**CHAPTER ONE**

1. **INTRODUCTION**

**1.1 BACKGROUND OF STUDY**

Plants have always been useful source of remedy to several ailments owing to us easy availability and affordability in traditional setting worldwide. Some of this plant that exhibit medicinal properties have been known to help in stabilizing different internal organs in human being and animals, while others have hard side effects on the organs probably due to the varying amounts or quantity of toxic matter present in such plants (Sofomora, 2003). Because of this, toxicity testing in animals is carried out to identify potential hazard of the plant/drugs. This toxicity helps in determining the upper limits of administration, because not all content of the plant’s extract usually have medical property (Ahmed *et al*., 2001).

Coronary heart diseases, hyperlipidemia and hypolipidemia have been a major dietary problem in the world. These have lead to many arterial and heart diseases thereby causing high mortality and morbidity rate over the time in the world (Kizer *et al.,* 2010).

These health effects have been occurred as a result of high lipid deposits in the blood vessels and the arterial organs of the body. This menace has been highly tolerated in the society as solutions to its treatment are yet to be discovered. The prevalence of Atherosclerosis continues to rise worldwide and treatment with synthetic drugs ends with numerous side effects and huge monetary expenditure. World is endowed with a rich wealth of medicinal plants. The major causes of these ill-health effect has been as a result of dietary intakes with high Cholesterols, lipoprotein of Low density, Chylomicrons, Very Low density lipoproteins, Triglycerides, and low deposit of High-density lipoprotein levels in the system (Katan et al 1992).

Over the years, medicinal plants have provided a large platform for treatment of wide varieties of disease. This is as a result of phytochemicals found in then some of which have been isolated and purified as pure drugs. Hyperlipedemia has been associated with primary disease such as diabetes and so several works have been done on plant extract to investigate the effect on lipid parameter. This project works among such research effort.

Diabetes mellitus is characterized by insufficient blood levels of the hormone insulin. When blood concentration of insulin is too low, muscle and liver cells do not absorb glucose form the blood which in turn leads to increased levels of blood glucose (hyperglycemia), impaired metabolism of fats and protein, Ketosis and possible diabetic Coma (William *et al*., 2009). According to World Health Organization (WHO), about 170 million people are currently affected by diabetes and the figure is expected to double by the year 2025 (Boyle *et al*., 2001).

Diabetes is also known to involve oxidative stress and changes in Lipid metabolism (Scoppola *et al*., 2001).

In 2010 it was estimated that the world prevalence of diabetes among adults (aged 20-79 years) was 6.4% (285 million adults). (Shau, 2010). Perhaps even more concerning is that diabetes is projected to affect approximately 7.7% of the world’s population (439 million adults by 2030).

Type 2 diabetes mellitus is characterized by a decline in B-Cell function and worsening insulin resistance (Fonseca, 2009). Therapy for type 2 diabetes mellitus focuses around improving glucose tolerance through diet, exercise and anti diabetic medications of the individuals affected with diabetes, approximately 95% are diagnosed with type 2 diabetes mellitus.

Despite the advances in modern medicine, DM continues to be the most common endocrine metabolic disorder and the disease is rapidly increasing worldwide affecting all parts of the world (Patel, 2012).

Type 1 diabetes mellitus (TIDM) is considered as an auto immune disease resulting in absolute insulin deficiency that is insulin producing B-cells of the pancreas are destroyed. Patients are prone to kedosis and dependent on insulin therapy. (David, *et al.,* 1999).

*Momordica Charantia* is complex plant medicine that has a remarkable long history of use, both as a food and as a medicine.

*Momordica Charantia* commonly known as bitter gourd is an economically important medicinal plant belonging to the family cucurbitaceae. It is also known as silter melon, balsam pear and karela. In Nigeria and many other parts of the world, fruties, leave and seeds of bitter gourd have used by traditional healer to treat ulcer, HIV, inflammation and cancer (Assubaie and EL-Garaluary, 2004). The plant is also famous for its traditional use in diabetes mellitus (Paul *et al*., 2011). Therefore this plant could be a good source of alternative treatment for diabetes mellitus.

**1.2 Aim and Objective of the Research**

*Momordia charantia* have been used in various forms by traditional medicinal practitioners in the treatment of diseases without properly investigating its anti-lipidamic level.

Hence, this experiment aimed to investigate the anti-lipidemic effect of *Momordica Charantia* leaf fractions 0n alloxan diabetic induced albino rats.

This was achieved through the following objectives;

1. Investigating the anti-lipidemic level of *Momordica charantia* of triglycerides on alloxan induced diabetic rats.
2. Investigating the anti-lipidemic level of *Momordica charantia* of total cholesterol on alloxan induced diabetic rats.
3. Investigating the anti-lipidemic level of *Momordica charantia* of high-density lipoprotein (HDL) on alloxan induced diabetic rats.
4. Investigating the anti-lipidemic level of *Momordica charantia* of low –density lipoprotein (LDL) on alloxan induced diabetic rats.

**CHAPTER TWO**

**2.0 LITERATURE REVIEW**

**2.1 OVERVIEW OF *MOMORDICA CHARANTIA***

*Momordica charantia*, known as bitter melon, bitter gourd bitter squaph, or bousam-pear, is a tropical and subtropical vine of the family cucurbitaceaey, widely grown in Asia, Africa, and the Caribbean for its edible fruit. Its many varieties differ substantially in the shape and bitterness of the fruit.

Bitter melon originated in India and was introduced into China in the 14th century. It is widely used in East Asian, South Asian, and Southeast Asian cuisine.

This herbaceous, tendril-bearing vine grows up to 5m (16ft) in length. It bears simple, alternate leaves 4-12cm (1.6-4.7) in across with three to seven deeply separated lobes. Each plant bears separate yellow male and female flowers. In the Northern Hemisphere flowering occurs during June to July and fruiting during September to November.

The fruit has a distinct warty exterior and an Oblong shape. It is hollowing in cross-section, with a relatively thin layer of flesh surrounding a central seed cavity filled with large, flat seeds and pith. The fruit is most often eaten green, or as it is beginning to turn yellow. At this state, the fruits flesh is crunchy and watery in texture, similar to cucumber, chayote or green bell pepper, but bitter. The skin is tender and edible seeds, and pith appear white in unripe fruits; they are not intensively bitter and can be removed before cooking.

**2.1.1 MEDICINAL USES**

Bitter melon has been used in various Asian and African herbal medicine systems for a long time. (Arnason, 2005). In Turkey, it has been used as a folk remedy for a variety of ailments, particularly stomach complaints. (Atanas, 2014).

*Momordica Charantia* has a number of purported uses including cancer prevention, treatment of diabete, fever HIV and Aids, and infections.

In 2017, the University of Penadeniya researches revealed that bitter gourd seeds can be potentially used to destroy cancer cells and they were successfully administered to patient in cancer unit.

*Momordica Charantia* with regard to use for diabetes, several animal studies and small-scale human studies has demonstrated a hypoglycemic effect of concentrated bitter melon extracts (Wang, 2011). In addition, a 2014 review shows evidence that *Momordica Charantia*, when consumed in raw or juice from, can be efficacious in lowering blood glucose levels. Bitter melon is contraindicated in pregnant women because it can induce bleeding, contractions, and miscarriage.

**2.1.2 TAXONOMY AND NOMENCLATURE**

**Taxonomy Tree**

Domain: Eukaryota

 Kingdom: Plantae

 Phylum: Spermatophyta

 Subphylum: Angiospermae

 Class: Dicotyledonae

 Order: Violales

 Family: *Cucurbitaceae*

 Genus: *Momordica*

 Species: *Momordica Charantia*



Fig. 1. Picture of *Momordica Charantia*

According to Schaefer and Renner (2010), genus *momordica* comprises 59 species distributed in the worm tropics, chiefly in Africa and with about 12 species in South-East Asia. *Momordica Charantia L*. is the most widely cultivated species of *momordica*. The generic name apparently derives from the Latin Mordeo (to bite), perhaps a reference to the jagged edges of the seeds, *charantia* is from the ancient Greek for beautiful flower. *Momordica* is in the sub tribe Thladianthinae, tribe joliffieae, subfamily cucurbitaceal. There are five other cultivated *momordica* species in addition to *M. charantia.* The other species are cultivated in Asia and some parts of Africa (Robinson and Decker Walters, 1997).

**2.1.3 DISTRIBUTION**

*M. Charantia* is widely distributed throughout tropical and subtropical regions on all continents. It appears to be native to the African and Australia continents, but its actual origin has been obscured by its spread as a food crop. Currently it can found cultivated and naturalized in North central and South America, the

West indices and on several Islands in the Pacific Ocean.

**2.1.4 HABITAT**

*M. Charantia* grows under a very wide range of conditions throughout the tropics and subtropics. Its rapid growth and maturation allow it to colonize any area where there is sufficient short-term soil moisture, whilst it can also survive as a perennial in condition of continulous soil moisture. It grows from sea level to over 1300m (Tjitosoedirdjo, 1990) and in area with annual rainfall as 10m as 480mm. It grows in soil with PH ranging from 4.3 to 8.7 (Holm *et al*., 1997). *M. Charantia* is a fast growing vine and quickly covers the supporting vegetation or structure. In general, this species can be found growing in coastal area, along Greeks and rivers, forest edges and disturbed sites (Hall *et al.,* 2012). For optimum growth, *M. Charantia* needs a story support, 1-4m tall, however, it will also grow as a malted ground cover.

**2.1.5 MEANS OF DISPERSAL**

*M. Charantia* spreads sexually by seeds and vegetatively by underground stems. The tips of the fruits split at maturing and the sections curl backwards to expose the woody seed embedded in sugary bright red rills. The arts are attractive to birds and animals which disperse the seeds. Seeds keep in dry storage remain viable for up to 24 months (HOLM *et al.,* 1997).

**2.2 DIABETES**

Diabetes mellitus, or simply diabetes, is a group of metabolic disease in which a person has high blood sugar, either because the pancreas does not produce enough insulin, or because cells do not respond to the insulin that is produced (David, 2011). This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increase thirst) and polyphagia (increases hunger).

Gestation diabetes occurs when pregnant women without a previous history of diabetes develop with blood sugar levels. This type affects 260-560 females during pregnancy some women have very high levels of glucose in their blood, and their bodies are unable to produce enough insulin to transport all of the glucose into their cells, resulting in progressively rising levels of glucose. This type of diabetes is fully treated, but requires carefully medical attention throughout the period of pregnancy. About 20%-50% of affected women develop type 2 diabetes later in life (Couri *et al.,* 2009).

**2.2.1 TYPES OF DIABETES MELLITUS**

**A. Type 1 diabetes mellitus**

Type 1 diabetes mellitus is characterized by loss of the insulin producing beta cells of the islets of langerhans in the pancreas, leading to insulin deficiency. This type can be further classified as immune-mediated or idiopathic. The majority of type 1 diabetes is of the immune-mediated nature in which beta cells loss is a T-cell-medicated autoimmune attack. (Rother, April 2009).

Type 1 diabetes typically appears in childhood or adolescence, but its oneset is also possible in adulthood.

Type 1 diabetes always requires insulin treatment and an insulin pump or daily injections will be lifelong requirement to keep blood sugar levels under control.

**B. Type 2 diabetes mellitus**

Type 2 diabetes mellitus characterized by insulin resistance, which may be combined with relatively reduced insulin secretion (David *et al.,* 2011).

Type 2 diabetes mellitus most commonly develops in adulthood and is more likely to occur in people who are overweight and physically inactive.

**2.2.2 SIGNS AND SYMPTOMS**

The classic symptoms of untreated diabetes are weight loss, polyuria (increased urination), polydipsia (increased thirst), and polyphagia (increased hunger). Symptoms may develop rapidly (week or months) in type I DM, while they usually develop much more slowly and may be subtle or absent in type 2 DM.

**2.2.3 TREATMEMT AND MANADEMENT OF DIABETES**

Diabetes mellitus is a chronic disease for which there is no known cure except in every specific situation. Management concentrates on keeping blood sugar levels as close to normal without causing low blood sugar. This can usually be accomplished with a healthy diet, exercise, weight lose, and use of appropriate medications (insulin in the case of type 1 diabetes, oral medications, as well as possibly insulin, in type 2 diabetes).

**2.2.3 Preventions**

There is no known preventive measure for type 1 diabetes. Type 2 diabetes which account for 85-90% of all cases can often be prevented or delayed by maintaining a normal body weight, engaging in physical activity, and consuming a healthy diet (Bantle *et al.,* 2006).

**2.3 LIPID PROFILE**

Lipid profile or lipid panel is a panel of blood tests that serves as an initial screening tools for abnormalities in lipids, such as cholesterol and triglycerides. The results of this test can identify certain genetic diseases and can determine approximate risk for cardiovascular disease, certain forms of pancreatitis, and other diseases. The extra cholesterol may be deposited in plagues on the malls of blood vessels.

Plagues can narrow or eventually block the opening of blood vessels, leading to hardening of the arteries (atherosclerosis) and increasing the numerous health problems, including heart disease and stroke.

Atherosclerosis is a disease in which the inside of an artery narrows due to the build in of plague (Ross, 1993).

Initially, there are generally no symptoms when sever, it can result in coronary artery disease, stroke, peripheral artery disease or kidney problems depending on the arteries affected. The exact cause is not known, risk factors include abnormal cholesterol levels, high blood pressure, diabetes, smoking, obesity, farming history and an unhealthy diet.

Atherosclerosis is the number one cause of death and disability in the developed world (Robert, 2007). There is evidence, however, that the condition occurred in people more than 5,000 years age (Allam, 2008).

**2.3.1 COMPONENT OF LIPID PROFILE**

Low-density Lipoprotein (LDL)

High-density Lipoprotein (HDL)

Triglycerides

Total cholesterol

High – Density Lipoprotein (HDL) is the smallest of the lipoprotein particles. It is the densest because it contains the highest proportion of protein to lipids. Its most abundant apolipoproteins are apo A-1 and apo A-11 (Jean-Pierre, 2009). HDL transports cholesterol mostly to the liver or steroidogenic organs such as adrenals, ovary, and testes by both direct and indirect pathways.

Low – Density Lipoprotein (LDL) is one of the major groups of lipoprotein which transport all fat molecules around the body in extracellular matter. These groups from less dense compared to shrouding water (Largest particles) to most dense LDL delivers fat molecules to the cells and can drive the progression of atherosclerosis if they become oxidize within the man’s of arteries.

Triglycerides are chemical compounds digested by the body to provide it with the energy for metabolism. Triglycerides are the most common form of fat in the body and also the most common that we digest. They are the main ingredient in vegetable oil and animal fats.

Elevated triglyceride levels are a risk factor for atherosclerosis. The narrowing of arteries with building of fatty plagues may lead to heart attack, stroke, and peripheral artery disease.

Cholesterol is found in every cell of the body and has important natural functions. It is manufactured by the body but can also be taken from food. It has max and fat like in appearance. Cholesterol is oil-based and so does not mix with the blood, which is water based. It is therefore carried around the body in the blood by lipoproteins. High cholesterol is a major risk factor for coronary heart disease, a cause of heart attacks.

**2.3.2 Lipids disorders**

Fats (lipids) are an important source of energy for the body. The body's store of fat is constantly broken down and reassembled to balance the body's energy needs with the food available. Over time, accumulations of these substances can be harmful to many organs of the body. Disorders caused by the accumulation of lipids are called lip doses. Some diseases that could arise in excessive accumulations of fats includes

1. **Gaucher’s Diseas****e**

Gaucher's disease is caused by a buildup of glucocerebrosides in tissues. Children who have the infantile form usually die within a year, but children and adults who develop the disease later in life may survive for many years.

In Gaucher's disease, glucocerebrosides, which are a product of fat metabolism, accumulate in tissues. Gaucher's disease is the most common lipidosis. The disease is most common among Ashkenazi (Eastern European) Jews. Gaucher's disease leads to an enlarged liver and spleen and a brownish Pigmentation of the skin. Accumulations of glucocerebrosides in the eyes cause yellow spots called pingueculae to appear. Accumulations in the bone marrow can cause pain and destroy bone.

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**Type 1**, the chronic form of Gaucher's disease, is the most common. It results in an enlarged liver and spleen and bone abnormalities. Most commonly diagnosed during adulthood, type 1 Gaucher's disease may lead to severe liver disease, including increased risk of bleeding from the stomach and esophagus and liver cancer. Neurologic problems can also occur.

**Type 2**, the infantile form, usually causes death in the first year of life. Affected infants have an enlarged spleen and severe neurologic problems.

**Type 3**, the juvenile form, can begin at any time during childhood. Children with type 3 disease have an enlarged liver and spleen, bone abnormalities, and slowly progressive neurologic problems. Children who survive to adolescence may live for many years.

Many people with Gaucher's disease can be treated with enzyme replacement therapy, in which enzymes are given by vein, usually every 2 weeks. Enzyme replacement therapy is most effective for people who do not have nervous system complications.

1. **Tay - Sachs disease**

Tay-Sachs disease is caused by a buildup of gangliosides in the tissues. This disease results in early death.

In Tay-Sachs disease, gangliosides, which are products of fat metabolism, accumulate in tissues. The disease is most common among families of Eastern European Jewish origin. At a very early age, children with this disease become progressively intellectually disabled and appear to have floppy muscle tone. Spasticity develops and is followed by paralysis, dementia, and blindness. These children usually die by age 3 or 4. The disease cannot be treated or cured.

Before conception, parents can find out whether they carry the gene that causes the disease. During pregnancy, Tay-Sachs disease can be identified in the fetus by chorionic villus sampling or amniocentesis.

1. **Niemann-Pick Disease**

Niemann-Pick disease is caused by a buildup of sphingomyelin or cholesterol in the tissues. This disease causes many neurologic problems.

In Niemann-Pick disease, the deficiency of a specific enzyme results in the accumulation of sphingomyelin (a product of fat metabolism) or cholesterol. Niemann-Pick disease has several forms, depending on the severity of the enzyme deficiency, which determines how much sphingomyelin or cholesterol accumulates.

Some forms of Niemann-Pick disease can be diagnosed in the fetus by chorionic villus sampling or amniocentesis. After birth, the diagnosis can be made by a liver biopsy (removal of a tissue specimen for examination under a microscope). None of the types of Niemann-Pick disease can be cured, and children tend to die of infection or progressive dysfunction of the central nervous system. Currently, some therapies that may slow or halt the progression of symptoms in types B and C are being studied.

1. **Fabry's Disease**

Fabry's disease is caused by a buildup of glycolipid in tissues. This disease causes skin growths, pain in the extremities, poor vision, recurrent episodes of fever, and kidney or heart failure.

In Fabry's disease, glycolipid, which is a product of fat metabolism, accumulates in tissues. Because the defective gene for this rare disorder is carried on the X chromosome, the full-blown disease occurs only in males ([X-Linked Inheritance](http://www.merckmanuals.com/home/fundamentals/genetics/inheritance_of_single-gene_disorders.html#v711661)). The accumulation of glycolipid causes noncancerous (benign) skin growths (angiokeratomas) to form on the lower part of the trunk. The corneas become cloudy, resulting in poor vision. A burning pain may develop in the arms and legs, and children may have episodes of fever. Children with Fabry's disease eventually develop kidney failure and heart disease, although most often, they live into adulthood. Kidney failure may lead to high blood pressure, which may result in stroke.

Fabry's disease can be diagnosed in the fetus by chorionic villus sampling or amniocentesis. The disease cannot be cured or even treated directly, but researchers are investigating a treatment in which the deficient enzyme is replaced by transfusion. Treatment consists of taking analgesics to help relieve pain and fever or anticonvulsants. People with kidney failure may need a kidney transplant.

1. **Fatty Acid Oxidation Disorders**

Fatty acid oxidation disorders are caused by a lack or deficiency of the enzymes needed to break down fats, resulting in delayed mental and physical development.

Several enzymes help break down fats so that they may be turned into energy. An inherited defect or deficiency of one of these enzymes leaves the body short of energy and allows breakdown products, such as acyl-CoA, to accumulate. The enzyme most commonly deficient is medium chain acyl-CoA dehydrogenase (MCAD). Other enzyme deficiencies include short chain acyl-CoA-dehydrogenase deficiency (SCAD), long chain-3-hydroxyacyl-CoA-

Deficiency (LCHAD) and trifunctional protein deficiency (TFP).

**2.4 ALLOXAN**

**2.4.1 Alloxan and its Mechanism of Action**

Alloxan is a toxic glucose analogue, which selectively destroys insulin producing cells in the pancreas (that is beta cells) when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus (called “alloxan diabetes”) in these animals, with characteristics similar to type 1 diabetes in humans. Alloxan is selectively toxic to insulin producing pancreatic beta cells because it preferentially accumulates in beta cells through uptake via the GLUT2 glucose transporter.

Alloxan in the presence of intracellular thiols, generates reactive oxygen species (Rosl in a cyclic reaction with its reduction product, dialuric acid. The beta cell toxic action of alloxam is initiated by free radicals formed in this redox reaction. Studies suggest that alloxan does not cause diabetes in humans (Lenzen, 2008). Other found a significant difference in allozan plasma levels in children with and without diabetes type 1 (Mroziki emicz *et al*., 1994).

**2.4.2 Impact upon Beta Cells**

Alloxan selectively kills the insulin producing beta cells found in the pancreas, alloxan is used to induced diabetes in laboratory animals (Danilova, 2014; Loreta *et al.,* 2009).

This occurs most likely because of selective uptake of the compound due to its structural similarity to glucose as well as the beta-cells highly efficient uptake mechanism (GLUT2). Furthermore, alloxan inhibits glucokinase, a SH-containing protein essential for insulin secretion induced by glucose.

Most studies have shown that alloxan is not toxic to the human beta-cell even in every dose, probably because of differing glucose uptake mechanisms in humans and rodents.

Alloxan is however, toxic to the liver and the kidney in high doses

Alloxan

NH

HN

O

O

O

Fig. 2. Structure of alloxan

**CHAPTER THREE**

**3.0 MATERIALS AND METHOD**

**3.1 MATERIALS**

Glucose strips

Insulin syringes

Injection bottles

Razor blade, plain bottles

Foil

What mann (no-1) filter paper

Latex gloves

Animal conges

Spatula

Hand towels

Glass wares

Steal basin

**3.1.2 Chemical and kits**

Alloxan

Methanol

Chloroform (anesthesia)

Ethyacetate

Hexane

Total cholesterol kit from Randonx Laboratories. 55 Diamond, Crumilin, Country Antrim, BT29 4OY, United Kingdom.

HDL-cholesterol kit from Randox Laboratories. 55 Diamond, Crumilin, Country Antrim, BT29, 4QY United Kingdom.

Triglyceride kit from Teco Diagnostics, 1268 Lakeview Avenue, Aniehiem, CA 92807 U.S.A.

All chemicals used in this study were of analytical grade and products of Randox, UK; Teco (TC), USA.

**3.1.3 Equipments**

Centrifuge (Model 800D, England)

Water Bath (Model DK, England)

Electrical Weighing Balance (Model No: Yp.502N)

Spectrophotometer (Spectrumlab 23A, England)

Adjusted Micropipette (Perfect, U.S.A)

Refrigerator (Kelvinator, Germany)

Glucometer (Accucheck Advantage II)

**3.1.4 Biological Material**

Fresh leaves of *Momordica* *charantia*

Forty adult female Albino rats

**3.2 METHOD**

**3.2.1 Collection and Identification of Sample**

Fresh leaf of *Momrodica charantia* was collected from Orba Nsukka in Udenu Local government Area of Enugu State. The botanical identification of the leaf was done by Prof. C.U. Okeke and kept at the Haberium Unit of Botany Department, Nnamdi Azikiwe University.

The plant was collected early morning and after sunset. These periods before sun rise and after sunset are the best periods because, plants activities such as photosynthesis are inactivated at these periods and plants chemical constituents are properly maintained.

The leaf were dried under the mild sun and blended into powder with an electronic blender, weighed and stored in airtight container until ready for the extraction.

**3.2.2 Sample Processing and Preparation of Extract**

The crude extract was obtained through cold extraction process. The coarse powdered sample were submerged in methanol and allowed to stand for 48 hours with occasional shaking and stirring. When the solvent become concentrated, the alcohol content was filtered through Muslim cloth and then through filter paper (Whatman filter paper no. 1). Then the solvent was allow to evaporate using water both at temperature 40-500C. Thus, the highly concentrated crude extract was obtained. The crude extract was then fractionated with Ethylacetate, Hexane, Aqueous, Methanol and Chloroform, then their respective fractions were collected. The fractions were then evaporated using water both at low temperature. The concentrated fractions of the extract were then preserved in the fridge for experimental uses.

**3.3 EXPERIMENTAL ANIMAL**

**3.3.1 Collection of Animals**

Forty-five (45) adult female albino rats were obtained from the Department of Pharmacology and Vertinary Medicine, University of Nigeria, Nsukka and used for the study. Prior to commencement of the experiment, all the rats were acclimatized to the new environmental condition for a period of one week. During the experimental period the rats were kept in a well ventilated animal house of Godfrey Okoye University Enugu. The animals were housed in plastic capes with covers, the animals were properly fed with growers feed from Vital Feed Company and the floor were covered with saw dust which sucks away the urine of the rats. This was changed on daily basis.

There was cross ventilation in the animal house and was kept clean throughout the study. Clean tap water was used for the rats’ drink in plastic plate while feed was provided in a plastic bowls.

**3.3.2 Animal Grouping**

Fourty (40) Albino Wister rats were randomly selected, weighed and assigned into 8 groups of 5 animals each. These animals were induced biabetic with alloxan and treated by oral administration with the different fractions of the extract and a standard drug according to their groups.

Group A: Treated with Gilbenclamide

Group B: Treated with Hexane fraction

Group C: Treated with Aqueous fraction

Group D: Treated with Methanol fraction

Group E: Treated with Ethylacetate fraction

Group F: Treated with Chloroform fraction

Group G: Diabetic not treated

Group H: Positive control (not induced)

**3.3.3 Collection of Blood Sample**

Blood sample of all the rats were collected by a snip-cut at the tip of the tail vein and blood sugar level was measured with an Accu-check Glucometer machine with strip. The strips were inserted one at a time in the glucometer.

**Procedure**

Ones blood is dropped in the yellow squared spot on the test strips inserted inside the glucometer, glucose in the blood reacts with the chemical present in the test strip. Glucometer test strip is based on double sequential enzyme reaction in which an enzymes, glucose oxidase (GOD) converts glucose to hydrogen peroxide and glucuronic acid while peroxides oxidizes the dye in the test strip to produce a color. The blood glucose level in Mg/dl will be displaced on the screen after 20 seconds. The reactions are shown below.

Glucose + 02 Glucose Oxidase Glucose + H202 + H20

H202 + dye Peroxidase Oxidized Glucose + H202 + H20

**3.3.4 Preparation of Extracts for Administration**

A quantity of 10g of the dried extracts was dissolved in 100ml of normal saline. This formed the stock solution volume of the five fractionated extracts administered to the animals according to their body weight except those in group H (see appendix).

**3.3.5 Experimental Induction of Diabetes and Collection of Serum**

Animals were allowed to fast for 12 hrs and were induced diabetic by intraperitoneal injection of a freshly prepared solution of alloxan monohydrate 100mg/kg bodyy weight (Qualikems India). In distilled water after glucose estimation was done, the alloxan induced animals were allowed to feed over night to overcome drug induced hypoglycemia. After 48 hours blood glucose level was measured again using blood sample from the tail vein of the rats. When the condition of diabetes was established (blood glucose level above I mmol/l) the drug Gilbenclamide and fractions of plant extract were administered to the respective groups of animals for a period of four weeks treatment interval. After completing the blood glucose level treatment, the blood glucose level was accessed again after which 3-5ml of blood was collected directly from the heart of each animal by syringes, centrifuged at 3, 500 r/mins for 15 minutes and the serum was obtained for biochemical analysis.

**3.3.6 Determination percentage yield of extract**

The percentage yield of the leaf extract of *Momordica Charantia* was determined by weighing the ground leaf before extraction and the concentrated extract obtained after extraction and then calculated using the formula.

% yield = weigh (g) of extract evaporated to dryness ÷ weigh (g) of ground sample × 100.

**3.4 Determination of lipid parameters**

The concentration of lipid profile level was determined by some kits which are High Density Lipoprotein (HDL) kit, Total Cholesterol kit, Low Density Lipoprotein (LDL) kit and Triglyceride kit (TRIGS) and results were reportedly in mg/dl.

**3.4.1 Determination of HDL-cholesterol by Randox kit**

**Principle**

Low density lipoproteins (LDL) and fractions were precipitated quantitatively by the action of phosphotungstic acid in the presence of magnesium ions. After centrifugations, the cholesterol concentration in the HDL (High density lipoprotein) fraction, which remains in the supernatant was determined.

**Procedure**

A total of 12 test tubes with the first two representing blank and standard respectively were used. The sample test tube (3-10) received 4ul of serum. The standard test tube received 4ul of standard solution. Finally 300ul of the direct HDL Cholesterol (R1) was added to all the test tubes. After thorough missing, the test tubes were the centrifuge for 10 minutes at 4000 rpm. The clear supernatant (sample and standard supernatants) were separated off. The sample supernatant (100ul) were separated into another separate test tubes while 100ul of standard supernatant was pipette into another test tube. Then 100ul of working reagent R1hd1 was added to both sample and standard text tube. Finally 100ul of working of distilled water was added to test tube labeled blank. After thorough missing the test tube were incubated for 5 minutes at 370C in a water bath. The absorbance of sample (Asample) and standard (Astandard) was read against that of reagent at 500nm.

**3.4.2 Determination of Total cholesterol by Randox kit**

**Principle**

Cholesterol is measured enzymatically is serum or plasma in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. One of the reaction by product H202 is measured quantitatively in perioxidase catalyzed reaction that produces a colour. Absorbance is measured at 500nm. The color intensity is proportional to cholesterol concentration the reaction sequence is as follows:

Cholesterol esters + H20 cholesterol esterase cholesterol + fatty acid

2H202 + 4-aminophenazone + chlorophenol peroxidase Quinoneimine + HCl + 4H20

**Procedure**

Eight (8) test tubes were labeled serially with 1st and 2nd representing blank and standard. The sample tubes 3-8 received 10ul of serum then 1000ul of working reagent R1 CHOL was added to the test tubes labeled standard and blank respectively. After thorough mixing, the test tubes were incubated in the water bath for 5mins at 370C. the absorbance of the sample (Asample) and standard (Astandard) were read against that of reagent blank at 500nm within 60 minutes.

**3.4.3 Determination of Triglycerides concentration by Randox kit**

Triglycerides was determined using Randox commercial kit

**Principles**

Triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide 4-aminophenazone and 4-chlorophenol under the enzymatic catalysis of peroxidase. Equation of reactions involved is shown below.

Triglceride lipase Gylcerol + fatty acids

Glycerol + ATP glycerol kinase Glycerol-1-phosphate + ADP

Glycerol-1-phosphate + 02 GIP DAP + H202

H202 + 4 AAP + DHBS perioxidase Quinoneimine Dye + 2H20

Kinase, glycerol-3-phosphate oxidase, peroxidas and sodium azide which was prepared as follow:

**Procedure**

Thirty-seven test tubes were labeled serially with 1st and 2nd representing the standard and blank respectively, while A-H represent samples. To each of labeled test tubes, 1.0 of working reagent R1trigs was added 10ul of standard solution (cal) while the lanktest tube was added 10ml of distilled water. After mixing thoroughly the test tubes were incubated in a water bath at 370C for four minutes. The absorbance of sample (Asample) and standard (Astandard) were read against that of reagent blank within 60 minutes.

R1 trigs=buffer (pipes buffer), 4-clorophenol and magnesium ions

**Determination of low density lipoprotein (LDL)**

Cholesterol LDL was determined using cholesterol LDL precipitating Reagent.

**CHAPTER FOUR**

**4.0 RESULTS**

**Key**

**1** = Gilbenclamide 200mg/kg

**2** = Hexane fraction 200mg/kg

**3** = Aqueous fraction 200mg/kg

**4 =** Methanol fraction 200mg/kg

**5 =** Ethylacetate fraction 200mg/kg

**6 =** Chloroform fraction 200mg/kg

**7 =** Diabetic not treated (negative control)

**8** = Positive control (not induced)

**4.1 Percentage yield of extract fractions**

Fig. 1. Shows the % yield of the solvents used in the extraction. Chloroform produced the highest amount of yield of the extract compared to the other solvent. This shows that chloroform is more polar with higher solubility ratio of absorbing the phytonutrients prsent in the sample.

**4.2 The triglyceride concentration (mg/dl) chat of the study groups**



Fig. 2 Anti-lipidemic effect of the extract fractions of *Momordica charantia* in alloxan induced diabetic rat showed that the difference in Triglyceride value of treated and untreated rats were significant (P < 0.05) when compared with control.

**4.2 The Total Cholesterol concentration (mg/dl) chat of the study groups**



Fig. 2. The results of anti-lipidemic effect of the extract fractions of *Momordica charantia* in alloxan induced diabetic rat showed a significant decrease in the level of cholesterol in rats fed with the extracts of *Momordic charantia* as compared to the control. The difference in total cholesterol value of treated and untreated rats were significant (P <0.05) when compared with the control.

**4.3 The High Density Lipoprotein concentration (mg/dl) chat of the study groups**



Fig. 3 Anti-lipidemic effect of the extract fractions of *Momordica charantia* in alloxan induced diabetic rat showed that there was a significant difference (P <0.05) in high density lipoprotein (HDL) level between the group treated with extracts compared to the control group.

**4.4 The Low Density Lipoprotein concentration (mg/dl) chat of the study groups**



Fig 4 anti-lipidemic effect of the extract fractions of *Momordica charantia* in alloxan induced diabetic rat showed a significant decrease (P < 0.05) in the level of serum low density lipoprotein (LDL) in the groups treated with the standard drug glibenclamide, hexane, aqueous and methanol fractions but showed a reduced level in relation to the positive control group.

**CHAPTER FIVE**

**DISCUSSION AND CONCLUSION**

**5.1 DISCUSSION**

The present research study was designed to investigate the effect of *Momordica charantia* (Bitter gourd) on lipid profile parameter on an alloxan induced diabetic rats. The result shown in Fig 1 with reference to the control group indicates that the *Mormordica charantia* extracts caused a decrease level of triglyceride in the animals as shown above. All the treated groups showed that *Momordica charantia* increased the triglyceride level of the treated animals compared to the control. From the above analysis, I infer that *Momordica charantia* which was administered to the treated groups serve as a management option for the diseases associated to increase or low triglyceride level of a patient.

Also, as seen in fig. 2, there was a decrease in cholesterol level of the treated groups in reference to the control. In the same view of cholesterol level, it was noticed in fig2 that, diabetic patients are highly subjected at high risk of cardiovascular diseases.

High density lipoprotein being good cholesterol which transports cholesterol from cells and tissues back to the liver and removes cholesterol deposited in the walls of blood vessels thereby limiting arterosclerosis, high blood pressure and other cardiovascular risk diseases, Fig. 3 indicates that *Momordica charantia* maintains a goodrole in increasing the level of HDL in the diabetic treated animals. Whereas a reduced level of HDL in the untreated group indicative sign of greater chances of cardiovascular risk disease associated to kidney problems as regards the hyperglycemic conditions of the untreated group.

The LDL level in this study, when compared with the control showed significant decrease and increase at different dosage. Experimental animals in groups treated with glibenclamide, hexane, aqueous, methanol fractions, Ethylacetate fraction and Chloroform fraction showed significantly decrease in LDL levels compared to the control group, whereas the untreated diabetic group showed significant increase in LDL level compared to the positive control group.

**5.2 CONCLUSION**

In conclusion, administration of *momordic charantia*, at doses and duration employed in this study, had dose-dependent cardio-protective properties via its effect on the blood cholesterol levels. However, there is an indication that higher doses should be discouraged. In addition, this study also revealed propensity of the plant extract to possess anti-hyperlipidemic principle.

**5.3 RECOMEMDATION**

From the above observation; it is evident that *Momordica charantia* (bitter gourd) had anti-lipidemic protective effects. However, further investigation is warranted to confirm the hepatoprotective effect of *Momordica charantia*.

**REFERENCES**

A. K. Shetty1, *(*2005) "Effect of bitter gourd (*Momordica charantia*) on glycaemic status in streptozotocin induced diabetic rats". *Plant Foods Human Nutrition*, 60(3):,109– 12.

A. K. Shetty1, G. Suresh Kumar1, K. Sambaiah1 and P. V. Salimath (2005). "Effect of bitter gourd (*Momordica charantia*) on glycaemic status in streptozotocin induced diabetic rats". *Plant Foods Human Nutrition,* 60 (3):, 109–12.

Ahmad N, Hassan M.R,and Halder H, (1999). Effect of *Momordica charantia* (Karolla) extracts on fasting and postprandial serum glucose levels in NIDDMpatients. *Bangladesh Medical Research Council Bull*.;25:11-13.

Ahmed I, Lakhani M.S, Gillett M, John A, Raza H (2001). Hypotriglyceridemic and hypocholesterolemic effects of anti-diabetic *Momordica charantia* (karela) fruit extract in streptozotocin-induced diabetic rats, Diabetes*.* *Research Clinical Practice*, 2001; 51(3): 155- 61.

Alam S, and Asad M, (2009). Antiulcer activity of methanolic extract of *Momordica charantia L.* in rats. *Journal of ethnopharmacology*.; 123(3): 464-9.

Balch, Phyllis A (2006). Prescription for nutritional healing. 4th ed. New York: Avery,. p. 54

Behrman E.J, Gopalan V (December 2005). "Cholesterol and Plants". *Journal of Chemistry Education* 82 (12): 1791.

Behrman, E. J, Gopalan, Venkat (2005). William M. Scovell, ed. "Cholesterol and Plants". *Journal of Chemical Education* 82 (12): 1791.

Brugts J.J, Yetgin T, Hoeks S.E, Gotto A.M, Shepherd J, Westendorp RG (2009). "The benefits of statins in people without established cardiovascular disease but with cardiovascular risk factors: meta- analysis of randomised controlled trials". *Biology and Medicine Journal* 338: pp 2376.

Brunzell J.D, Davidson M, Furberg C.D, Goldberg R.B, Howard B.V,& Stein J.H (2008). "Lipoprotein management in patients with cardiometabolic risk: consensus statement from the American Diabetes

Dashti M, and Kulik W (2011). "A phospholipidomic analysis of all defined human plasma lipoproteins.". *Science Reproduction* 1(139).

Dashty M, (2014). "Proteome of human plasma very low-density lipoprotein and low- density lipoprotein exhibits a link with coagulation and lipid metabolism.". *Thromb Haemostostasis*. 23 (111): 518–530.

Davidson, Michael H. (28 January 2008). "Pharmacological Therapy for Cardiovascular Disease". In Davidson, Michael H.; Toth, Peter P.; Maki, Kevin C. Therapeutic Lipidology. Contemporary Cardiology. Cannon, Christopher P.; Armani, Annemarie M. Totowa, New Jersey: Humana Press, Incorporation. pp. 141–142.

Dhalla, N.S. Gupta, K.C. Sastry, M.S. and Malhotra, C.L. *Chemical composition of the fruit of Momordica charantia Linn*. *Indian Journal of Pharmacology* 23, 1961, 128 therapeutic uses

Dubois C, Armand M, Mekki N, Portugal H, Pauli A.M ,and Bernard P.M (1994). "*Effects of increasing amounts of dietary cholesterol on postprandial lipemia and lipoproteins in human subjects*". Journal of LIPID Research 35 (1994): 1993–2007.

Durrington P (August 2003). "*Dyslipidaemia*". *Lancet laboratories* 362 (9385): 717–31.

Espenshade P.J, &Hughes A.L (2007). "Regulation of sterol synthesis in eukaryotes". *Annual Revision Genet* 41: 401–27.

Fernandez ML, Webb D (2008). *The LDL to HDL cholesterol ratio as a valuable tool to evaluate coronary heart disease risk*. *Journal of American Collaborative Nutrition,* 2008; 27(1):1-5.

Gosain S, Ircchiaya R, Sharma C.P, Tharejad S, Kalra A, Deep A,and Bhardwaj Y (2010). Hypolipidemic effect of ethanolic extract from the leaves of Hibiscus sabdariffa L. in hyperlipidemic rats. *Acta Pol Pharm-Drug Research* 67 (2 ) : 179-184.

Hajizadeh R.M,and Mirzajani E (2011). The effects of consumption of raw garlic serum lipid level, blood sugar and a number of effective hormones on lipid and sugar metabolism in hyperglycemic and/or hyperlipidemic individuals. *Advanced Biolology and Chemistry*; 1(2):29-33.

Hanukoglu I (Dec 1992). "*Steroidogenic enzymes: structure, function, and role in regulation of steroid hormone biosynthesis*." *Journal of Steroid Biochemistry and Molecular Biology* 43 (8): 779–804.

"*High cholesterol levels by NHS*". National Health Service. Retrieved2010-09-14.

 <http://momordica-charantia.101herbs.com/> "Antidiabetic and hypolipidemic effects of *Momordica cymbalaria* Hook. fruit powder in alloxan-diabetic rats*"*. *Journal of Ethnopharmacology*: 67 (1): 1999, 103–9.

Huang C. X, Zhang Y. L (2013). "*The target of regulating the ATP-binding cassette A1 protein (ABCA1*): promoting ABCA1-mediated cholesterol efflux in different cells". Journal of *Current Pharmaceutical Biotechnology* 14 (6): 623– 31.

Incardona J.P,and Eaton S (April 2000). "Cholesterol in signal transduction". *Current Opinion Cell Biology* 12 (2): 193–203.

Indian Medicinal Plants, A Compendium of 500 species, Orient Longman Ltd., Madras, 4, 1995, 48-51.

Jaspreet Virdia, S (2003). Sivakamia, S. Shahanib, A. C. Sutharc, M. M. Banavalikar, M. K. Biyanic *"Antihyperglycemic effects of three extracts from Momordica charantia".* *Journal of Ethnopharmacology*, 88 (1):, 107– 11.

John S, Sorokin A.V, and Thompson P.D (February 2007). "Phytosterols and vascular disease". Currernt Opin. Lipidol. 18 (1): 35–40.

Joshi S.C (2005). Plant and plant products used as hypolipidaemic/antiatherosclerotic agents: an overview. *Proceeding of Zoological Society of India*.; 4: 27–32.

Kendrick M (May 2007). "Should women be offered cholesterol lowering drugs to prevent cardiovascular disease? No". *Biology and Medicine Journal* 334 (7601): 983–983.

Kizer J.R, Madias C, Wilner B, Vaughan C.J, Mushlin A.I, and Trushin P (2010). "Relation of different measures of low-density lipoprotein cholesterol to risk of coronary artery disease and death in a meta- regression analysis of large-scale trials of statin therapy.". *The American journal of cardiology* 105 (9): 1289–96.

Kwiterovich P.O (Dec 2000). "The metabolic pathways of high-density lipoprotein, low- density lipoprotein, and triglycerides: a current review". *The American Journal of Cardiology* 86 (12A): 5L–10L.

Lampe, M. A A L Burlingame, J Whitney, M L,Williams, B.E and Brown, E Roitman, and M. Elias (1983). "Human stratum corneum lipids: characterization and regional variations". *Journal of Lipid Research* 24: 120–130.

Leatherdale B.A, Panesar R.K,and Singh G, (1981). Improvement in glucose tolerance due to *Momordica charantia* (karela). *Br Medicine Journal (Clinical Research Edition).* ;282:1823-1824.

 Lecerf J.M, and Lorgeril M (2011). "Dietary cholesterol: from physiology to cardiovascular risk". *Br Journal of Nutrition* 106 (1): 6–14.

 Lewington S, Whitlock G, Clarke R, Sherliker P, Emberson J, and Halsey J (2007). "Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths". *Lancet laboratories* 370 (9602): 1829–39.

Lotlikar, M.M. and Rajarama Rao, M.R. Pharmacology of a hypoglycaemic principle isolated from the fruits of *Momordica charantia* Linn. *Indian Journal of Pharmacology*, 28, 1966, 129.

Lotlikar, M.M. and Rajarama Rao, M.R. Pharmacology of a hypoglycaemic principle isolated from the fruits of *Momordica charantia* Linn. *Indian Journal of Pharmacology*, 28, 1966, 129.

M.J. Sorrentino (2011), "Non-HDL-Cholesterol", Hyperlipidemia in Primary Care, pp. 86–87.

Mahmood Z.A, Sualeh M, Mahmood S.B,and Karim (2010). Herbal treatment for cardiovascular disease the evidence based therapy. *Pakistan Journal of Pharmaceutical Science*. 23(1):119– 124.

Matthew Lui,and Ross Garberich, Craig Strauss, Thomas Davin, and Thomas Knickelbine, *Usefulness of Lipid Apheresis in the Treatment of Familial Hypercholesterolemia Journal of Lipids*, vol. 2014, Article ID 864317, 6 p2014.

Mensink R.P,and Katan M.B (1992). "Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials". *Arteriosclerosis Thrombosis* 12 (8): 911–9.

Nadkarni K. M. (1993) Indian Materia Medica, Vol. 1, Popular Prakashan, 805- 806.

National Cholesterol Education Program (Ncep) Expert Panel On Detection, E. (2002). "Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report". Circulation 106 (25): 3143–3421.

National Health and Nutrition Examination Survey" (2012). *United States Center for Disease* Control. Retrieved 2012-01-28.

Nelson, D LCox, M M "*Lehninger, Principles of Biochemistry*" 3rd Ed. Worth Publishing: New York, 2000.

Nerurkar P.V, (2006). Lipid lowering effects of *Momordica charantia* (Bitter Melon) in HIV-1- protease inhibitor-treated human hepatoma cells, HepG2. Br *Journal of Pharmacology;* 148(8): 1156-64.

Nicholls S.J, (August 2008). "Rosuvastatin and progression of atherosclerosis". *Exper*t *Revised Cardiovascular Therapy* 6 (7): 925–33.

Ohvo-Rekilä H, Ramstedt Leppimäki P,and Slotte (January 2002). "Cholesterol interactions with phospholipids in membranes". Prog. Lipid Res. 41 (1): 66– 97.

Olson R.E, (February 1998). "Discovery of the lipoproteins, their role in fat transport and their significance as risk factors". *Journal of Nutrition* 128 (2): 439S–443S.

Parks, E.J. (2002). *"*Dietary carbohydrate’s effects on lipogenesis and the relationship of lipogenesis to blood insulin and glucose concentrations*"*. *British Journal of Nutrition* 87: S247–S253.

Puri M,(2009). *Ribosome inactivating proteins (RIPs) from Momordica charantia for anti viral therapy*. Curr Mol Med.; 9(9): 1080-94

Rahman I. U, Bashir M, Salman M, Idrees M,and Khan Bitter Melon *(Momordica Charantia) Reduces Serum Sialic Acid in Type2 Diabetics: Evidence to Delay the Process of Atherosclerosis*. Chinese Medicine, 2011; 2(4):125-29.

Razin S, and Tully JG (1970). "*Cholesterol Requirement of Mycoplasmas*".Journal of Bacteriology 102 (2): 306–310.

Rhodes C.M Stryer L,and Tasker R (1995). *Biochemistry* (4th ed.). San Francisco: W.H. Freeman. pp. 280, 703.

Sadava D, Hillis D.M, Heller H.C, Berenbaum M.R (2011). Life: The Science of Biology 9th Edition. San Francisco: Freeman. pp. 105–114.

Segrest J.P, (September 2001). "Structure of apolipoprotein B-100 in low density lipoproteins". *Journal of Lipid Research* 42 (9): 1346–67.

Shepherd J, Cobbe S.M, Ford I, Isles C.G, Lorimer A.R,and MacFarlane P.W (November 1995). "Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group". N. *England Journal Medicine* 333 (20): 1301– 7.

Sidhu, D.; Naugler, C. (2012). "Fasting Time and Lipid Levels in a Community- Based Population<subtitle>A Cross-sectional Study</subtitle><alt- title>Fasting Time and Lipid Levels</alt-title>". *Archives of Internal Medicine* 172 (22): 1–4.

Sirtori, Cesare R. (October 2006). "*HDL and the progression of atherosclerosis: new insights*". European Heart Journal Supplements.

Smith L.L (1991). "*Another cholesterol hypothesis: cholesterol as antioxidant*".Free Radic. Biol. Med. 11 (1): 47–61.

Srivastava Y,  *(*1993). Antidiabetic and adaptogenic properties of *Momordica charantia* extract: an experimental and clinical evaluation. *Phytother Res*. 1993;7:285-289

Stephens N.A, (2012). "*Trypanosome resistance to human innate immunity: targeting Achilles' hee*l". Trends in Parasitology 28 (12): 539–45.

Superko H.R, Nejedly M, Garrett B (2002). "*Small LDL and its clinical importance as a new CAD risk factor: a female case study*". Progress in Cardiovascular Nursing 17 (4): 167–73.

Sutharc, M. M. Banavalikar, M. K. Biyanic (2003) "*Antihyperglycemic effects of three extracts from Momordica charantia*". *Journal Ethnopharmacology*, 88 (1): 107– 11.

Tymoczko, John ,Stryer Berg, Tymoczko, Stryer, Lubert Berg, and Jeremy Mark (2002). *Biochemistry*. San Francisco: W.H. Freeman. pp. 726–727.

"*USDA National Nutrient Database for Standard Reference*,"(2008). United States Department of Agriculture. Retrieved 2008-10-24.

Vance D E and Vance (Eds.) J E *Biochemi.stly ~/Lipids. LipoproteiHs aml Membranes* (4th Edn. ) ~ 2002 Elsevier Science B.V. 18:483-485

Virdi J, Sivakami S, (2003). *Antihyperglycemic effects of three extracts from Momordica charantia*. *Journal Ethnopharmacology*; 88(1): 107-11. 11. Khan BB & Flier JS *"Obesity and insulin resistance* *J.Clin.Investig*; 106: 2000, 473- 481

Warnick G.R, Knopp , Fitzpatrick V,and Branson L (January 1990). "*Estimating low- density lipoprotein cholesterol by the Friedewald equation is adequate for classifying patients on the basis of nationally recommended cutpoints*". Clin. Chem.36 (1): 15–9.

Weingärtner O, Böhm M, Laufs U (February 2009). "*Controversial role of plant sterol esters in the management of hypercholesterolaemia*". Eur. Heart J. 30 (4): 404–9.

Weingärtner O, Ulrich C, Lütjohann D, Ismail K, Schirmer S.H,and Vanmierlo T (February 2011). "*Differential effects on inhibition of cholesterol absorption by plant stanol and plant sterol esters in apoE-/- mice*". Cardiovasc Res 90 (3): 484–92.

Welhinda J, and Karunanayake E.H. *Effect of* *Momordica charantia* on the glucose tolerance in maturity onset diabetes. *Journal Ethnopharmacology.* 1986;17:277-282.

Yeagle P.L, (October 1991). "*Modulation of membrane function by cholesterol*".Biochimie 73 (10): 1303–10.

**APPENDIX**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Weight in gram** |  | **Glucose level before alloxan** | **Alloxan in gram** | **Alloxan in ml** | **Alloxan in microlitre** | **2 days after induction** | **3 days after induction** |
| **Group A** | **Glibenclamide** |
|  | 121.1 | No mark  | 90mg/dl | 0.012 | 0.6 | 24ul | 103 | 211 |
|  | 119.47 | Tail | 97mg/dl | 0.012 | 0.6 | 24ul | 115 | 230 |
|  | 93.28 | Head | 77mg/dl | 0.010 | 0.5 | 20ul | 111 | 221 |
|  | 138.37 | Stomach  | 90mg/dl | 0.012 | 0.6 | 24ul | 114 | 236 |
|  | 141.44 | Back | 96mg/dl | 0.014 | 0.7 | 28ul | 115 | 241 |
| **Group B** | **Hexane**  |
|  | 117.95 | No mark | 211mg/dl | 0.012 | 0.6 | 24ul | 213 | 303 |
|  | 87.47 | Head | 72 mg/dl | 0.009 | 0.45 | 18ul | 101 | 188 |
|  | 96.7 | Back | 73mg/dl | 0.01 | 0.5 | 20ul | 98 | 203 |
|  | 116.57 | Tail  | 73mg/dl | 0.012 | 0.6 | 24ul | 113 | 233 |
|  | 147.45 | Stomach  | 73mg/dl | 0.015 | 0.75 | 30ul | 99 | 199 |
| **Group C** | **Aqueous Fraction**  |
|  | 143.51 | No mark | 59 | 0.014 | 0.7 | 28ul | 89 | 189 |
|  | 176.07 | Tail | 93 | 0.017 | 0.85 | 34ul | 111 | 168 |
|  | 139.57 | Stomach  | 180 | 0.14 | 0.7 | 28ul | 199 | 285 |
|  | 120.75 | Back  | 106 | 0.012 | 0.6 | 24ul | 201 | 300 |
|  | 124.95 | Head  | 103 | 0.01 | 0.5 | 20ul | 198 | 238 |
| **Group D**  | **Methanol**  |
|  | 128.65 | No mark  | 76 | 0.013 | 0.65 | 26ul |  |  |
|  | 163.72 | Tail  | 88 | 0.02 | 1.0 | 40ul |  |  |
|  | 115.45 | Back  | 93 | 0.012 | 0.6 | 24ul |  |  |
|  | 134.31 | Stomach  | 85 | 0.013 | 0.65 | 26ul |  |  |
|  | 132.62 | Head  | 97 | 0.013 | 0.65 | 26ul |  |  |
| **Group E** | **Et. Ac** |
|  | 133.57 | No mark | 93 | 0.013 | 0.65 | 26ul | 122 | 297 |
|  | 79.75 | Stomach  | 74 | 0.008 | 0.4 | 16ul | 97 | 173 |
|  | 129.57 | Tail  | 111 | 0.013 | 0.65 | 26ul | 103 | 333 |
|  | 120.25 | Back  | 103 | 0.012 | 0.6 | 24ul | 117 | 252 |
|  | 156.57 | Head  | 55 | 0.02 | 1.0 | 40ul | 88 | 151 |
| **Group F** | **Chlorofoam**  |
|  | 165.84 | Back  | 80 | 0.02 | 1.0 | 40ul | 115 | 205 |
|  | 164.45 | No mark | 85 | 0.02 | 1.0 | 40ul | 119 | 265 |
|  | 102.03 | Head | 157 | 0.010 | 0.5 | 20ul | 105 | 232 |
|  | 131.50 | Stomach  | 84 | 0.013 | 0.65 | 26ul | 110 | 209 |
|  | 137.00 | Tail  | 77 | 0.014 | 0.7 | 28ul | 113 | 213 |
| **Group G** | **Alloxan No Treatment** |
|  | 129.28 | Tail  | 87 | 0.013 | 0.65 | 26ul | 106 | 299 |
|  | 157.49 | Back  | 93 | 0.016 | 0.8 | 32ul | 128 | 334 |
|  | 103.28 | Head  | 90 | 0.010 | 0.5 | 20ul | 115 | 241 |
|  | 98.81 | Stomach  | 78 | 0.01 | 0.5 | 20ul | 111 | 190 |
|  | 144.85 | No mark  | 79 | 0.014 | 0.7 | 28ul | 98 | 234 |
| **Group H** | **No Alloxan**  |
|  | 152.37 | Tail  | 79mg/dl |  |  |  |  |  |
|  | 151.01 | Head  | 98mg/dl |  |  |  |  |  |
|  | 172.61 | No mark  | 111mg/dl |  |  |  |  |  |
|  | 147.22 | Back  | 96mg/dl |  |  |  |  |  |
|  | 140.25 | Stomach  | 93mg/dl |  |  |  |  |  |

**Administration of Alloxan**

100mg/kg pose

**Group A**

**121 gram No mark rat**

1000 g = 100 mg

121 g =?

Mg = $\frac{121 ×100}{1000}$

$$\frac{12.1}{1000}$$

= 0.012g

**119.41 g Tail**

1000 g = 100 mg

119.41 g = ?

Mg = $\frac{119.41 ×100}{1000}$

$$\frac{11.9}{1000}$$

= 0.0119g $≅0.012g$

**93g Head**

1000 g = 100 mg

93g = ?

Mg = $\frac{93 ×100}{1000}$

$$\frac{9.3}{1000}$$

= 0.009g $≅0.01g$

**93g Stomach**

1000 g = 100 mg

138.37g = ?

Mg = $\frac{138.37 ×100}{1000}$

$$\frac{13.837}{1000}$$

= 0.012g

**141.44g Head**

1000 g = 100 mg

141g = ?

Mg = $\frac{141.44 ×100}{1000}$

$$\frac{14.144}{1000}$$

= 0.014g

**Group B**

**117.95 gram No mark rat**

1000 g = 100 mg

117.95 g = ?

Mg = $\frac{117.95 ×100}{1000}$

$$\frac{11.7}{1000}$$

= 0.012g

**87.47 g Head**

1000 g = 100 mg

87.47 g = ?

Mg = $\frac{87.47 ×100}{1000}$

$$\frac{8.7}{1000}$$

= 0.009g

**96.76g Back**

1000 g = 100 mg

96.76g = ?

Mg = $\frac{96.76 ×100}{1000}$

$$\frac{9.6}{1000}$$

= $0.01g$

**116.57 Tail**

1000 g = 100 mg

116.57g = ?

Mg = $\frac{116.57 ×100}{1000}$

$$\frac{11.6}{1000}$$

= 0.012g

**147.45g Stomach**

1000 g = 100 mg

147.45g = ?

Mg = $\frac{147.45 ×100}{1000}$

$$\frac{14.7}{1000}$$

= 0.015g

**Group C**

**143.51 gram No mark rat**

1000 g = 100 mg

143.51 g = ?

Mg = $\frac{143.51 ×100}{1000}$

$$\frac{14.3}{1000}$$

= 0.014g

**176.07 Tail**

1000 g = 100 mg

176.07 g = ?

Mg = $\frac{176.07 ×100}{1000}$

$$\frac{17.6}{1000}$$

= 0.017g

**139.57g Stomach**

1000 g = 100 mg

139.57g = ?

Mg = $\frac{139.57 ×100}{1000}$

$$\frac{13.9}{1000}$$

= $0.14g$

**120.75 Back**

1000 g = 100 mg

120.75g = ?

Mg = $\frac{120.75 ×100}{1000}$

$$\frac{12.0}{1000}$$

= 0.012g

**124.95g Head**

1000 g = 100 mg

124.95g = ?

Mg = $\frac{124.95 ×100}{1000}$

$$\frac{12.4}{1000}$$

= 0.01g

**Group D**

**128.65 gram No mark rat**

1000 g = 100 mg

128.65 g = ?

Mg = $\frac{128.65 ×100}{1000}$

$$\frac{12.8}{1000}$$

= 0.013g

**163.72 g Tail**

1000 g = 100 mg

163.72 g = ?

Mg = $\frac{163.72 ×100}{1000}$

$$\frac{16.4}{1000}$$

= 0.02g

**115.43g Back**

1000 g = 100 mg

115.43g = ?

Mg = $\frac{115.43 ×100}{1000}$

$$\frac{11.5}{1000}$$

= $0.012g$

**134.31 Stomach**

1000 g = 100 mg

134.31g = ?

Mg = $\frac{134.31 ×100}{1000}$

$$\frac{13.4}{1000}$$

= 0.013g

**147.45g Head**

1000 g = 100 mg

132.62g = ?

Mg = $\frac{132.62 ×100}{1000}$

$$\frac{13.3}{1000}$$

= 0.013g

**Group E**

**133.57 gram No mark rat**

1000 g = 100 mg

133.57 g = ?

Mg = $\frac{133.57 ×100}{1000}$

$$\frac{13.3}{1000}$$

= 0.013g

**79.75 g Stomach**

1000 g = 100 mg

79.75 g = ?

Mg = $\frac{79.75 ×100}{1000}$

$$\frac{7.9}{1000}$$

= 0.008g

**129.57g Tail**

1000 g = 100 mg

129.57g = ?

Mg = $\frac{129.57 ×100}{1000}$

$$\frac{12.9}{1000}$$

= $0.013g$

**120.25 Back**

1000 g = 100 mg

120.25 g = ?

Mg = $\frac{120.25 ×100}{1000}$

$$\frac{12.1}{1000}$$

= 0.012g

**156.57 Head**

1000 g = 100 mg

156.57 = ?

Mg = $\frac{156.57 ×100}{1000}$

$$\frac{15.7}{1000}$$

= 0.02g

**Group F**

**165.84 Back**

1000 g = 100 mg

165.84 g = ?

Mg = $\frac{165.84 ×100}{1000}$

$$\frac{16.6}{1000}$$

= 0.02g

**No mark= 164.45**

1000 g = 100 mg

164.45 g = ?

Mg = $\frac{164.45 ×100}{1000}$

$$\frac{16.5}{1000}$$

= 0.02g

**Head =102.3**

1000 g = 100 mg

102.3g = ?

Mg = $\frac{102.3 ×100}{1000}$

$$\frac{10.3}{1000}$$

= $0.01g$

**Stomach 131.50**

1000 g = 100 mg

131.50 g = ?

Mg = $\frac{131.50 ×100}{1000}$

$$\frac{13.2}{1000}$$

= 0.013g

**Tail 137.00**

1000 g = 100 mg

137.00 = ?

Mg = $\frac{137.00 ×100}{1000}$

$$\frac{13.7}{1000}$$

= 0.014g

**Group G**

**Tail 129.28**

1000 g = 100 mg

129.28 g = ?

Mg = $\frac{129.28 ×100}{1000}$

$$\frac{12.9}{1000}$$

= 0.013g

**No mark= 157.49**

1000 g = 100 mg

157.49 g = ?

Mg = $\frac{157.49 ×100}{1000}$

$$\frac{15.7}{1000}$$

= 0.016g

**Head =102.3**

1000 g = 100 mg

102.3g = ?

Mg = $\frac{102.3 ×100}{1000}$

$$\frac{10.3}{1000}$$

= $0.01g$

**Stomach 131.50**

1000 g = 100 mg

131.50 g = ?

Mg = $\frac{131.50 ×100}{1000}$

$$\frac{13.2}{1000}$$

= 0.013g

**Tail 137.00**

1000 g = 100 mg

137.00 = ?

Mg = $\frac{137.00 ×100}{1000}$

$$\frac{13.7}{1000}$$

= 0.014g