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Hypoglycaemic and Hypolipidemic Effects of Aqueous Extract of *Annona muricata* Stem bark on Alloxan Induced Diabetes in Albino Wistar Rats

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ABSTRACT
The importance of the active constituents of plants in medicine has stimulated significant scientific interests in the biochemical activities of these substances. This study investigated the hypoglycaemic and hypolipidemic effects of the stem bark of *Annona muricata* (Soursop). Fourty albino rats of both sexes (aged 3 months, 140-170g) were divided into eight groups. Group A was male control, Group H female control while Groups B to G were test groups. Diabetes was induced in the test rats with a single intraperitoneal injection of 2.5mg/ml/kg alloxan and the rats treated with oral administration of aqueous extract of *Annona muricate* stem bark of 100, 120, 140, 160, 180 and 200mg/kg respectively for 7days. The blood glucose, total cholesterol(TC), High density lipoprotein(HDL), Low density lipoprotien(LDL), Very low density lipoprotein(VLDL) and Triglyceride (TG) levels were assayed by standard methods before and after alloxan injection and on the 8th day after treatment with the extract. After induction, blood glucose increased from a control value of 4.97±0.7mmol/l to 12.4±6.4mmol/l, TC from 0.98±0.4mmol/l to01.25±0.2mmol/l, LDL from 0.68±0.14mmol/l to 4.12±0.64mmol/l, VLDL from 0.18±0.04mmol/l to 0.21±0.04mmol/l and TG from 0.27±0.06mmol/l to 0.32±0.19mmol/l. HDL decreased from 0.44±0.04mmol/l to 0.21±0.12mmol/l. These parameters were significantly elevated($P<0.05$) after induction with alloxan compared to control values but decreased significantly ($p<0.05$) after treatment with aqueous extract of *Annona muricata* stem bark. The extract of *Annona muricata* stem bark had the capacity to lower high blood glucose level and regulated lipid profile balance.
Key words: *Annona muricata*, stem bark, hypoglycaemic, hypolipidemic, capacity.

INTRODUCTION
Natural products especially those derived from plants have been used to help mankind sustain its health since the dawn of medicine. The attainment of a reasonable perception of natural products necessitates comprehensive investigations on the biological and biochemical activities of these plants and their key phytochemicals. One of such plants in traditional use is *Annona muricata*, also known as Soursop (Alexander *et al.*, 2014). Annonaceae are a family of flowing plants commonly known as the custard apple family or Soursop family. They have 108 accepted genera and about 2400 known species (Arikawe *et al.*, 2012). Several genera produce edible fruit notably Annona, Anonidium, Asimina, Rollinia and Uvaria. Seven Annona species and one hybrid are grown for domestic or commercial use, mostly for the edible and nutritious fruits. Many of the species are used in traditional medicines for the treatment of a variety of disease, though their efficacy is yet to be validated scientifically. Annona species are tap rooted, evergreen or semidecidous (seasonally shed leaves, petals and fruits after flowering, usually in autum) tropically trees or shrups.

Annona muricata is also called graviola or guyabano and in Spanish, guanabana. It is a small, upright, evergreen tree that can grow to about 30feet (9.1m) tall. It is native to the warmest tropical areas in South and North American and now widely distributed

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throughout tropical and subtropical parts of the world including Indian, Malaysia and Nigeria (Bradly *et al.*, 2012). It is adapted to areas of high humidity and relatively warm winters. Its young branches are hairy with oblong to oval leaves, 8 centimeters (3.1inches) to 16 centimeters (6.3inch) long and 3 centimeters (1.2inch) to 7 centimeters (2.8inch) wide. The fruits are dark green and prickly. Their flesh is juicy, acid, whitish and aromatic (Ahalya *et al.*, 2014). The fruit is usually called Soursop due to its slightly acidic taste when ripe with an aroma similar to pineapple (Devi *et al.*, 2012). The peak period of Soursop trees is between May and June but they bear fruit all year round (Adewole *et al.*, 2016).

The term "Diabetes" and "Mellitus: were derived from Greek. "Diabetes" denotes a passer through; a siphon" whereas the Mellitus denotes "sweet". The Greeks named it because the excessive amounts of urine produced by the diabetics attracted flies and bees (Petal, 2011). Although the prevalence of both type I and type II DM is increasing worldwide, the prevalence of type II DM is rising much more rapidly, presumably because of increasing obesity, reduced activity levels as countries become more industrialized and the ageing of the population.

Diabetes mellitus is a group of metabolic diseases characterized by increased level of blood glucose which results from defects in insulin secretion, insulin action or both. Several distinct types of diabetes mellitus are caused by a complex interaction of genetics and environment factors. Depending on the aethiology, the metabolic deregulation associated with diabetes mellitus causes a secondary pathophysiologic change in multiple organ systems that impose a tremendous burden on the individual with diabetes and on the health care systems (Anderson et al., 2011). The main types of diabetes are types 1 and type II. Type 1 diabetes results from an irreversible loss of pancreatic β-cells while type II is primarily caused by impaired Insulin action (Lensen, 2013). Several pathogenic processes are involved in the development of diabetes. These range from auto immune destruction of the pancreatic β-cells with consequent insulin deficiency to abnormalities that result in resistance to insulin action.

Diabetes is a disease characterized by abnormal glucose metabolism (Adeyemi *et al.*, 2015). Generally, all forms of diabetes are due to the beta cells of the pancreas being unable to produce sufficient insulin which causes hyperglycemia. One major role of insulin is to stimulate the storage of food energy following consumption of a meal. This energy storage is in the form of glycogen in hepatocytes and skeletal muscle. Additionally, Insulin stimulates hepatocytes to synthesize triglyceride(triacylglycerol) and storage of triglycerides in adipose tissue (Casy *et a*l., 2014).

Alloxan is a urea derivative which causes selective necrosis of the beta-cells of the pancreatic islets. It is used to induce experimental diabetes in animals such as rabbits, rats, dogs and mice. The diabetogenic effects is by the destruction of beta cells caused by reactive oxygen species (ROS) in a cyclic redox reaction. Alloxan selectively inhibits glucose induced Insulin secretion through its ability to inhibit the beta cell glucose sensor

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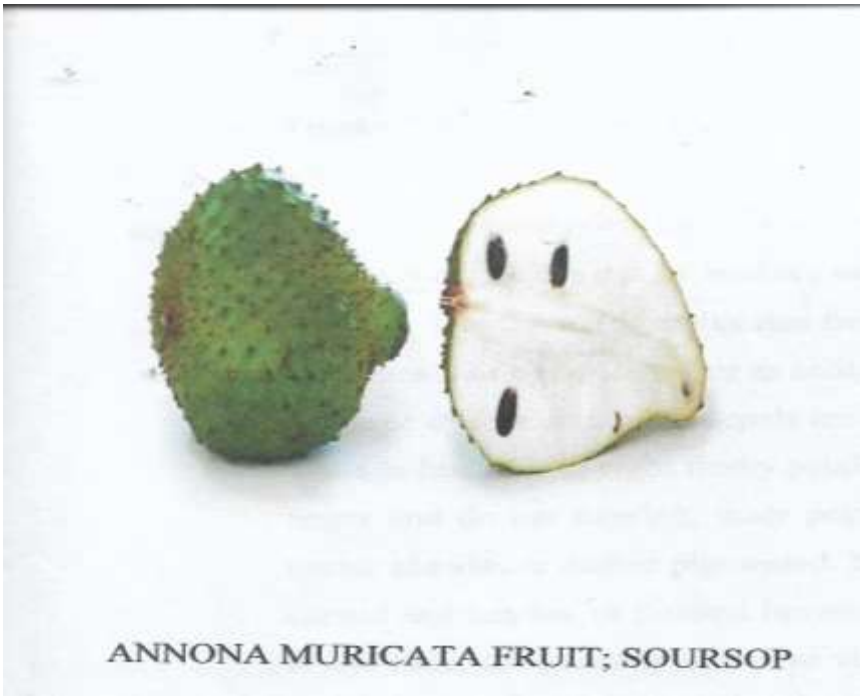
glucokinase (Argoudelis *et al.,* 2014). A lipid profile is a measurement of various lipids that are found in the blood. It is a collective term given to the estimation of typically, total cholesterol, high-density lipoprotein , low-density lipoprotein and triacylglycerol. An extended lipid profile includes very low-density lipoprotein. These are used to identify hyperlipidemia and the risk of heart disease (Chawla, 2014).



ANNONA MURICATA FRUIT; SOURSOP

ANNONA MURICATA LEAVES AND FRUIT

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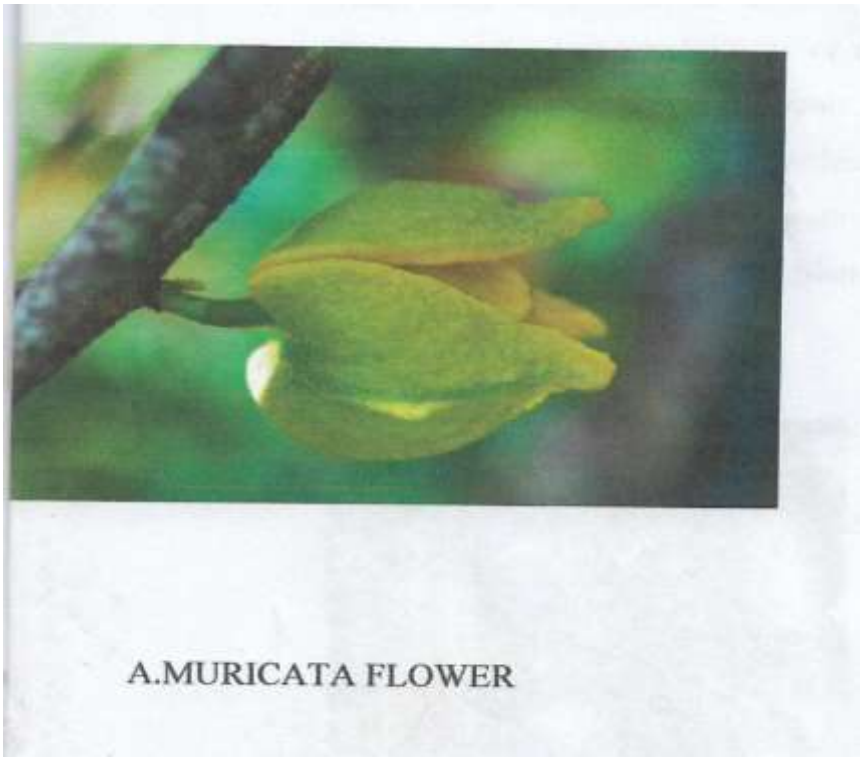

RATIONALE OF STUDY

The attainment of a reasonable perception of plants natural products necessitates comprehensive investigations on the biochemical activities of these plants and their key phytochemicals. Plants with a long history of use in ethno medicine are a rich source of active phytoconstituents that provide medicinal or health benefits against various aliments and diseases (Bolton *et al.,* 2011). One of such plants in traditional use is *Annona muricata* from the family of Annonaceae. Diabetes is a disease characterized by abnormal glucose metabolism, a risk of developing microvascular complications specific to diabetes (Adeyimi *et al.,* 2013). Diabetes plays an important role in oxidative stress and abnormal lipid metabolism that leads to dyslipidemia, a frequent complication of diabetes (Ahalya *et al.,* 2014). The Chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction and failure of different organs especially the eyes, kidneys, nerve, heart and blood vessels. Previous studies have been focused on the biological activities of *Annona muricata* extract, further investigation on the biochemical and physiological functions of active compounds and the detailed mechanisms underlying these activities are completely pivotal for the development of pharmaceutical and agricultural products (Beuges *et al.,* 2016). DM if left untreated can lead to serious problems like macrovascular and microvascular complications (Ferris *et al.,* 2012).Therefore, drugs and plants that have hypoglycaemic and hypolipidemic effects are very useful in the management of diabetes and its complications. Thus, it was the aim of this study to ascertain the hypoglycaemic and hypolipidemic effects of aqueous extract of *Annono muricata* stem bark in the management of diabetes in albino rats.

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MATERIALS AND METHODS

Forty albino rats of both sexes (aged 3months, 140-170g) were obtained from Animal House, University of Nigeria, Nuskka. They were housed in laboratory cages under standard laboratory conditions and fed with standard rat pallets (Chikun palleted finisher feed) and given tap water for 2weeks before experimental procedures. The rats were divided into 8 groups of 5 rats each. Group A was control group (male) while Group H was control group (female). Groups B, C, D, E,F and G were test groups.

INDUCTION OF EXPERIMENTAL DIABETES

The test animals were left to fast for 48hours without food and water. Diabetes was induced in the test Group B to G with a single intraperitoneal injection of 2.5mg/ml of alloxan. The test animals became diabetic within 72hours of alloxan administration as confirmed by blood glucose measurements before and 72hours after alloxan induction. Diabetes and hyperlipidemia were allowed to develop and stabilize in the induced rats for 6 days. All animals in all the groups were maintained under laboratory conditions and were allowed free access to food and water.

PREPARATION OF PLANT MATERIALS

The stem bark of *Annona muricata* plant was freshly removed and washed thoroughly with tap water and air dried at room temperature for 3weeks. The air dried stem-bark was ground into fine powder with electric grinding machine. The powdered stem-bark (20g) was soaked in 400ml distilled water for 72hours. It was filtered twice, with moselyn sheet and secondly with whatman filter paper to obtain a clear solution of the plant sample. The aqueous solution was concentrated in a water-bath at a temperature of 70°C for 48hours to obtain a paste-like extract. During the administration of the extract to the test rats, 30ml of distilled water was added to form an aqueous solution.

DOSAGE FOR ADMINISTRATION

The aqueous plant extract was orally administered every morning for 7days. Dosage of extract administered to each rat was calculated using the formula

$$\text{Volume to be administered (ML)} = \frac{\text{weight of rat (kg)} \times \text{Dose of Extract (mg/kg)}}{\text{Concentration of Extract (mg/kg)}}$$

The test groups of B, C, D, E, F and G were treated with 100, 120, 140,160, 180 and 200mg/kg of the extract for 7 consecutive days.

COLLECTION OF BLOOD SAMPLES

Blood samples were collected from the test rats though eye puncture using non-heparinized capillary tubes into fluoride and plain bottles, blood samples were collected before alloxan induction, after alloxan induction and after administration of aqueous extract of *Annona muricata* stem bark. Fluoride samples were assayed for blood glucose by glucose oxidase method while plain bottle samples were allowed to clot for one hour at room temperature and serum samples separated into sample tubes for the assay of total

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cholesterol, HDL, LDL, VLDL and triglyceride using Randox Kits and Chemwell Auto analyzer.

STATISTICAL ANALYSIS

Results were expressed as mean ± standard deviation and analysed using one-way analysis of variance (ANOVA). P value ($P<0.05$) was considered significant.

TABLE 1: Mean values before and after induction of diabetes and after Extract Administration

	Blood glucose (mmol/l)	TC (mmol/l)	HDL (mmol/l)	LDL (mmol/l)	VLDL (mmol/l)	TG (mmol/l)
Before induction (control)	4.97±0.7 (4.27-5.66)	0.98±0.40 (0.58-1.38)	0.44±0.04 (0.4-0.48)	0.68±0.14 (0.54-0.82)	0.18±0.04 (0.14±0.28)	0.27±0.06 (0.21±0.33)
After Induction (Diabetes)	12.4±6.46 (5.94±18.87)	1.25±0.20 (1.05±1.45)	0.21±0.12 (0.09±0.33)	4.12±0.64 (3.48±4.76)	0.21±0.04 (0.17±0.25)	0.32±0.10 (0.13±0.51)
After Extract of (*Annona muricata*)	2.66±0.78 (1.88±3.44)	0.96±0.40 (0.56±1.36)	0.39±0.12 (0.27±0.51)	1.26±0.2 (1.06±1.46)	0.19±0.04 (0.15±0.23)	0.28±0.02 (0.26±0.3)

$P<0.05$

Table 2: Percentage variations of mean values after induction of Diabetes

Parameters	Percentage increase (%)	Percentage decrease (%)
Glucose	59.9	-
TC	21.6	-
HDL	-	52
LDL	83.5	-
VLDL	14.3	-
TG	15.6	-

$P<0.05$

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Table 3: Percentage variations of mean values after administration of aqueous extract of *Annona muricata* stem-bark

Parameters	Percentage increase (%)	Percentage decrease (%)
Glucose	-	78.5
TC	-	23
HDL	46	_
LDL	-	69.4
VLDL	-	9.5
TG	-	12.5

P<0.05

DISCUSSION

The use of plants, plant extracts and active components from plants to cure diseases is a very potential step in new drug discovery (Ftuk *et al.*, 2014). This study investigated the efficacy of the aqueous extract of *Annona muricata* stem-bark on the lipid profile and blood glucose levels in alloxan induced diabetes in albino rats. The test rats were deprived of food for 48hrs and diabetes was successfully induced with alloxan. Diabetes is increase in blood glucose level (hyperglycaemia) due to lack or Insulin insufficiency (Cambel *et al.*, 2014). Blood glucose concentration was significantly increased by 59.9% (P<0.05) in the test rats after induction of diabetes with alloxan. Alloxan ignites a rise in glucose concentration by destroying insulin producing cells in the pancrease. Alloxan is selectively toxic to insulin producing beta cells in the presence of intracellular thiols. In pancreatic beta cells, the reduction process occurs in the presence of reducing agents such as reduced glutathione (GSH), cystein, ascorbate and protein-bound sulphydryl(-SH) groups. Alloxan reacts with two SH groups in the sugar binding site of glucokinase and result in inactivation of the enzyme. Dialuric acid is formed as a result and is re-oxidized back to alloxan establishing a redox cycle and generates reactive oxygen species (ROS) and superoxide radicals. The superoxide radicals liberate ferric ion from ferritin and reduce them to ferrous ions and also undergo dismutation to yield hydrogen peroxide (Argoudelis *et al.*, 2014).

Beta cells toxic action of alloxan is initiated by free radicals formed in this redox reaction. Alloxan action in the pancreas is precded by its rapid uptake by pancreatic beta cells and this selective uptake is due to its structural similarity to glucose and also because of high efficient uptake mechanism of beta cells (Chichowska *et al.*, 2012). Persistent hyperglycemia causes glycosylation of all proteins, especially collagen cross linking and matrix proteins of arterial wall. This eventually causes endothelial cells dysfunction . The chronic hyperglycaemia of diabetes is associated with long term damage, dysfunction and disturbance in failure of various organs, especially the eyes, kidneys, nerves, heart and

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blood vessels (Lachi *et al.,* 2012). Several pathogenic processes are involved in the developing of diabetes. These range from autoimmune destruction of the cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat and protein metabolism in diabetes is deficiency of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue response to insulin at one or more points in the complex pathways of hormone actions (Patel *et al.,* 2011).

The body responds to hyperglycaemia by drawing water out of the cells and into the blood stream. The excess sugar is excreted in the urine. This is why diabetes presents constant thirst, drinking large amounts of water and polyuria as the cells try to get rid of the extra glucose. This subsequently leads to glucosuria. As hperghycaemia prolongs the body cells are devoid of glucose due to lack of Insulin. This forces the cells to seek alternative mobilizable energy sources. In this situation, the cells turn to fatty acids stored in the adipose tissue (Arsianian *et al.,* 2013). Fats are not fuel sources for the red blood cells, kidney cortex and the brain. The red blood cells lack mitochondria in which beta-oxidation pathway rest. The fatty acids cannot pass the bloods-brain banner. To provide energy to such cells and tissue, the acetyl-CoA arising from catabolism of fatty acids is diverted to ketogenesis to generate ketone bodies which serve as alternative fuel sources for such cells and tissues. These ketone bodies are also passed in the urine, causing ketonuria. Build up of ketone bodies in the blood gives rise to Ketosis. Ketone bodies are acidic in nature and their accumulation in the blood lowers blood pH leading to acidosis. A combination of ketosis and acidosis leads to keto-acidosis and if left untreated might result in coma and death.

Glucose is an osmotic diuretic and an increase in renal loss of glucose is accompanied by loss of water and electrolytes and termed polyuria. The result of the loss of water (and overall volume) leads to the activation of thirst mechanism (Polydipsia). The negative caloric balance which results from the glucosuria and tissue catabolism leads to an increase in appetite and food in-take (polyphagia) (Chung *et al.,* 2012). Lipids are a heterogeneous group of water-insoluble (hydrophobic) organic molecules that can be extracted from tissues by non-polar solvents. Because of their insolubility in aqueous solution, body lipids are generally compartmentalized, as in the case of membrane associated lipids or droplets of triacylglycerol in white adipocytes or transported in plasma in association with protein as in lipopotein particles or on albumin. Lipids are a major source of energy for the body and they also provide the hydrophobic barrier that permits partitioning of the aqueous contents of the cells and subcellular structures. Lipids serve additional functions in the body for example, some fat soluble vitamins have regulatory or co-enzyme functions and prostaglandins and steroid hormones play major roles in the control of body homeostasis. Deficiencies or imbalances of lipid metabolism lead to some major clinical conditions such as hyperglycaemia and lipidemia (Craighead, 2014).

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A lipid panel comprises total cholesterol (TC), high density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL) and triglyceride (TG). Following induction of diabetes in this study, TC increased by 21.6%, from 0.98± 0.40mmol/l to 1.25± 0.20mmol/l as shown in table 1. Cholesterol is a necessary molecule in human metabolism. It is a component of all membranes and a building block of bile, estrogen and testosterone. It is a precursor of bile acids, steroid hormones and vitamin D and therefore of critical importance to cells of the body which require appropriate supply of cholesterol (Goldner and Gomori, 2013). To meet this need, a complex series of transport, biosynthetic and regulatory mechanisms are in place. The liver plays a central role in the regulation of the body's cholesterol homeostasis, for example, cholesterol enters the liver's cholesterol pool from a number of sources including dietary cholesterol as well as cholesterol synthesized de novo by extrahepatic tissues and by the liver itself. Cholesterol is eliminated from the liver as unmodified cholesterol in the bile or converted to bile salts that are secreted into the intestinal lumen. In humans, the balance between cholesterol influx and efflux is not precise, resulting in a gradual deposition of cholesterol in the tissues particularly in the endothelial linings of blood vessels. This is a partially life-threatening occurrence when the lipid deposition leads to plaque formation, causing narrowing of blood vessels (atherosclerosis) and increased risk of cardiovascular disease (Chawla *et al.*, 2014).

HDL cholesterol is cholesterol that is packaged for delivery to the liver where the cholesterol level is removed from the body. High-density lipoprotien was significantly ($P<0.05$) decreased after induction of diabetes by 52% compared to control value. HDL is called "good Cholesterol" since it removes cholesterol from the liver. The intestine and the liver release HDL and HDL particles contains APO-C and APO-E which can be transferred to VLDL and cylomicrons to allow the metabolism of these particles. HDL also contains APO-A-1 which functions as an activator of lecithin cholesterol acyltransferase (LCAT). LCAT transfer acyl chains from phospholipids and concentrates cholesterol from both tissues and other lipoproteins. APO-A is a ligand for the HDL receptor. HDL binds to its receptor in the liver and transfer accumulated cholesterol and cholesterol esters to the liver for processing and the HDL is then either released or degraded. High levels of HDL are associated with reduced risk of heart disease due to increased cholesterol scavenging and in the process lower LDL and total cholesterol. Exercise is associated with an increase in HDL levels and females have higher HDL until menopause. This is strongly corrected with lower risk of heart disease and an increased risk as HDL level falls after menopause. This is due to estradiol levels.

Low density lipoprotein, termed "bad cholesterol" markedly increased by 83.5%, from 0.68± 0.14mmol/l to 4.12± 0.68mmol/l during the diabetic phase of this study. It contains the highest amount of cholesterol. It is a major transport form of cholesterol and cholesterol esters. The rate of LDL release by the liver depends on the availability of cholesterol. If the regulatory pathways signal the liver to increase its cholesterol output, then the liver increase the LDL production. High levels of LDL are associated with elevated risk of heart disease. Very low-density lipoprotein was elevated after induction of

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diabetes from 0.18±0.04mmol/l to 0.21±0.04mmol/l. This is a 14.3 percent increase in concentration while mean triglyceride value increased from 0.27±0.06mmol/L to 0.32±0.19mmol/L. Triglyceride is the most common type of lipid formed in animals. Triglycerides or triacylglycerol are the simplest lipids composed of three fatty acids each in ester linkage with a single glycerol molecule. They are non-polar, hydrophobic and essentially, insoluble in water since the polar hydroxyls of glycerol and the polar carboxylates of fatty acids are bound in ester linkage. Triglyceride constitutes the main source of energy for the body and in the human system. It is the most reliable energy reserve and is stored in adipose tissue. High triglyceride level is called hypertriglyceridemia and indicates insulin resistance in the metabolism of triglyceride, also leading to hyperglycaemia (Mendez and Ramos, 2013).

Lipid transport is a continuous varying process. During the absorption of nutrients from the diet, lipids must be transported to the tissues for use. When lipids are not absorbed they must be transported from adipose stores to maintain metabolism (Lachin *et al.,* 2012). Four major groups of lipoprotein are recognized; Chylomicrons transport lipids resulting from digestion and absorption, very low density lipoprotein transports triacylglycerol from the liver, LDL delivers cholesterol to the tissues while HDL removes cholesterol from the tissues in the process known as reverse cholesterol transport. Chylomicrons and VLDL are metabolized by hydrolysis of their triacylglycerol and lipoprotein remnants and left in the circulation. These are taken up by the liver, some of the remnants (IDL) resulting from VLDL form LDL which is taken up by the liver via the LDL receptor.

In table 1, after the administration and treatments with aqueous extract of *Annona muricata* stem bark for 7 days, it resulted in a significant 78.5% reduction ($P<0.05$) in glucose level, from12.4±6.4mmol/l to 2.66±0.78mmol/l. Also the levels of total cholesterol, LDL, VLDL and triglyceride decreased to baseline while HDL that was decreased following induction of diabetes reverted back to normal level. These results explained the beneficial effect of *Annona muricata* stem bark on pancreatic tissues especially pancreatic beta cells, and ultimately improved glucose and lipid metabolism. Generally, this study revealed the anti-diabetic and anti-lipidemic properties of *Annona muricata* stem bark. It is believed that the government, pharmaceutical and agricultural companies will take advantage of this study for the benefit of mankind.

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