15590 51391 117251	018344-37-1 000629-50-5 1000382-54-8	81 80 78

Key: Pk= Peak, RT= Retention Time, CAS= Chemical Abstract Service, Qual=Qualitative

4.3: % Composition of Different Diet Components of *Sesamum indicum* Seed

Table 4.3 showed the result of composition of different diet components of the compounded feed. From the table, the composition of maize for the four different diets (Normal, 15%, 30% and 60%) is 65.50%, 50.50%, 35.50%, 5.50% respectively. The composition of groundnut cake for the four different diet is 19.99%, composition of wheat offal is 10.01%, fish meal is 2.5%, common salt is 0.25%, bone meal is 0.5%, elephant grass is 1.00%, premix is 0.25% while the composition of *Sesamum indicum* is 0%, 15.00%, 30.00% and 60.00% for normal feed, 15% feed, 30% feed and 60% feed respectively.

Table 4.3: Result of % Composition of Different Diet Components on the Compounded Feed

S/No	Component	% Composition			
		Normal	**15%**	**30%**	**60%**
1	Maize	65.50	50.50	35.50	5.50
2	Groundnut Cake	19.99	19.99	19.99	19.99
3	Wheat Offal	10.01	10.01	10.01	10.01
4	Fish Meal	2.5	2.5	2.5	2.5
5	Common Salt	0.25	0.25	0.25	0.25
6	Bone Meal	0.5	0.5	0.5	0.5
7	Elephant Grass	1.00	1.00	1.00	1.00
8	Premix	0.25	0.25	0.25	0.25
9	*Sesamum indicum*	0.00	15.00	30.00	60.00

4.4: Effects of the *Sesamum indicum* Compounded Feed on Blood Glucose Level (mg/ul)

Fig 4.1 showed the result of the effect of the compounded feed on blood glucose level for days 3, 7, 10 and 14. The result was presented in a histogram form.

The result showed that the blood glucose level of group 1 which served as the control group remained less than 100 for day 3, 7, 10 and 14. While the blood glucose level of group 2 which served as diabetic untreated group was observed to be above 200 for day 3, 7, 10 and 14. The blood glucose level of group 3 that was treated with diabetic standard drug was observed to be above 200 at day 3 when the diabetics was induced but began to reduce below 200 for day 7, 10 and 14 as the treatment progresses.

The histogram showed that the same trend observed in group 3 was also observed for group 4, 5, and 6 where their blood glucose level reduced below 200 as the feeding with the compounded feed progresses from day 3 to day 14

For group 7, 8 and 9 that was fed with the diet for 14 days before inducing diabetes, it was observed that the blood glucose level remained less than 200 even after the induction of diabetes.

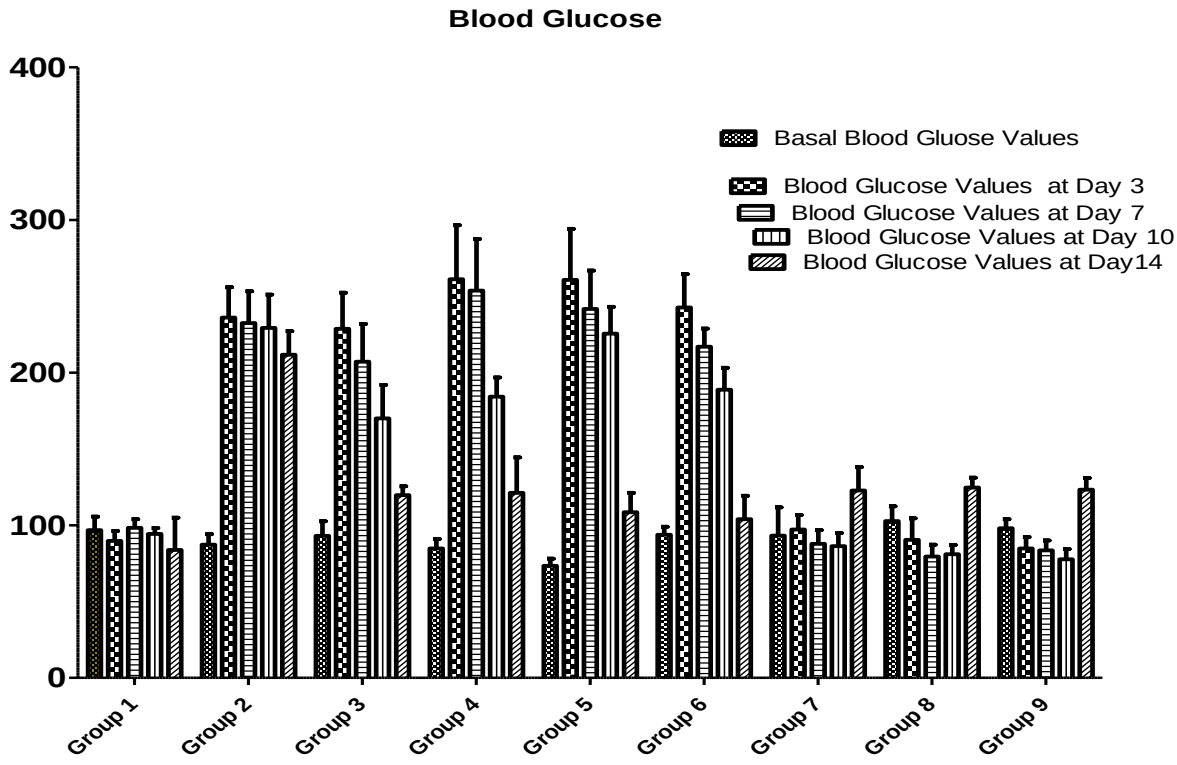


Fig 4.1: Effect of treatment on blood glucose (mg/ul) level of albino rats at 3rd 7^{th} 10^{th} and 14^{th} day

X axis = Glucose Level, Y axis = Groups

4.5: Relative Body Weight (%) of Albino Rat for Days 7 and 14

Table 4.4 shows the result of relative body weight of Albino Rat for day 7 and day 14. The results were presented in percentage. From the result it was observed that the relative body weight of group 1 increased from 14.9 to 37.5, group 2 decreased from -1.4 to -2.4, group 3 increased from 3.0 to 3.9, group 4 increased from 0.4 to 8.2, group 5 increased from 0.5 to 11.5, group 6 increased from 1.0 to 13.5, group 7 increased from 8.1 to 12.2, group 8 increased from 14.1 to 18.2 and group 9 increased from 22.1 to 25.6

Table 4.4: Relative Body Weight (%) of Albino Rats at Day 7 and 14

Groups	Relative Body Weight Value (%)	
	Day 7	Day 14
1	14.9	37.5
2	-1.4	-2.4
3	3.0	3.9
4	0.4	8.2
5	0.5	11.5
6	1.0	13.5
7	8.1	12.2
8	14.1	18.2
9	22.1	25.6

Group 1: normal control, Group 2: diabetic untreated, Group 3: standard drug treated, Group 4, 5 and 6: 15%, 30% and 60% compounded diet fed group respectively. Group 7, 8 and 9: fed 15%, 30% and 60% compounded diet respectively before inducing.

4.6: Relative Weight (%) of Liver, Kidney and Pancreas of Albino Rats After Sacrifice

Table 4.5 showed the result of relative weight of Liver, Kidney and Pancreas after the albino rats were sacrificed. From the table, it was observed that the Liver for group 2 (diabetic untreated group) has the highest relative weight of 5.2% when compared with the liver of groups 1, 3, 4, 5, 6, 7, 8, and 9 that has relative weight of 4.2%, 4.9%, 3.9%, 4.2%, 4.3%, 3.8%, 4.0% and 3.8% respectively.

The same trend was observed for the relative weight of Kidney, were the relative weight of group 2 (diabetic untreated group) was observed to be higher (1.3%) than that of the group 1 (0.7%), group 3 (0.9%), group 4 (0.8%) and groups 5, 6, 7, 8, and 9 (0.7%, 0.9%, 0.9%, 0.9% and 0.8% respectively).

Reverse was observed to be the case of the relative weight of pancreas. Were the pancreas weight of the group 2 was observed to be lower (0.1%) than that of both the group 1 (0.3%), group 3 (0.2%), group 4 (0.3%) and the groups 5, 6, 7, 8 and 9 (0.2%, 0.2%, 0.3%, 0.2% and 0.2% respectively) fed with the diet.

Table 4.5 Relative Organ Weight of Albino Rats after Sacrifice

Groups	Relative Organ Weight Value %		
	Liver	Kidney	Pancreas
1	4.2	0.7	0.3
2	5.7	1.3	0.1
3	4.9	0.9	0.2
4	3.9	0.8	0.3
5	4.2	0.7	0.2
6	4.3	0.9	0.2
7	3.8	0.9	0.3
8	4.0	0.9	0.2
9	3.8	0.8	0.2

Group 1: normal control, Group 2: diabetic untreated, Group 3: standard drug treated, Group 4, 5 and 6: 15%, 30% and 60% compounded diet fed group respectively. Group 7, 8 and 9: fed 15%, 30% and 60% compounded diet respectively before inducing.

4.7: Effect of Compounded Diet on Liver Function Parameters of Albino Rats

Table 4.6 showed the effect of the compounded diet on Liver Function parameters of albino rats studied. The results are presented in mean ± standard error of mean deviation and values bearing different superscript letters are significantly different ($P<0.05$). The Liver function parameters presented are AST, ALT, ALP, Albumin, Protein and Bilirubin.

The result showed that the AST for the groups 4, 5, 6, 7, 8 and 9 which are the groups feed with the diet were not significantly different (36 ± 1.5^a, 30 ± 4.6^a, 33 ± 0.9^a, 37 ± 1.5^a, 42 ± 1.5^a, and 37 ± 1.6^a respectively) when compared with control group 1 (35 ± 1.4^a) and standard treated group 3 (47 ± 1.6^a). But were significantly different when compared with diabetic untreated group 2 (99 ± 1.8^b).

For ALT, the groups 4, 5, 6, 7, 8 and 9 which are the groups feed with the diet were not significantly different (30 ± 4.7^a, 19 ± 0.8^a, 22 ± 0.9^a, 27 ± 3.0^a, 41 ± 0.8^a, and 36 ± 1.9^a respectively) when compared with control group 1 (23 ± 0.7^a) and standard treated group 3 (44 ± 2.2^a). But were significantly different when compared with diabetic untreated group 2 (57 ± 3.9^b).

The result of ALP showed that the groups 4, 5, 6, 7, 8 and 9 which are the groups feed with the diet were not significantly different (46 ± 2.3^a, 47 ± 2.3^a, 42 ± 1.4^a, 40 ± 2.4^a, 66 ± 4.4^a, and 50 ± 5.7^a respectively) when compared with control group 1 (74 ± 5.2^a) and standard treated group 3 (45 ± 3.9^a). but were significantly different when compared with diabetic untreated group 2 (164 ± 5.2^b).

The same trend was observed for Albumin were the groups 4, 5, 6, 7, 8 and 9 which are the groups feed with the diet were not significantly different (46 ± 1.7^a, 46 ± 2.8^a, 45 ± 1.4^a, 38 ± 3.1^a, 37 ± 2.4^a, and 44 ± 1.7^a respectively) when compared with control group 1 (33 ± 1.4^a) and standard treated group 3 (52 ± 1.1^a). But were significantly different when compared with diabetic untreated group 2 (73 ± 4.4^b).

For Protein, the groups 4, 5, 6, 7, 8 and 9 which are the groups feed with the diet were not significantly different (78±1.7[a], 72±2.6[a], 66±2.0[a], 66±3.2[a], 82±3.0[a], and 72±3.8[a] respectively) when compared with control group 1 (68±3.5[a]) and standard treated group 3 (84±1,8[a]). but were significantly different when compared with diabetic untreated group 2 (94±4.1[b]).

For Bilirubin, the groups 4, 5, 6, 7, 8 and 9 which are the groups feed with the diet were not significantly different (0.66±0.11[a], 0.25±0.014[a], 0.29±0.014[a], 0.51±0.14[a], 0.22±0.041[a], and 0.33±0.014[a] respectively) when compared with control group 1 (0.73± 0.17[a]) and standard treated group 3 (27±0.019[a]). but were significantly different when compared with diabetic untreated group 2 (1.3±0.025[b]).

Table 4.6: Effect of Compounded Diet on Liver Function Parameters of Albino Rat

GROUPS	AST	ALT	ALP	ALBUMIN	PROTEIN	BILIRUBIN
1	35±1.40[a]	23±0.70[a]	74±5.20[a]	33±1.40[a]	68±3.50[a]	0.73±0.17[a]
2	99±1.80[b]	57±3.90[b]	164±5.20[b]	73±4.40[b]	94±4.10[b]	1.3±0.03[b]
3	47±1.60[a]	44±2.20[a]	45±3.90[a]	52±1.10[a]	84±1.80[a]	27±0.02[a]
4	36±1.50[a]	30±4.70[a]	46±2.30[a]	46±1.70[a]	78±1.70[a]	0.66±0.11[a]
5	30±4.60[a]	19±0.80[a]	47±2.30[a]	46±2.80[a]	72±2.60[a]	0.25±0.01[a]
6	33±0.90[a]	22±0.90[a]	42±1.40[a]	45±1.40[a]	66±2.00[a]	0.29±0.01[a]
7	37±1.50[a]	27±3.00[a]	40±2.40[a]	38±3.10[a]	66±3.20[a]	0.51±0.14[a]
8	42±1.50[a]	41±0.80[a]	66±4.40[a]	37±2.40[a]	82±3.00[a]	0.22±0.04[a]
9	37±1.60[a]	36±1.90[a]	50±5.70[a]	44±1.70[a]	72±3.80[a]	0.33±0.014[a]

Data are presented as means ± standard deviation; n= 4 for each group. For each parameter, values bearing different superscript letters are different ($P<0.05$). Group 1: normal control, Group 2: diabetic untreated, Group 3: standard drug treated, Group 4, 5 and 6: 15%, 30% and 60% compounded diet fed group respectively. Group 7, 8 and 9: fed 15%, 30% and 60% compounded diet respectively before inducing.

4.8: Effect of Compounded Diet on Kidney Function Parameters of Albino Rats

Table 4.7 showed the effect of the compounded diet on Kidney Function parameters of albino rats studied. The results are presented in mean ± standard error of mean deviation and values bearing different superscript letters are significantly different ($P<0.05$). The Kidney function parameters presented are Urea, Creatinine, Chloride, Sodium, Potassium and Bicarbonate.

The result showed that the Urea for the groups 4, 5, 6, 7, 8 and 9 which are the groups feed with the diet were not significantly different (8.5 ± 0.99^a, 98 ± 0.76^a, 78 ± 0.70^a, 8.9 ± 1.4^a, 12 ± 0.55^a, and 6.2 ± 0.31^a respectively) when compared with control group 1 (27 ± 5.2^a) and standard treated group 3(9.5 ± 0.68^a). But were significantly different when compared with diabetic untreated group 2 (55 ± 2.5^b).

For Creatinine, the groups 4, 5, 6, 7, 8 and 9 which are the groups feed with the diet were not significantly different (0.045 ± 0.0029^a, 0.073 ± 0.016^a, 0.10 ± 0.025^a, 0.093 ± 0.015^a, 0.11 ± 0.016^a, and 0.085 ± 0.0065^a respectively) when compared with control group 1 (1.0 ± 0.065^a) and standard treated group 3 (0.08 ± 0.019^a). But were significantly different when compared with diabetic untreated group 2 (1.6 ± 0.11^b).

The result of Chloride showed that the groups 4, 5, 6, 7, 8 and 9 which are the groups feed with the diet were not significantly different (91 ± 2.0^a, 95 ± 1.6^a, 75 ± 1.8^a, 60 ± 5.7^a, 95 ± 1.1^a, and 98 ± 4.0^a respectively) when compared with control group 1 (102 ± 1.8^a) and standard treated group 3 (80 ± 2.4^a). but were significantly different when compared with diabetic untreated group 2 (110 ± 1.0^b).

The same trend was observed for Sodium were the groups 4, 5, 6, 7, 8 and 9 which are the groups feed with the diet were not significantly different (136 ± 1.4^a, 138 ± 3.3^a, 136 ± 1.2^a, 139 ± 1.5^a, 142 ± 0.88^a, and 140 ± 3.8^a respectively) when compared with control group 1 (139 ± 0.81^a) and standard treated group 3 (137 ± 0.58^a). But were significantly different when compared with diabetic untreated group 2 (161 ± 4.2^b).

For Potassium, the groups 4, 5, 6, 7, 8 and 9 which are the groups feed with the diet were not significantly different (4.2 ± 0.29^{a}, 3.3 ± 0.084^{a}, 4.5 ± 0.16^{a}, 1.4 ± 0.068^{a}, 1.8 ± 0.070^{a}, and 1.5 ± 0.041^{a} respectively) when compared with control group 1 (3.7 ± 0.10^{a}) and standard treated group 3 (3.6 ± 0.29^{a}). but were significantly different when compared with diabetic untreated group 2 (6.9 ± 0.40^{b}).

For Bicarbonate, the groups 4, 5, 6, 7, 8 and 9 which are the groups feed with the diet were not significantly different (18 ± 2.2^{a}, 27 ± 1.1^{a}, 24 ± 0.24^{a}, 20 ± 1.4^{a}, 23 ± 0.61^{a}, and 25 ± 1.4^{a} respectively) when compared with control group 1 (26 ± 0.68^{a}) and standard treated group 3 (25 ± 0.51^{a}). but were significantly different when compared with diabetic untreated group 2 (31 ± 1.1^{b}).

Table 4.7: Effect of treatment on kidney function parameters of albino rat

Groups	Urea	Creatinine	Chloride	Sodium	Potassium	Bicarbonate
1	27±5.20[a]	1.0±0.06[a]	102±1.80[a]	139±0.81[a]	3.7±0.10[a]	26±0.68[a]
2	55±2.50[b]	1.6±0.11[b]	110±1.00[b]	161±4.20[b]	6.9±0.40[b]	31±1.10[b]
3	9.5±0.68[a]	0.08±0.02[a]	80±2.40[a]	137±0.58[a]	3.6±0.29[a]	25±0.51[a]
4	8.5±0.99[a]	0.045±0.01[a]	91±2.00[a]	136±1.40[a]	4.2±0.29[a]	18±2.20[a]
5	9.8±0.76[a]	0.073±0.02[a]	95±1.60[a]	138±3.30[a]	3.3±0.08[a]	27±1.10[a]
6	78±0.70[a]	0.10±0.03[a]	75±1.80[a]	136±1.20[a]	4.5±0.16[a]	24±0.24[a]
7	8.9±1.40[a]	0.093±0.01[a]	60±5.70[a]	139±1.50[a]	1.4±0.06[a]	20±1.40[a]
8	12±0.55[a]	0.11±0.02[a]	95±1.10[a]	142±0.88[a]	1.8±0.07[a]	23±0.61[a]
9	6.2±0.31[a]	0.085±0.02[a]	98±4.00[a]	140±3.80[a]	1.5±0.04[a]	25±1.40[a]

Data are presented as means ± standard deviation; n= 4 for each group. For each parameter, values bearing different superscript letters are different ($P<0.05$). Group 1: normal control, Group 2: diabetic untreated, Group 3: standard drug treated, Group 4, 5 and 6: 15%, 30% and 60% compounded diet fed group respectively. Group 7, 8 and 9: fed 15%, 30% and 60% compounded diet respectively before inducing.

4.9: Effect of Compounded Diet on Lipid Profile Parameters of Albino Rats

Table 4.8 showed the effect of the compounded diet on Lipid Profile parameters of albino rats studied. The results are presented in mean ± standard error of mean deviation and values bearing different superscript letters are significantly different ($P<0.05$). The Lipid Profile parameters presented are Cholesterol, Triglyceride, HDL and LDL.

The result showed that the Cholesterol for the groups 4, 5, 6, 7, 8 and 9 which are the groups feed with the diet were not significantly different (105 ± 26^a, 156 ± 26^a, 128 ± 25^a, 140 ± 12^a, 133 ± 8.6^a, and 93 ± 8.4^a respectively) when compared with control group 1 (144 ± 5.9^a) and standard treated group 3(105 ± 12^a). But were significantly different when compared with diabetic untreated group 2 (245 ± 12^b).

For Triglyceride, the groups 4, 5, 6, 7, 8 and 9 which are the groups feed with the diet were not significantly different (97 ± 9.0^a, 106 ± 8.5^a, 78 ± 1.4^a, 106 ± 1.3^a, 124 ± 1.8^a, and 70 ± 4.0^a respectively) when compared with control group 1 (137 ± 3.4^a) and standard treated group 3 (101 ± 1.7^a). But were significantly different when compared with diabetic untreated group 2 (165 ± 3.0^b).

The result of HDL showed that the groups 4, 5, 6, 7, 8 and 9 which are the groups feed with the diet were not significantly different (46 ± 4.0^a, 52 ± 3.7^a, 54 ± 1.5^a, 52 ± 3.3^a, 54 ± 2.1^a, and 55 ± 8.0^a respectively) when compared with control group 1 (54 ± 1.5^a) and standard treated group 3 (46 ± 3.0^a). but were significantly different when compared with diabetic untreated group 2 (34 ± 3.0^b).

The same trend was observed for LDL were the groups 4, 5, 6, 7, 8 and 9 which are the groups feed with the diet were not significantly different (115 ± 1.5^a, 121 ± 2.6^a, 101 ± 1.5^a, 112 ± 3.4^a, 81 ± 2.2^a, and 96 ± 1.8^a respectively) when compared with control group 1 (104 ± 1.7^a) and standard treated group 3 (113 ± 1.0^a). But were significantly different when compared with diabetic untreated group 2 (156 ± 1.9^b).

Table 4.8 Effect of treatment on lipid profile function parameters of albino rat

Groups	Cholesterol	Triglyceride	HDL	LDL
1	144± 5.90[a]	137± 3.40[a]	54± 1.50[a]	104±1.70[a]
2	245±12[b]	165± 3.00[b]	34± 3.00[b]	156±1.90[b]
3	105± 12[a]	101± 17[a]	46±3.00[a]	113±1.00[a]
4	105± 26[a]	97± 9.00[a]	46± 4.00[a]	115± 1.50[a]
5	156± 26[a]	106± 8.50[a]	52± 3.70[a]	121± 2.60[a]
6	128± 25[a]	78± 14[a]	54± 1.50[a]	101± 1.50[a]
7	140± 12[a]	106± 13[a]	52± 3.30[a]	112± 3.40[a]
8	133± 8.60[a]	124± 1.80[a]	54± 2.10[a]	81± 2.20[a]
9	93± 8.40[a]	70± 4.00[a]	55± 8.00[a]	96± 1.80[a]

Data are presented as means ± standard error mean; n= 4 for each group. For each parameter, values bearing different superscript letters are different (P<0.05). Group 1: normal control, Group 2: diabetic untreated, Group 3: standard drug treated, Group 4, 5 and 6: 15%, 30% and 60% compounded diet fed group respectively. Group 7, 8 and 9: fed 15%, 30% and 60% compounded diet respectively before inducing.

4.10: Photomicrograph of Pancreas for Groups 1 to 9

Plate A and B showed the Photomicrograph of the Pancreas of Group 1, 2, 3, 4, 5, 6, 7, 8, and 9. The photomicrograph was presented in plate form.

Plate 1 shows the Photomicrograph of the Pancreas of Group 1 which served as Control group. From the plate, it shows that the pancreas is histologically normal with Pancreatic Islet (PI) containing pancreatic Islet cells ($\alpha\ \beta\ \delta$). Intact Pancreatic Acini (PA), Blood Vessels (BV), Pancreatic Duct (D) and Interlobular Connective Tissue (CT).

Plate 2 shows the Photomicrograph of the Pancreas of Group 2 which served as diabetic untreated group. From the plate, it shows that the pancreas is histologically distorted with damaged Pancreatic Islet (PI), Intact Pancreatic Acini (PA), Patent Blood Vessels (BV), and Patent Pancreatic Duct (D).

Plate 3 shows the Photomicrograph of the Pancreas of Group 3 which served as Standard drug treated group. it shows that the pancreas is histologically normal with Pancreatic Islet (PI) containing pancreatic Islet cells ($\alpha\ \beta\ \delta$). Intact Pancreatic Acini (PA), Blood Vessels (BV), Pancreatic Duct (D) and Interlobular Connective Tissue (CT).

Plate 4, 5, and 6 shows the Photomicrograph of the Pancreas of Group 4, 5 and 6 which served as 15%, 30% and 60% compounded diet fed group respectively. it shows that the pancreas is histologically normal with Pancreatic Islet (PI) containing pancreatic Islet cells ($\alpha\ \beta\ \delta$). Intact Pancreatic Acini (PA), Blood Vessels (BV), Pancreatic Duct (D) and Interlobular Connective Tissue (CT).

Plate 7, 8, and 9 shows the Photomicrograph of the Pancreas of Group 7, 8 and 9 which served as the group that was fed 15%, 30% and 60% compounded diet for 14 days and afterwards induced with diabetes respectively. it shows that the pancreas is histologically normal with

Pancreatic Islet (PI) containing pancreatic Islet cells (α β δ). Intact Pancreatic Acini (PA), Blood Vessels (BV), Pancreatic Duct (D) and Interlobular Connective Tissue (CT).

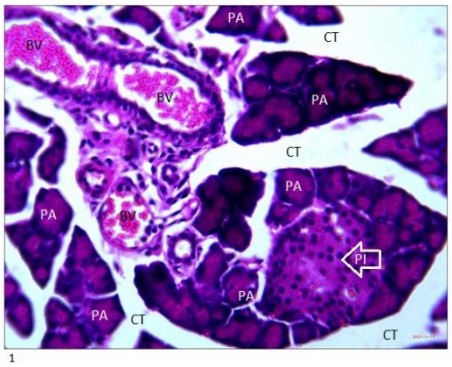


Plate 1: Photomicrograph of Pancreas of rat in grp 1 normal control (X 400) Stain: H&E. Histologically normal pancreas with pancreatic islet (PI), Pancreatic acini (PA), blood vessel (BV), and connective tissues (CT)

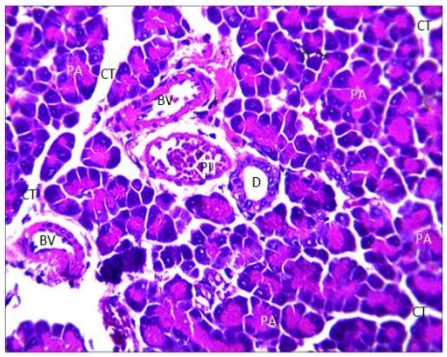


Plate 2: Photomicrograph of Pancreas of rat in grp 2 diabetic untreated (X 400) Stain: H&E. Histologically distorted pancreas Atrophic pancreatic islet (PI) with scanty islet cells

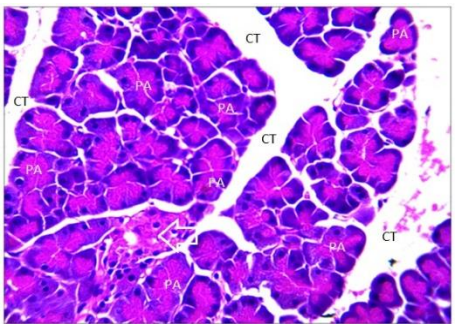


Plate 3: Photomicrograph of pancreas of rat in group 3 treated with Standard (Glibenclamide drug) for 21 days, (X400), Stain: H&E. Histologically normal pancreas with pancreatic islet (PI), Pancreatic acini (PA), blood vessel (BV), and connective tissues (CT)

Plate A: Photomicrography of Pancreas for Grp 1-6

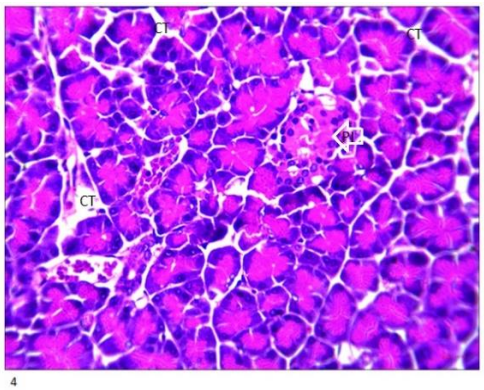


Plate 4: Photomicrograph of pancreas of diabetic rat in grp 4 fed 15% Sesamum indicum compounded diet for 14 days, (X 400), Stain: H&E. Histologically normal pancreas with pancreatic islet (PI), Pancreatic acini (PA), blood vessel (BV), and connective tissues (CT)

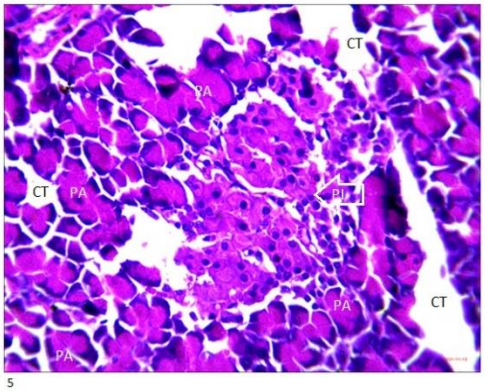


Plate 5: Photomicrograph of pancreas of diabetic rat in grp 5 fed 30% Sesamum indicum compounded diet for 14 days, (X 400), Stain: H&E. Histologically normal pancreas with pancreatic islet (PI), Pancreatic acini (PA), blood vessel (BV), and connective tissues (CT)

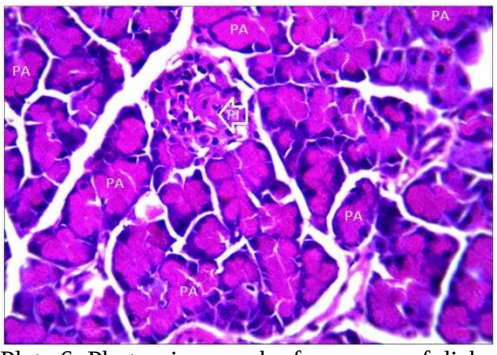


Plate 6: Photomicrograph of pancreas of diabetic rat in grp 6 fed 60% Sesamum indicum compounded diet for 14 days, (X 400), Stain: H&E Histologically normal pancreas with pancreatic islet (PI), Pancreatic acini (PA), blood vessel (BV), and connective tissues (CT)

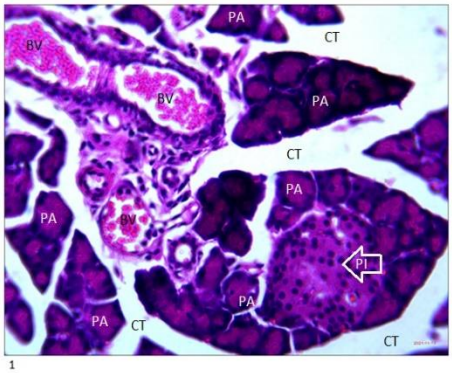


Plate 1: Photomicrograph of Pancreas of rat in grp 1 normal control (X 400) Stain: H&E. Histologically normal pancreas with pancreatic islet (PI), Pancreatic acini (PA), blood vessel (BV), and connective tissues (CT)

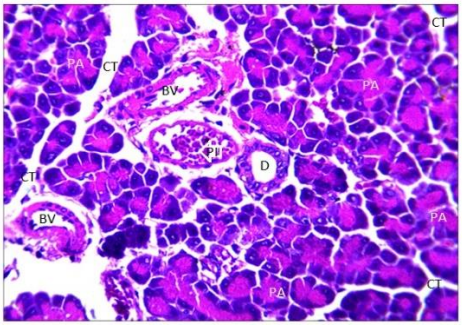


Plate 2: Photomicrograph of Pancreas of rat in grp 2 diabetic untreated (X 400) Stain: H&E. Histologically distorted pancreas Atrophic pancreatic islet (PI) with scanty islet cells

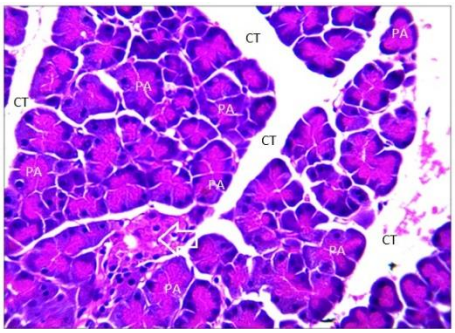


Plate 3: Photomicrograph of pancreas of rat in group 3 treated with Standard (Glibenclamide drug) for 21 days, (X400), Stain: H&E. Histologically normal pancreas with pancreatic islet (PI), Pancreatic acini (PA), blood vessel (BV), and connective tissues (CT)

Plate B: Photomicrography of Pancreas for grp 1-3, 7-9

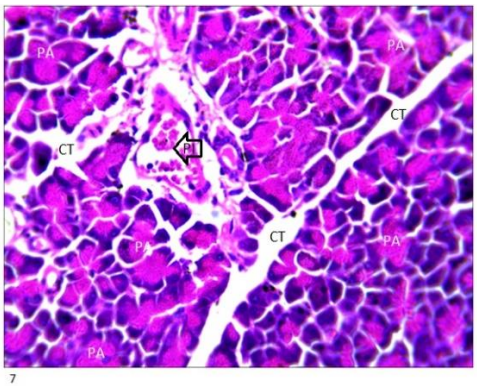


Plate 7: Photomicrograph of pancreas of rat in grp 7 fed 15% Sesamum indicum compounded diet for 14 days and induced with diabetic afterwards, (X 400), Stain: H&E. Histologically normal pancreas with pancreatic islet (PI), Pancreatic acini (PA), blood vessel (BV), and connective tissues (CT)

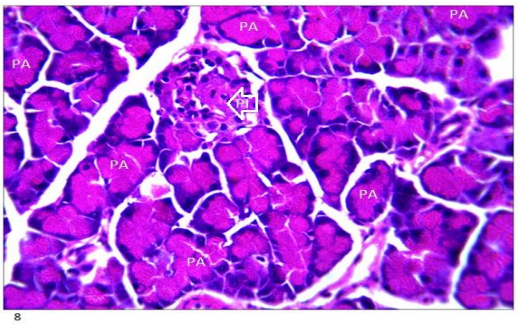


Plate 8: Photomicrograph of pancreas of rat in grp 8 fed 30% Sesamum indicum compounded diet for 14 days and induced with diabetic afterwards, (X 400), Stain: H&E. Histologically normal pancreas with pancreatic islet (PI), Pancreatic acini (PA), blood vessel (BV), and connective tissues (CT)

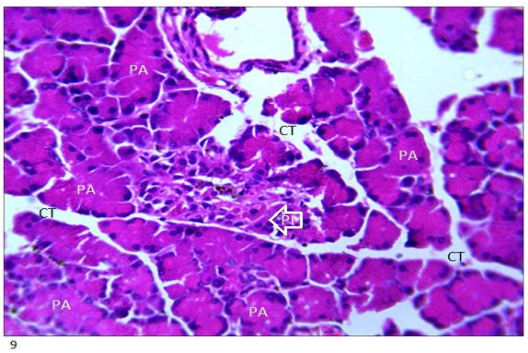


Plate 9: Photomicrograph of pancreas of rat in grp 9 fed 60% Sesamum indicum compounded diet for 14 days and induced with diabetic afterwards, (X 400), Stain: H&E. Histologically normal pancreas with pancreatic islet (PI), Pancreatic acini (PA), blood vessel (BV), and connective tissues (CT)

4.11: Photomicrograph of Kidney for Group 1-9

Plate A and B showed the Photomicrograph of the Kidney of Group 1, 2, 3, 4, 5, 6, 7, 8, and 9. Plate 1 shows the Photomicrograph of the Kidney of Group 1 which served as Control group. From the plate, it shows that the Kidney is histologically normal with intact Glomerular Tuft(G) containing Mesangial cells, Mesangial Matrix and Capillaries. Patent Bowmans Capsular (C) space. Renal tubule is lined with simple epithelia cells

Plate 2 shows the Photomicrograph of the Kidney of Group 2 which served as diabetic untreated group. From the plate, it shows that the Kidney is histologically distorted with Interstitial inflammation (INF), intact Glomerular Tuft (G) containing Mesangial cells, Mesangial Matrix and Capillaries. Patent Bowmans Capsular (C) space. Renal tubule is lined with simple epithelia cells.

Plate 3 shows the Photomicrograph of the Kidney of Group 3 which served as Standard drug treated group. it shows that the Kidney is histologically normal with intact Glomerular Tuft(G) containing Mesangial cells, Mesangial Matrix and Capillaries. Patent Bowmans Capsular (C) space. Renal tubule is lined with simple epithelia cells

Plate 4, 5, and 6 shows the Photomicrograph of the Kidney of Group 4, 5 and 6 which served as 15%, 30% and 60% compounded diet fed group respectively. it shows that the Kidney is histologically normal with intact Glomerular Tuft(G) containing Mesangial cells, Mesangial Matrix and Capillaries. Patent Bowmans Capsular (C) space. Renal tubule is lined with simple epithelia cells.

Plate 7, 8, and 9 shows the Photomicrograph of the Kidney of Group 7, 8 and 9 which served as the group that was fed 15%, 30% and 60% compounded diet for 14 days and afterwards induced with diabetes respectively. it shows that the Kidney is histologically normal with intact

Glomerular Tuft(G) containing Mesangial cells, Mesangial Matrix and Capillaries. Patent Bowmans Capsular (C) space. Renal tubule is lined with simple epithelia cells

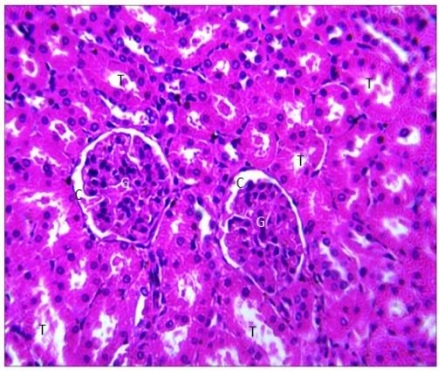


Plate 1: Photomicrograph of Kidney of rat in grp 1 normal control (X 400) Stain: H&E. Histologically normal kidney with Glomerular tuft (G), patent bowman's capsular space (C), and renal tubules (T)

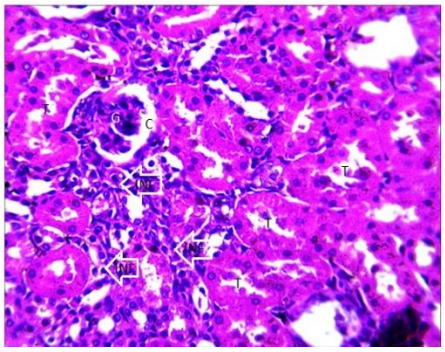


Plate 2: Photomicrograph of Kidney of rat in grp 2 diabetic untreated (X 400) Stain: H&E. Histologically distorted kidney with interstitial inflation (INF)

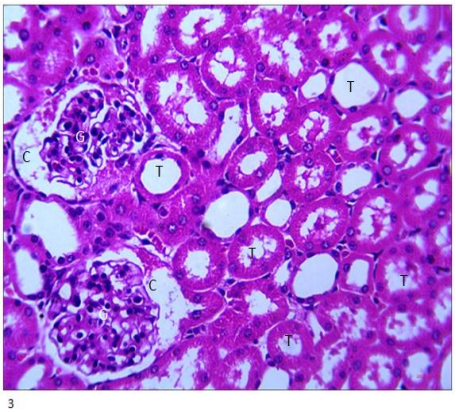


Plate 3: Photomicrograph of Kidney of rat in group 3 treated with Standard (Glibenclamide drug) for 21 days, (X400), Stain: H&E Histologically normal kidney with Glomerular tuft (G), patent bowman's capsular space (C), and renal tubules (T)

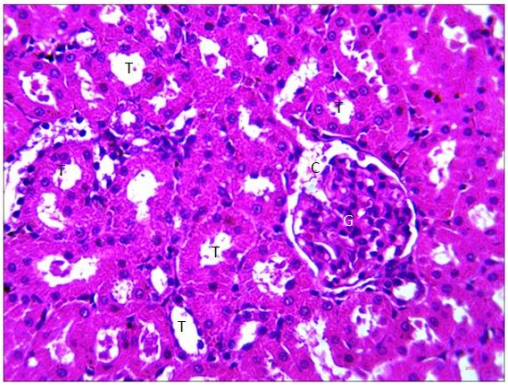


Plate 4: Photomicrograph of Kidney of diabetic rat in grp 4 fed 15% Sesamum indicum compounded diet for 14 days, (X 400), Stain: H&E. Histologically normal kidney with Glomerular tuft (G), patent bowman's capsular space (C), and renal tubules (T)

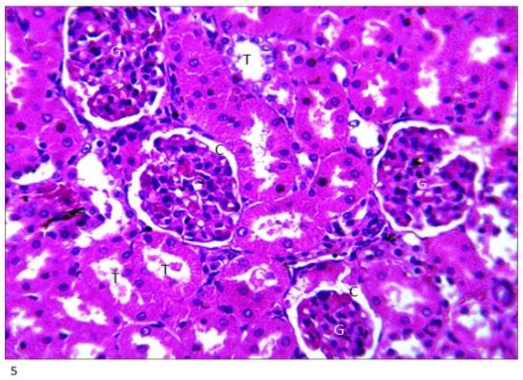


Plate 5: Photomicrograph of Kidney of diabetic rat in grp 5 fed 30% Sesamum indicum compounded diet for 14 days, (X 400), Stain: H&E. Histologically normal kidney with Glomerular tuft (G), patent bowman's capsular space (C), and renal tubules (T)

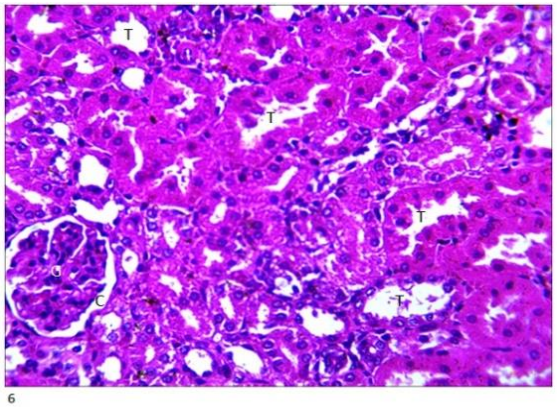


Plate 6: Photomicrograph of pancreas of diabetic rat in grp 6 fed 60% Sesamum indicum compounded diet for 14 days, (X 400), Stain: H&E. Histologically normal kidney with Glomerular tuft (G), patent bowman's capsular space (C), and renal tubules (T)

Plate C: Photomicrography of Kidney for Grp 1-6

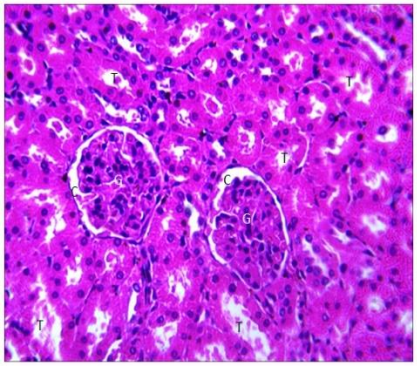


Plate 1: Photomicrograph of Kidney of rat in grp 1 normal control (X 400) Stain: H&E. Histologically normal kidney with Glomerular tuft (G), patent bowman's capsular space (C), and renal tubules (T)

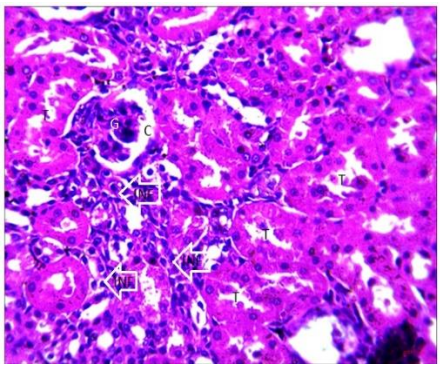


Plate 2: Photomicrograph of Kidney of rat in grp 2 diabetic untreated (X 400) Stain: H&E. Histologically distorted kidney with interstitial inflation (INF)

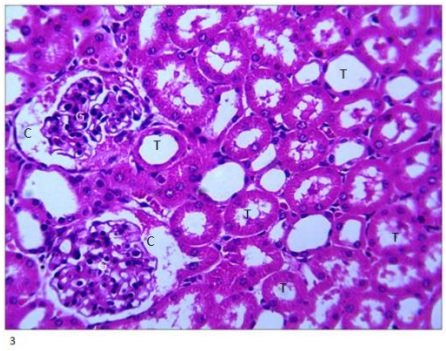


Plate 3: Photomicrograph of Kidney of rat in group 3 treated with Standard (Glibenclamide drug) for 21 days, (X400), Stain: H&E Histologically normal kidney with Glomerular tuft (G), patent bowman's capsular space (C), and renal tubules (T)

Plate D: Photomicrography of Kidney for grp 1-3, 7-9

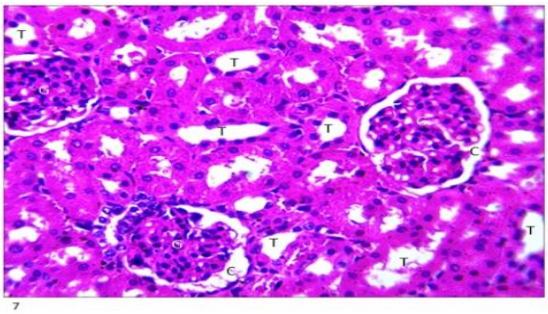


Plate 7: Photomicrograph of Kidney of rat in grp 7 fed 15% Sesamum indicum compounded diet for 14 days and induced with diabetic afterwards, (X 400), Stain: H&E Histologically normal kidney with Glomerular tuft (G), patent bowman's capsular space (C), and renal tubules (T)

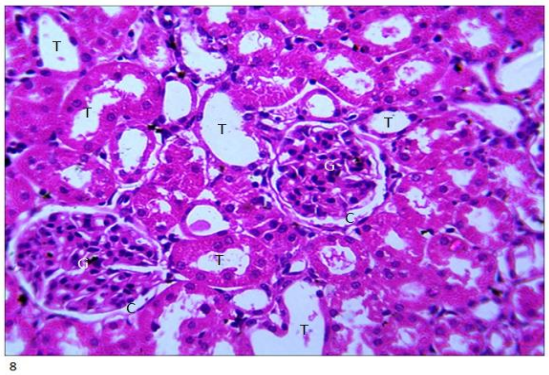


Plate 8: Photomicrograph of Kidney of rat in grp 8 fed 30% Sesamum indicum compounded diet for 14 days and induced with diabetic afterwards, (X 400), Stain: H&E Histologically normal kidney with Glomerular tuft (G), patent bowman's capsular space (C), and renal tubules (T)

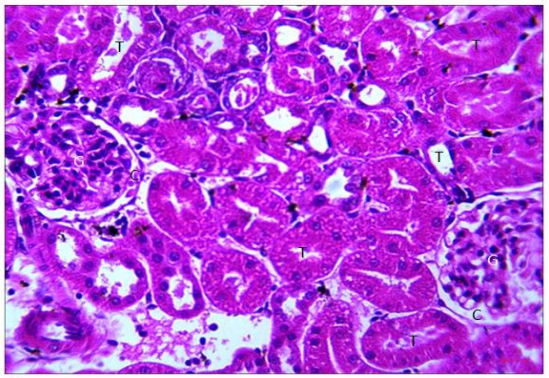


Plate 9: Photomicrograph of Kidney of rat in grp 9 fed 60% Sesamum indicum compounded diet for 14 days and induced with diabetic afterwards, (X 400), Stain: H&E Histologically normal kidney with Glomerular tuft (G), patent bowman's capsular space (C), and renal tubules (T)

4.12: Photomicrograph of Liver of Group 1-9

Plate A and B showed the Photomicrograph of the Liver of Group 1, 2, 3, 4, 5, 6, 7, 8, and 9. The photomicrograph was presented in plate form.

Plate 1 shows the Photomicrograph of the Liver of Group 1 which served as Control group. From the plate, it shows that the Liver is histologically normal with Intact Hepatocytes (H), Sinusoid (S) containing Kuffer cells, Patent Central Vein (CV) and intact Hepatic Artery.

Plate 2 shows the Photomicrograph of the Liver of Group 2 which served as diabetic untreated group. From the plate, it shows that the Liver is histologically distorted with Periportal Inflammation (INF), Congested Hepatic Artery, intact Hepatocytes (H) and Sinusoid (S) containing Kupffer cells.

Plate 3 shows the Photomicrograph of the Liver of Group 3 which served as Standard drug treated group. it shows that the Liver is histologically normal Intact Hepatocytes (H), Sinusoid (S) containing Kuffer cells, Patent Central Vein (CV) and intact Hepatic Artery.

Plate 4, 5, and 6 shows the Photomicrograph of the Liver of Group 4, 5 and 6 which served as 15%, 30% and 60% compounded diet fed group respectively. it shows that the Liver is histologically normal with Intact Hepatocytes (H), Sinusoid (S) containing Kuffer cells, Patent Central Vein (CV) and intact Hepatic Artery.

Plate 7, 8, and 9 shows the Photomicrograph of the Liver of Group 7, 8 and 9 which served as the group that was fed 15%, 30% and 60% compounded diet for 14 days and afterwards induced with diabetes respectively. it shows that the Liver is histologically normal with Intact Hepatocytes (H), Sinusoid (S) containing Kuffer cells, Patent Central Vein (CV) and intact Hepatic Artery.

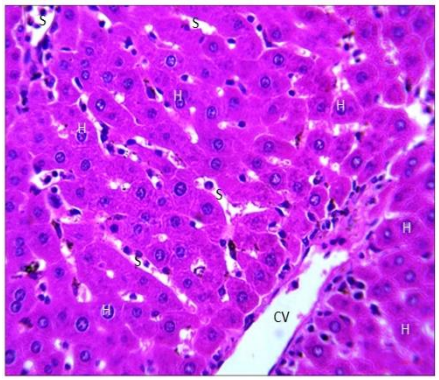


Plate 1: Photomicrograph of Liver of rat in grp 1 normal control (X 400) Stain: H&E. Histologically normal liver, intact hepatocytes (H), sinusoid (S) and patent central vein (CV)

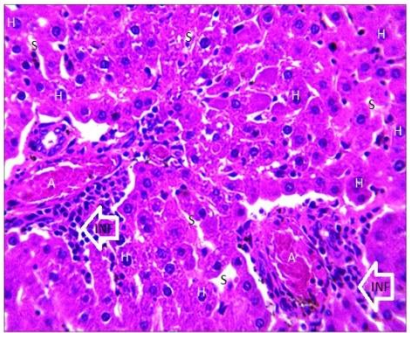


Plate 2: Photomicrograph of Liver of rat in grp 2 diabetic untreated (X 400) Stain: H&E. Histologically distorted liver, periportal inflammation (INF) and patent central vein

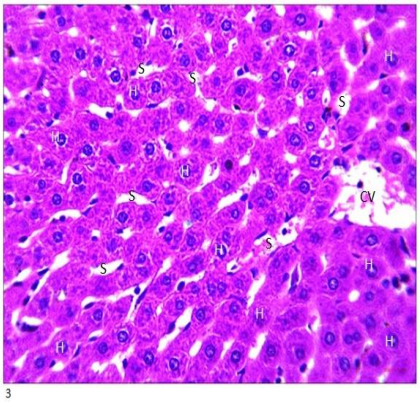


Plate 3: Photomicrograph of Liver of rat in group 3 treated with Standard (Glibenclamide drug) for 21 days, (X400), Stain: H&E. Histologically normal liver, intact hepatocytes (H), sinusoid (S) and patent central vein (CV)

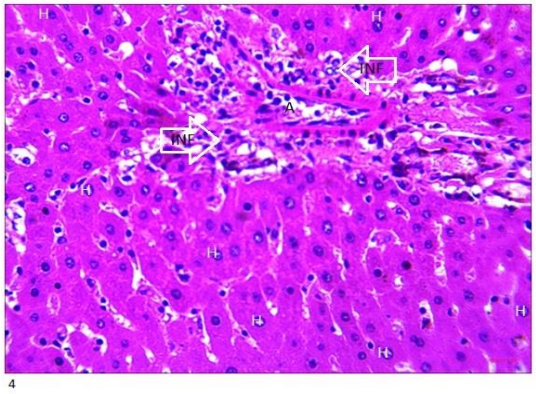


Plate 4: Photomicrograph of Liver of diabetic rat in grp 4 fed 15% Sesamum indicum compounded diet for 14 days, (X 400), Stain: H&E. Histologically normal liver, intact hepatocytes (H), sinusoid (S) and patent central vein (CV)

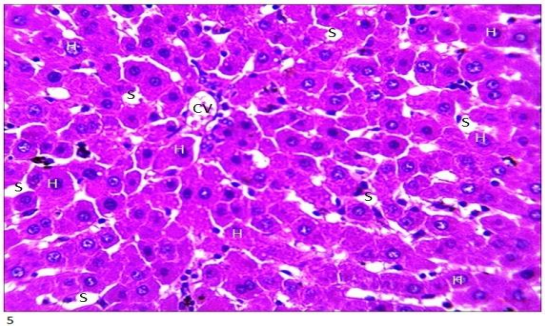


Plate 5: Photomicrograph of Liver of diabetic rat in grp 5 fed 30% Sesamum indicum compounded diet for 14 days, (X 400), Stain: H&E. Histologically normal liver, intact hepatocytes (H), sinusoid (S) and patent central vein (CV)

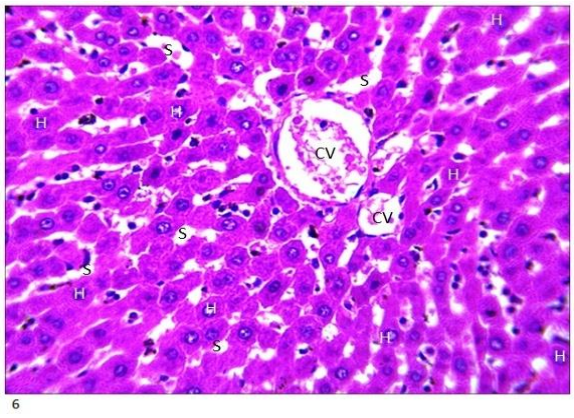


Plate 6: Photomicrograph of Liver of diabetic rat in grp 6 fed 60% Sesamum indicum compounded diet for 14 days, (X 400), Stain: H&E. Histologically normal liver, intact hepatocytes (H), sinusoid (S) and patent central vein (CV)

Plate E: Photomicrography of Liver for Grp 1-6

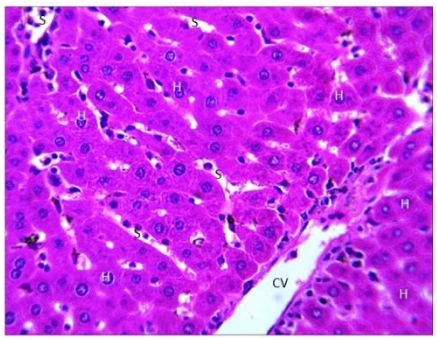


Plate 1: Photomicrograph of Liver of rat in grp 1 normal control (X 400) Stain: H&E. Histologically normal liver, intact hepatocytes (H), sinusoid (S) and patent central vein (CV)

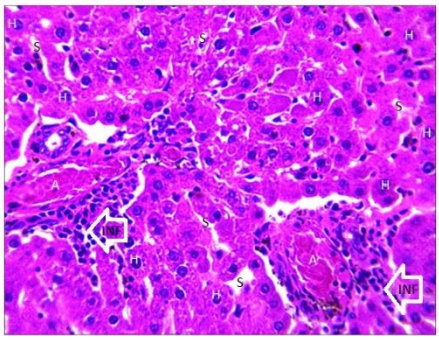


Plate 2: Photomicrograph of Liver of rat in grp 2 diabetic untreated (X 400) Stain: H&E. Histologically distorted liver, periportal inflammation (INF) and patent central vein

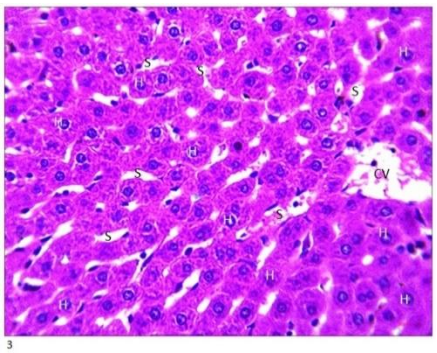


Plate 3: Photomicrograph of Liver of rat in group 3 treated with Standard (Glibenclamide drug) for 21 days, (X400), Stain: H&E. Histologically normal liver, intact hepatocytes (H), sinusoid (S) and patent central vein (CV)

Plate F: Photomicrography of Liver for grp 1-3, 7-9

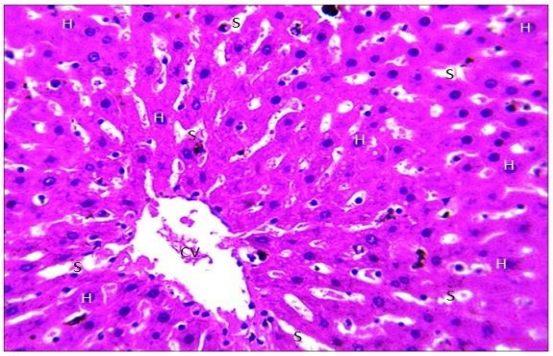


Plate 7: Photomicrograph of Liver of rat in grp 7 fed 15% Sesamum indicum compounded diet for 14 days and induced with diabetic afterwards, (X 400), Stain: H&E. Histologically normal liver, intact hepatocytes (H), sinusoid (S) and patent central vein (CV)

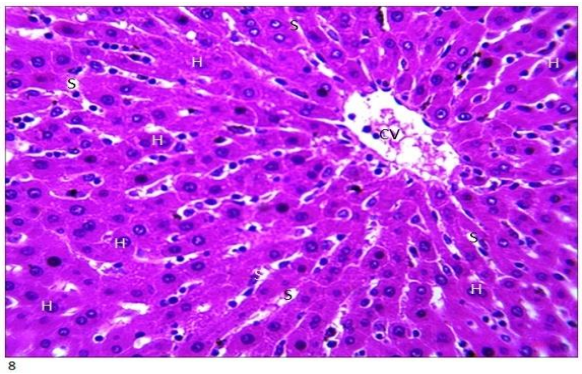


Plate 8: Photomicrograph of Liver of rat in grp 8 fed 30% Sesamum indicum compounded diet for 14 days and induced with diabetic afterwards, (X 400), Stain: H&E. Histologically normal liver, intact hepatocytes (H), sinusoid (S) and patent central vein (CV)

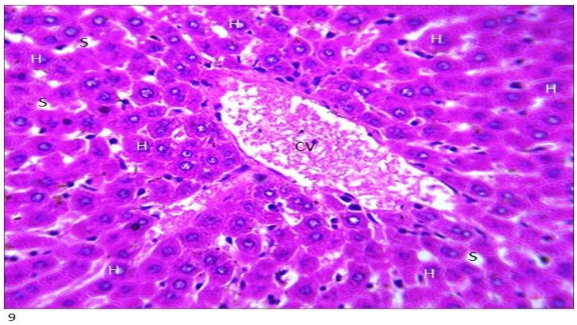


Plate 9: Photomicrograph of Liver of rat in grp 9 fed 60% Sesamum indicum compounded diet for 14 days and induced with diabetic afterwards, (X 400), Stain: H&E. Histologically normal liver, intact hepatocytes (H), sinusoid (S) and patent central vein (CV)

CHAPTER FIVE

DISCUSSION AND RECOMMENDATION

5.1 Discussion

The low moisture content and ash content found in Sesamum indicum seed fall within the range reported for most seeds and nuts (FAO, 1968; Oyenuga, 1968). This low moisture content is an index of stability, quality, shelf life and also high yield (Levander, 1990). The presence of the ash is a reflection of inorganic matter in a food sample. The result of the lipid suggest that the seed is a rich source of oil.

The presence of saturated and unsaturated fatty acids discovered by the GC/MS analysis justifies the use of this plant to treat many ailments in folk and traditional medicine. The presence of phenolic compounds and unsaturated fatty acid are possible contributor for anti-oxidant activity of the seed extract.

Increased level of blood glucose which were observed after the induction of diabetes is in line with literature. It has been known from literature that induction of diabetes with alloxan partially damages the pancreatic insulin and as a result lead to diabetes (Kar, 2007). Groups fed with the compounded feed, whose blood glucose level were observed to significantly reduce may be attributed to the presence of some phytochemicals present in the seed. From literature, the seed has been known to contain Sesamin, Alkaloids, Tannins, Flavonoids, Saponins (Ramesh at al., 2007). Sesamin and Flavonoid has hypoglycemic properties (Gougeon *et al.,* 2000). This hypoglycemic property brought about reduction in blood glucose level of the groups fed with the compounded diet. The standard drug (Glibenclamide) works by activating the pancreas to release more insulin and it works effectively in the presence of some pancreatic beta cell activity (Gougeon *et al.,* 2000).

In the development of disorder or disease in the body, Lipids plays a very important role. For diabetes, elevation of blood glucose level is normally followed by elevation of Low-density Lipoprotein (LDL), Triglyceride and Cholesterol with a reduction in the level of High-density Lipoprotein (HDL). This trend was observed in the result of this research; where the diabetic untreated group has elevated Cholesterol, LDL and Triglyceride with a decrease in HDL level while the opposite was observed for the groups fed with the compounded diet or treated with the standard drug. This observation is in line with the report of Ashraduzzaman et al (2001) on the effect of *Vigna inguiculata* seed oil in diabetic rats. It is also in conformity with the report of Khosla et al (1995) on administration of Fenugreek seed extract on diabetic rats.

It has been proven by the report of Shih et al (1997) on Acipinox attenuates hypertriglyceridemia in diabetic patients, that the elevation in triglyceride of diabetic animals may be as a result of insulin deficiency which results in hyperglycemia; when fatty acids from the adipose tissues are mobilized for purpose of energy, excess of it are accumulated in the liver, which are then converted to Triglyceride (Shih *et al.,* 1997).

The decrease in the Cholesterol level observed in the groups fed with the compounded diet may be as a result of the presence of saponin in the seed, which have been reported to form complexes with Cholesterol and bile acids preventing them from being absorbed through the small intestine thereby decreasing the Cholesterol level in the blood and liver (Shih *et al.,* 1997).

Report has also shown that insulin elevates the number of Low-Density Lipoprotein receptors. Therefore, insulin deficiency might be linked to a decrease level of LDL receptors that yields more LDL particles and as a result increases the level of LDL-Cholesterol in diabetes. The oil of *Sesamum indicum* has been reported to maintain good Cholesterol (HDL) and decrease bad

Cholesterol (Sirato *et al.,* 2001). Hence the increase in HDL observed in this research work is in line with literature.

Increase in the activities of AST, ALP and ALT in diabetic rats indicates that diabetes might have been induced due to liver dysfunction which might result to the leakage from the cytosol of hepatic cells into the blood stream. These enzymes are used as markers in hepatic injury.

Reduction in the level of these enzymes in the treated groups may be an indication of a reversal of the effect of high blood glucose in the diabetic rats and resultantly alleviate liver damage caused by alloxan.

The effect of sesamum diet in protecting group 7, 8 and 9 from elevated blood glucose and increased liver enzymes can be attributed to the presence of sesamin. Sesamin has been reported to protect the liver from oxidation damage (Sirato *et al.,* 2001). Hence the reason for decrease blood sugar and liver enzyme markers in group 7, 8 and 9.

The anti-diabetic effect of the compounded sesamum indicum diet was further concluded by the histopathological examinations. The results showed significant increase and improved liver, kidney and pancreas for the groups fed with the compounded diet when compared with the tissues of the diabetic untreated group.

The histopathological study of diabetes treated groups indicates increase in the volume density of islets, % of beta cells and size of islets which may be a sign of regeneration along with beta cell repairs.

Photo-micro graphical data in this study suggest that healing of pancreas by the diet may be a plausible mechanism of the anti-diabetic activity of sesamum indicum seed.

5.2 Conclusion

This research has shown that *Sesamum indicum* compounded diet has the ability to reduce blood sugar level, cholesterol, triglyceride, LDL, regulate the kidney function parameters and Liver biomarkers that are high in diabetes and as well protect the liver from damage by alloxan.

These properties could be exploited in the management and remedy of diabetes. It is possible that *Sesamum indicum* diet can be useful in the management of other related diseases especially those associated with hyper-lipidemia.

The use of natural anti-diabetic Sesamum compounded diet might be useful against pancreatic damage effect.

More so, the result showed that consumption of Sesame seed over time will protect one from possible diabetic condition that might arise in future.

5.3 Contribution to Knowledge

This research work has contributed to the knowledge by proving that inclusion of *Sesamum indicum* in the diet of diabetic patients will result to a decrease in their blood sugar level, Lipid and Liver Biomarkers. Also, that consumption of this seed has both protective and ameliorative effect on diabetes.

5.4 Recommendation

This research work should be carried out using streptozotocin and also higher concentration of the diet can be used as well to evaluate its effect on the diabetic albino rats.

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APPENDIX

Parameter	
Table Analyzed	Blood Glucose
Two-way RM ANOVA	Matching by rows

Source of Variation	% of total variation	P value
Interaction	31.46	< 0.0001
Groups	42.23	< 0.0001
Blood Glucose Values	21.75	< 0.0001
Subjects (matching)	2.1704	< 0.0001

Source of Variation	P value summary	Significant?
Interaction	***	Yes
Groups	***	Yes
Blood Glucose Values	***	Yes
Subjects (matching)	***	Yes

Source of Variation	Df	Sum-of-squares	Mean square	F
Interaction	32	246776	7712	44
Groups	8	331181	41398	66
Blood Glucose Values	4	170564	42641	245
Subjects (matching)	27	17023	630	3.6
Residual	108	18777	174	

Number of missing values	0

Bonferroni posttests

Basal Blood Gluose Values vs Blood Glucose Values at Day 3

Groups	Basal Blood Gluose Values		Blood Glucose Values at Day 3	Difference	95% CI of diff.
Group 1	97	90	-7.0	-38 to 24	
Group 2	87	236	149	118 to 179	
Group 3	93	229	136	105 to 166	
Group 4	85	261	177	146 to 207	
Group 5	74	261	187	157 to 218	
Group 6	94	243	149	118 to 180	
Group 7	93	97	4.0	-27 to 35	
Group 8	103	91	-12	-43 to 18	
Group 9	98	85	-13	-44 to 17	

Groups	Difference	t	P value	Summary
Group 1	-7.0	0.75	P > 0.05	ns
Group 2	149	16	P<0.001	***
Group 3	136	15	P<0.001	***
Group 4	177	19	P<0.001	***

Group 5	187	20	P<0.001	***
Group 6	149	16	P<0.001	***
Group 7	4.0	0.43	P > 0.05	ns
Group 8	-12	1.3	P > 0.05	ns
Group 9	-13	1.4	P > 0.05	ns

Basal Blood Gluose Values vs Blood Glucose Values at Day 7

Groups	Basal Blood Gluose Values			Blood Glucose Values at Day 7
	Difference	95% CI of diff.		
Group 1	97	98	1.5	-29 to 32
Group 2	87	233	145	115 to 176
Group 3	93	207	114	84 to 145
Group 4	85	254	169	138 to 200
Group 5	74	242	168	138 to 199
Group 6	94	217	123	93 to 154
Group 7	93	88	-5.5	-36 to 25
Group 8	103	80	-23	-54 to 7.4
Group 9	98	84	-15	-45 to 16

Groups	Difference	t	P value	Summary
Group 1	1.5	0.16	P > 0.05	ns
Group 2	145	16	P<0.001	***
Group 3	114	12	P<0.001	***
Group 4	169	18	P<0.001	***
Group 5	168	18	P<0.001	***
Group 6	123	13	P<0.001	***
Group 7	-5.5	0.59	P > 0.05	ns
Group 8	-23	2.5	P > 0.05	ns
Group 9	-15	1.6	P > 0.05	ns

Basal Blood Gluose Values vs Blood Glucose Values at Day 10

Groups	Basal Blood Gluose Values			Blood Glucose Values at Day 10
	Difference	95% CI of diff.		
Group 1	97	94	-2.5	-33 to 28
Group 2	87	229	142	111 to 173
Group 3	93	170	77	46 to 108
Group 4	85	184	100	69 to 130
Group 5	74	226	152	121 to 183
Group 6	94	189	95	64 to 126
Group 7	93	86	-7.0	-38 to 24
Group 8	103	81	-22	-52 to 8.9
Group 9	98	78	-20	-51 to 10

Groups	Difference	t	P value	Summary
Group 1	-2.5	0.27	P > 0.05	ns
Group 2	142	15	P<0.001	***
Group 3	77	8.3	P<0.001	***
Group 4	100	11	P<0.001	***

Group 5	152	16	P<0.001	***
Group 6	95	10	P<0.001	***
Group 7	-7.0	0.75	P > 0.05	ns
Group 8	-22	2.3	P > 0.05	ns
Group 9	-20	2.2	P > 0.05	ns

Basal Blood Gluose Values vs Blood Glucose Values at Day14

Groups	Basal Blood Gluose Values		Blood Glucose Values at Day14	
	Difference	95% CI of diff.		
Group 1	97	84	-13	-44 to 18
Group 2	87	212	125	94 to 155
Group 3	93	120	27	-3.9 to 57
Group 4	85	121	37	5.9 to 67
Group 5	74	109	35	4.4 to 66
Group 6	94	104	10	-20 to 41
Group 7	93	123	30	-1.1 to 60
Group 8	103	125	22	-8.6 to 53
Group 9	98	123	25	-5.4 to 56

Groups	Difference	t	P value	Summary
Group 1	-13	1.4	P > 0.05	ns
Group 2	125	13	P<0.001	***
Group 3	27	2.9	P < 0.05	*
Group 4	37	3.9	P<0.01	**
Group 5	35	3.8	P<0.01	**
Group 6	10	1.1	P > 0.05	ns
Group 7	30	3.2	P < 0.05	*
Group 8	22	2.4	P > 0.05	ns
Group 9	25	2.7	P > 0.05	ns