EFFECT OF ORAL ADMINISTRATION OF AQUEOUS FRUIT PULP EXTRACT OF *TAMARINDUS INDICA* ON DIABETES MELLITUS ON DIABETES MELLITUS AND HYPERLIPIDEMIA IN RATS

BY

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BEING A DISSERTATION SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY, FACULTY OF SCIENCE, BAYERO UNIVERSITY, KANO IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (M.SC) DEGREE IN BIOCHEMISTRY

MAY, 2013

DECLARATION

I hereby declare that this work is a product of my own efforts; under the supervision of Prof. M. S. Sule and has been presented and will not be presented elsewhere for the award of a Degree or Certificate. All sources have been dully acknowledged.

ZINAT SULEIMAN MOHAMMED **DATE**

CERTIFICATION

This is to certify that the research work for this dissertation and the subsequent preparation of this thesis by Zinat Suleiman Mohammed SPS/10/MBC/00014, were carried out under my supervision in the Department of Biochemistry, Bayero University, Kano, Nigeria.

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DEDICATION

This research project is dedicated to my beloved husband Alhaji Muhammad Sani.

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ABSTRACT

The aim for this study is to access the scientific basis of the utility of the aqueous fruit pulp extract of *Tamarindus indica*(AFPETI) for management of hyperglycaemia in alloxan induced diabetic and hypercholesterolemic group of rats. A total of sixty four rats were used in this study of which forty eight of the rats were used for testing the anti-diabetic effect of AFPETI and were grouped into six of eight rats each. Group I was used as normal control, groups II-VI was induced with diabetes using alloxan monohydrate (150mg/kg body weight). Group III, IV and V were daily administered with 200, 400 and 600mg/kg body weight respectively of AFPETI while group VI was daily administered with chlorpropamide (84mg/kg) for 2 weeks. Sixteen rats placed into four groups of four rats each for testing the anti-hypercholesterolemic effect of AFPETI. G II –IV were concomitantly fed with cholesterol rich diet and AFPETI for five weeks to induce hypercholesterolemia. *Tamarindus indica* fruit pulp extract was orally administered daily at 200, 400mg/kg body weight for two weeks to GIII - IV. The effect of the extract on Fasting Blood Glucose (FBG) level and total protein (TP) was determined weekly. All rats in the diabetic groups had FBG levels within the diabetic range ($p<5.5$mmol/L) at the initial stage of the experiment and the fasting blood glucose level significantly ($p<0.05$) dropped in dose-dependent manner after two weeks of treatment, with 600mg/kg dosage being most significant ($p<0.05$). The serum total protein increased, after treatment with AFPETI, which was significant ($p<0.05$) when compared with the diabetic control group II after two weeks. However, the glucose lowering effect of AFPETI is less than the standard drug (chlorpropamide). The decrease in triglycerides, LDL-cholesterol and total cholesterol in hypercholesterolemic rats was significant ($p<0.05$), with concomitant increase in serum HDL-cholesterol at a dose of 200 and 400mg/kg body weight after five weeks. The significant ($p<0.05$) increase in the levels of LDH and CK recorded in the hypercholesterolemic control group were prevented in the extract treated groups, the decrease was statistically significant ($p<0.05$). The results suggest that the AFPETI may reduce the metabolic changes in alloxan-induced diabetic rats and prevent hypercholesterolemia.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

In the ancient Sanskrit literature, diabetes mellitus was described as "honey-urine disease," associated with gross emaciation and wasting. The Greek physician Aretaeus described diabetes as a condition in which "melting of the flesh into urine" occurred. Sir William Osler, almost 100 years ago, described the disease in terms of "progressive emaciation," involving massive urinary losses of both glucose and urea. The rapid increase in the number of people with Diabetes Mellitus (DM) worldwide is quite alarming (Modak *et al*., 2007). At least, 177 million people worldwide live with diabetes and this figure is likely to increase by 2030 (WHO, 2000). The International Diabetes Federation (IDF) estimated that 10.8 million people have diabetes in sub-Saharan Africa in 2006 and that this would rise to 18.7 million by 2025 (Haque *et al*., 2011). In Nigeria, about 3% of adult were reported to have Diabetes Mellitus (Bakari *et al*., 1999).

There are two forms of diabetes. The Type 1 diabetes includes the cases which can be attributed to an autoimmune process and/or those with β-cell destruction for which has unknown pathogenesis. Type 1 diabetes is the consequence of an autoimmune- mediated destruction of pancreatic β-cells, leading to insulin deficiency. Patients require insulin treatment for survival. Type 2 diabetes is characterized by insulin resistance and relative, rather than absolute, insulin deficiency. Type 2 diabetes usually occurs in obese individuals and is associated with hypertension and dyslipidaemia. The Type 2 includes the common major form of diabetes which results from defects in insulin secretion or rather insulin resistance (WHO,

1999). The only therapy of Type 1 diabetes is the substitution of insulin. Many and diverse therapeutic strategies for the treatment of Type 2 diabetes are known. Conventional treatments include the reduction of the demand for insulin, stimulation of endogenous insulin secretion, enhancement of the action of insulin at the target tissues and the inhibition of degradation of oligo- and disaccharides (Groop *et al.,* 1997, Perfetti *et al.,* 1998).

It has been reported that diabetic patients have significant defects of antioxidant protections and generation of reactive oxygen species (oxidative stress) which may play an important role in the etiology of diabetic complications (Opara, 2002). In diabetes mellitus (DM), the disorders of carbohydrates, lipids and proteins metabolism play predominant role in diabetic complications. Hypercholesterolemia (CHOL) and hypertriglyceridemia (TG) are mostly observed and related largely to the degree of diabetic control (Paterson *et al.,* 1991). Serum high density lipoprotein-cholesterol (HDL-C) was reported to be low in diabetic patients of both types of DM (Lopes-Virella and Virella,1992). Hyperglycemia may alter lipoproteins to a form that promotes atherogenesis. Low-density lipoprotein-cholesterol (LDL-C) levels are frequently altered in diabetic patients. Lipid peroxidation products, which increase in clinical and experimental diabetes, are important results of oxygen-derived free radicals stress. These products may be important in the pathogenesis of vascular complications in DM (Velazquez *et al.,* 1991).

Hyperglycaemia is known to play a pathogenic role due to the excess formation of glucose-derived endproducts (Mani *et al.,* 1987). As a devastating illness with significant morbidity and mortality, diabetes mellitus has increased steadily worldwide (Luo and Luo, 2006). It is estimated that 25% of the world population is affected by this disease (Maiti *et al*., 2004). Diabetes mellitus, an endocrine and metabolic disorders characterized by chronic

hyperglycemia produces multiple biochemical impairments and oxidative stress especially an increased susceptibility to lipid peroxidation that play role in the progression of the symptoms of diabetes (Giugliano *et al.,* 1996). Several hypotheses have beenpostulated to explain the development of free radicals in diabetes which include auto oxidation of glucose, enzymatic and non-enzymatic glycation of proteins with increased formation of glucose derived advanced glycosylation end products (AGEs), enhanced glucose flux through polyol pathway (Oberley, 1988) and reduction of anti-oxidant defence (Kuyvenhoven *et al.,* 1999). Despite progress in the management of diabetes mellitus by synthetic drugs most of these drugs have side effects in the long run especially drug resistance is also noted (Rao and Apparao, 2001. So, the search for improved and safe natural antidiabetic agents is ongoing and World Health Organization has also recommended the development of herbal medicine in this concern (Upathaya and Pandy, 1984; WHO, 2002).

In modern medicine no satisfactory effective therapy is still available to cure diabetes mellitus (Mallick *et al*., 2006). Though insulin therapy is also used for the management of diabetes mellitus but there are several drawbacks like insulin resistance (Piedrola *et al*., 2001), anorexia nervosa, brain atrophy and fatty liver (Yaryura- Tobias *et al*., 2001) after chronic treatment. Recently, the search for appropriate hypoglycemic agents has been focused on plants used in traditional medicine partly because of leads provided by traditional medicine to natural products that may be better treatments than currently used drugs (Ugochukwu and Babady, 2003), because they are more harmonious with biological systems (Erasto *et al*., 2005).

Medicinal plants are the back bone of traditional medicine (Farnsworth, 1994). Medicinal plants have been used in various systems, as they have potential against numerous diseases. Every part of the plant from root to leaf tips is useful for human needs. Contrary to pharmaceuticals, it is often freely and readily available (Kumar *et al.,* 1991). Costs are significantly low; herbs have usually little or no toxicity during long-term oral administration and are relatively available at large scale (Ramos *et al*., 2003).

Tamarindus indica Linn is tree-type of plant belonging to the Caesalpiniaceae family (Maiti *et al*., 2005) grows naturally in tropical and subtropical regions and now is one of the most important plant resources as food materials and is accepted as herbal medicine in parts of the world (Siddhuraju, 2007). *Tamarindus indica* is rich in nutrients and plays an important role in human nutrition, mainly in the developing countries. *Tamarindus indica* Linn was used as a traditional medicine for the management of diabetes mellitus in human and experimental animals (Maiti *et al*., 2004;Maiti *et al*., 2005; Martinello *et al*., 2006; Chatterjee *et al*., 2009;).

The most outstanding characteristics of tamarind is its sweet acidic taste, the acid is due to mostly tartaric acid (10%). The sweet taste of the fruit is due to the increase in the amount of reducing sugars (30-40%). As a result, tamarind is known to be simultaneously the most acidic and sweetest fruit (El-Siddig *et al*., 2006). The sticky pulp of the brown pods is edible and has a sweet and sour flavor. It is used in dishes for a tangy component and in desserts, beverages, syrups, sauces and candy. Tamarind has been a long time folk remedy with a long list of uses, including treatment of sore throats and sunstroke. It may also have antibiotic properties. Animal studies have shown that tamarind can lower cholesterol and blood sugar levels (Martinello *et al*., 2006).

Tamarindus indica is a monotypic genus and belongs to the subfamily Caessalpinioideae of the family Leguminosae (Fabaceae). *Tamarindus indica L.,* commonly known as tamarind tree, is one of the most important multipurpose tropical fruit tree species in Nigeria. In northern Nigeria, it is known as *tsamiya* in Hausa language. Tamarind pods come from the tamarind tree, which originally came from Africa, but can now be commonly found and used in the cuisine of Asia, Arabia, Australia, Mexico and South America. Tamarind fruit is highly valued for its pulp. Tamarind fruit pulp has a sweet acidic taste due to a combination of high content of tartaric acid and reducing sugars. The pulp is used for seasoning, in prepared foods, to flavour confections, curries and sauces, and as a major ingredient in juices. Vitamin B content is quite high; carotene and Vitamin C contents are low. It is used traditionally in abdominal pain, diarrhea and dysentery, helminthes infections, wound healing, malaria and fever, constipation, inflammation, cell cytotoxicity, gonorrhea, and eye diseases. It has numerous chemical values and is rich in phytochemicals, and hence the plant is reported to possess anti-diabetic activity (Maiti *et al.,*2004;), antimicrobial activity, antioxidant activity, anti-malarial activity, hepato-protective activity, anti-asthmatic activity, laxative activity, and anti-hyperlipidemic activity (Rimbau *et al.,* 1999; Ramos *et al.,* 2003).The anti-oxidant properties, could be beneficial during diabetes. The seed coat extract has also been shown to contain polyphenolic flavonoid with strong anti-oxidant properties (Komutarin, 2004).

Hyperlipidaemia is a major cause of the chain of events leading to atherosclerotic plaques. A prime cause of hyperlipidaemia is the over consumption of fat and particularly saturated fatty acids in the diet. If these postulates are accepted, it follows that: dietary modification should lead to reduced blood lipid concentrations: and lowering blood lipid concentrations should

reduce morbidity and mortality from Ischaemic Heart Disease. Studies have suggested that total dietary fat intake is linked to an increased risk of obesity (Astrup *et al.,* 2005) and diabetes.
Total lipid profile of an individual is a principle resulting from blood cholesterol along with its associated varieties of lipoproteins i.e., high-density lipoproteins (HDL), low-density lipoproteins (LDL), very-low density lipoproteins (VLDL) and triglycerides. Disposition of blood pressure and coronary heart disease has been found to be in strong correlation with lipid profile particularly with blood cholesterol level (Ajayi *et al.,* 2006).

In hypercholesterolemic hamsters, the effect of the crude extract from the pulp was investigated on serum lipid levels and atherosclerotic lesions. Lowering the serum lipid level through dietary or drug therapy seems to be associated with a decrease in the risk of vascular disease (Scott and Grundy, 1999). Tamarind extract has a high potential in diminishing the risk of atherosclerosis in humans. Another experimental study on hamster has shown that the hydroalcoholic extract of Tamarind pulp influenced the mediator system of inflammation (Ushanandini *et al.,* 2006). Tamarind fruits have shown some effect on cardiovascular system and blood. Fruits of *Tamarindus indica* were evaluated for their effects on lipid profile, systolic and diastolic blood pressure, and the body weight of humans (Ifetekhar *et al.,* 2006). Linoleic acid present in tamarind is undoubtedly one of the most important polyunsaturated acids in human food because of its association in the reduction or prevention of heart vascular diseases. Dietary fat rich in linoleic acids is, apart from preventing cardiovascular disorders such as coronary heart diseases and atherosclerosis, also associated with preventing high blood pressure (Ajayi *et al.,* 2006). Humans and other mammals have a dietary requirement for certain essential fatty acids, such as linoleic acid (an omega-6 fatty acid) and alpha-linolenic acid (an

omega-3 fatty acid) because they cannot be synthesized from simple precursors in the diet (Stryer *et al.*, 2007). Both of these fatty acids are 18-carbon polyunsaturated fatty acids differing in the number and position of the double bonds.The American Diabetes Association recommends a post-meal glucose level of less than 10 mmol/L (180 mg/dL) and a fasting plasma glucose of 5 to 7.2 mmol/L (90–130 mg/dL) (ADA, 2007).

Many of the chronic complications of diabetes involve changes in structural proteins. It is thus possible that changes in protein metabolism are responsible for many of the chronic complications of diabetes mellitus, because even a minor imbalance between protein synthesis and degradation can potentially have a profound effect over the long term on cell viability and metabolism. Alterations in protein synthesis and degradation can also adversely affect the repair of tissue after injury or infection.The capacity of nutrients to stimulate insulin release from thepancreatic β-cell reflects their capacity to augment oxidative fluxes in the islet cells (Malaisse, 1982). Also, oxidant stress associated with insulin resistance and non-insulin-dependent diabetes mellitus (Gopaul *et al.,* 1995) contributes to poor insulin action (Paolisso *et al.,* 1994). Thus, the treatment aims to reduce insulin resistance (diet, exercise and drug therapy) and to stimulate insulin secretion. In DM, oxidative stress seems mainly to be due to an increased production of free radicals and/or a sharp reduction of antioxidant defenses (Cross *et al.,* 1987; Oberley, 1988). Oxygen-derived free radicals have been implicated in the pathophysiology of various disease states, including diabetes mellitus (Giuliano *et al.,* 1996). It is well known that superoxide anion is the primary radical formed by the reduction of molecular oxygen that may lead to secondary radicals or reactive oxygen species (ROS)such as hydrogen peroxide and hydroxyl radical (Katusic, 1996). Also, Jang *et al* (2000) found that

increased oxidative stresshas been suggested to be involved in the pathogenesis and progression of diabetic tissue damage. On the otherhand, there is evidence that diabetes induces changes in the activities of antioxidant enzymes in various tissues.Diabetes mellitus is characterized by increased generation of glyco-oxidation products associated with the advance doxidative stress (Mullarkey *et al.,* 1990). The presence of higher glucose or glycated protein concentration enhances lipid peroxidation (Kawamura *et al.,* 1994) and reversely, lipid peroxides may increase the extent of advanced glycation end-products (Hicks *et al.,* 1989). The causes of enhanced free radical production are hyperglycemia (Hammes *et al.,*1997) and hyperinsulinemia (Paolisso and Giugliano, 1996). Hyperglycemia is a widely known cause of enhanced plasma free radical concentrations (Hammes *et al.,* 1997). Free radical production caused by hyperglycemia may occur via at least four different routes: i) increased glycolysis (Vaag *et al.,* 1992); ii) intercellular activation of sorbitol (polyol) pathway (Williamson *et al.,* 1993); iii) autooxidation of glucose (Wolff *et al.,* 1991) and iv) non-enzymatic protein glycation (Ceriello *et al.,* 1992).

Some plant extracts have effect on diagnostic markers of myocardial infarction such as creatine phosphokinase (CK), and lactate dehydrogenase (LDH) (Chatterjea and Shinde2002). These enzymes are tightly bound to the contractile apparatus of the cardiac muscle tissue and any serious insult to the heart muscle will evoke the release of these enzymes into the serum. The integrity of the cardiac apparatus in drug biotransformation and metabolism could be assessed by evaluating the levels of CK, and LDH in serum.

1.2 JUSTIFICATION

Diabetes is on the rise worldwide, with about 25 percent of the world's population suffering from the disease. This disease is characterized by high blood sugar levels, which can lead to complications ranging from nerve damage, stroke and coronary artery disease. More study on effective dosages and considerations like possible side effects is needed before tamarind, a potent amylase inhibitor, can be recommended for use in managing or preventing diabetes. A herbal antidiabetic drug with antioxidant potential will certainly be beneficial and valuable for treatment of diabetes. Thus, this study was undertaken to evaluate the antihyperglycemic and antihyperlipidemic effect of the aqueous extract of *Tamarindus indica* fruit pulp in alloxan induced diabetic rats as a way of validating its traditional usage. Modern synthetic antihyperglycemic agents were reported to produce undesirable and unwanted side effects such as**;** weight gain**,** lactic acidosis and hypoglycaemia and are not easily or cheaply available**.** Thus, more alternative therapy and approach with less or no side effects are needed to manage Diabetes Mellitus and its complications.

Hypercholesterolemia has long been recognized as a major risk factor for coronary artery disease and myocardial infarction, Coronary heart disease (CHD) is the cause of mortality in 50% of people around the world. Atherosclerosis and related heart disease is strongly associated with elevated blood levels of total (and LDL) cholesterol. Due to the widespread incidence as well as severity of this pathological condition, this study was undertaken to validate the use of *Tamarindus indica* as a hypocholesteroleamic agent for maintaining low blood lipid profile and blood cholesterol.

1.3 AIM AND OBJECTIVES

The aim of this research is to determine the effect of *Tamarindus indica* pulp on Serum Lipid Profile, Serum Glucose Levels, Cardiac Enzymes (LDH and Creatine Kinase) and Serum Total protein.

The objectives of carrying out this research are to:

- Determine a relation between *Tamarindus Indica* pulp and serum lipids profile in rats.
- Demonstrate the effect of oral administration of *Tamarindus indica* pulp on serum glucose and total protein levels in alloxan induced diabetic rats.
- Study the effect of oral administration of *Tamarindus indica* pulp on cardiac enzymes (LDH and Creatine Kinase) in hyperlipidemic rats.
- Demonstrate that effect of oral administration of *Tamarindus indica* pulp is dose dependent.

CHAPTER TWO

LITERATURE REVIEW

2.1 MEDICINAL PLANTS

Plants continue to be a major source of medicines, as they have been throughout human history. In addition to providing the basis for between 30 and 40 percent of today's conventional drugs, the medicinal and curative properties of various plants are also employed in herbal supplements, botanicals, nutraceuticals and teas. Studies have shown that phytochemicals isolated from plant sources have been used for the prevention and treatment of cancer, heart disease, diabetes mellitus, and high blood pressure (Waltner-Law *et al.,* 2002*)*. Herbal medicine, or Botanical Medicine, is the use of herbs for their therapeutic or medicinal value. Herb is a plant or plant part valued for its medicinal, aromatic or savory qualities. Plants are well known in traditional herbal medicine for their hypoglycaemic activities, and available literature indicate that there are more than 800 plant species showing hypoglycaemic activity (Rajagopal and Sasikala, 2008).

Many familiar medications of the twentieth century were developed from ancient healing traditions that treated health problems with specific plants. WHO notes that of 119 plant-derived pharmaceutical medicines, about 74% are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by native cultures. The World Health Organization has recommended the evaluation of the effectiveness of plants in conditions, where safe orthodox drugs are scarce (WHO, 1980).

In addition to active ingredients, plants contain minerals, vitamins, volatile oils, glycosides, alkaloids, bioflavanoids, and other substances that are important in supporting a particular herb's medicinal properties. These elements also provide an important natural safeguard. Isolated or synthesized active compounds can become toxic in relatively small doses; it usually takes a much greater amount of a whole herb, with all of its components, to reach a toxic level. Herbs are medicines, however, and they can have powerful effects. Over the past few years, the use of medicinal plants has been rapidly increasing and therapeutic benefits of these plants are often attributed to their antioxidant properties (Arora *et al.,*2005). Plants have played a major role in the introduction of new therapeutic agents. Every ancient ethnic culture has its own treasure house of herbal medicines. At least, drugs from higher plants are being used throughout the world. Even now, almost 75-80% of world population depends on crude plant drug preparations to tackle their health problems (Monique and Simmonds, 1995).

Many plants derived medicines were reported to demonstrate a bright future in therapy and management of DM due to their less toxic effects and are cheaply available (Azadbakht *et al*., 2010). Natural product medicines from plant source of wide diversity have long been used effectively in the treatment of blood pressure and higher lipid level. Hypolipidemic activity was observed from tamarind fruit extract in hypercholesterolemic hamsters (Martinello *et al.,* 2006). Treatment of hypercholesterolemic hamsters with tamarind fruit pulp extract (5%) led to a decrease in the levels of serum total cholesterol (50%), non-high-density lipoprotein cholesterol (73%) and triglyceride (60%). The study of Martinello *et al* (2006) indicates the potential of tamarind extracts in diminishing the risk of atherosclerosis development in humans, which is the main contributor for the pathogenesis of myocardial and cerebral infarction.

Some studies have predicted that *Tamarindus indica* shows antiasthmatic and hepatoprotective effect. The methanolic extract of leaves of *Tamarindus indica* Linn, exhibited significant antihistaminic, adaptogenic, and mast cell stabilizing activity in laboratory animals (Tayade *et al.,* 2009). Protective effect of *Tamarindus indica* Linn. (Caesalpiniaceae) was evaluated by injecting the rats with paracetamol aqueous extracts of different parts of *Tamarindus indica*, such as fruits, leaves (350mg/kg), and unroasted seeds (700mg/kg) and significant hepatoregenerative effect was observed for the aqueous extracts of tamarind leaves, fruits, and unroasted seeds as judged from the parameters studied (Pimple *et al.,* 2007). *Tamarindus indica* is also used for treating diarrhea and dysentery. When diarrhea is not treated properly, the patient has risks of dehydration and death. The tamarind pulp with lemon is used to treat diarrhea (Bhat *et al.,* 1990) and the root is used to treat dysentery.

2.2 *TAMARINDUS INDICA*

Tamarind fruit was at first thought to be produced by an Indian palm, as the name tamarind comes from a Persian word "tamar-I-hind," meaning date of India. Its name "Amlika" in Sanskrit indicates its ancient presence in India. *Tamarindus indica* is used as traditional medicine in India, Pakistan, Bangladesh, Nigeria and other tropical countries (Havinga, *et al.,* 2010). The medicinal properties of the tree and particularly its fruits have been widely noted in the traditionalmedicinal systems of many countries. In the Indian sub-continent, traditional practitioners prescribe the fruits for constipation, indigestion and flatulency, while the seeds are prescribed for diabetes. In the Bangladesh folk medicinal system, both fruits and seeds are prescribed for diabetes. The fruits are used as laxative or febrifuge throughout the Sahel and Sudan. The bark and leaves are used in the treatment of wounds in central West Africa. In West Africa, the bark is used to treat diarrhea, while the leaves are used for this purpose in East

Africa. In Trinidad and Tobago, the plant is used to treat hypertension (Lans, 2006). In the traditional medicinal system of Burkina Faso, Africa the plant is used to treat kidney diseases (Lengani *et al.,* 2010).

Tamarind has been a long time folk remedy with a long list of uses as a medicinal plant. Tamarind is valued mostly for its fruit, especially the pulp, which is used for a wide variety of domestic and industrial purposes (Kulkarni *et al.,* 1993). In traditional practice, the pulp is applied on inflammations, is used in a gargle for sore throat and, mixed with salt, as a cream for rheumatism. Tamarind use as laxative refers to the use of its fruit, and also the use of a macerate of its leaves with potash has been reported in northern Nigeria. Soaked fruits are also eaten by rural Fulani in Nigeria, to relieve constipation (Lockett and Grivetti, 2000).

The pulp is said to aid the restoration of sensation in cases of paralysis. In Colombia, an ointment made of tamarind pulp, butter, and other ingredients is used to rid domestic animals of vermins (Morton, 1987). Tamarind pulp has been reported to be used in the treatment of a number of ailments, including the alleviation of sunstroke, datura poisoning (Gunasena and Hughes, 2000), and the intoxicating effects of alcohol and cannabis. It can be gargled for sore throats, dressing of wounds (Chaturvedi, 1985) and is said to aid the restoration of sensation in cases of paralysis. The fiber in tamarind fruit pulp binds to toxins in food, thereby help protect the colon mucus membrane from cancer causing chemicals. Tamarind pulp is also said to aid in the cure of malarial fever. It has been suggested that polymeric tannins with their high molecular weight and the proximity of many aromatic rings and hydroxyl groups are also very important for the free radical scavenging (Siddhuraju, 2007). The whole plant of tamarind is

used extensively for medicinal and industrial purpose; hence it is very beneficial to human being.

2.2.1 Morphology

A typical tamarind fruit contains 40% -55% pulp (El-Siddig *et al.,* 1999), 34% seeds, and 11% shell (pod) and fibers (Kumar and Bhattacharya, 2008).The tamarind is a long-lived, medium-growth, bushytree, which attains a maximum crown height of 12.1 to 18.3 metres (40 to 60 feet). The crown has an irregular, vase-shaped outline of dense foliage. Leaves are evergreen, bright green in color, elliptical ovular, arrangement is alternate, of the pinnately compound type, with pinnate venation and less than 5 cm (2 inches) in length. The branches drop from a single, central trunk as the tree matures and is often pruned in human agriculture to optimize tree density and ease of fruit harvest. At night, the leaflets close up. The tamarind does flower, though inconspicuously, with red and yellow elongated flowers. Flowers are 2.5 cm wide (one inch), five-petalled, borne in small racemes, and yellow with orange or red streaks. Buds are pink as the four sepals are pink and are lost when the flower blooms.The fruit is an indehiscentlegume, sometimes called a pod, 12 to 15 cm (3 to 6 inches) in length, with a hard, brown shell (Gunasena andHughes,2000).

The fruit has a fleshy, juicy, acidulous pulp. It is mature when the flesh is coloured brown or reddish-brown. The tamarinds of Asia have longer pods containing six to 12 seeds, whereas African and West Indian varieties have short pods containing one to six seeds. The seeds are somewhat flattened and glossy brown. As a tropical species, it is frost sensitive. The pinnate leaves with opposite leaflets give a billowing effect in the wind. Tamarind timber consists of

hard, dark red heartwood and softer, yellowish sapwood.The seeds are dicotyledonous and brownish black in color, though the kernels are white (El-Siddig *et al.,* 2006).

2.2.2 CLIMATE AND DISTRIBUTION

Tamarind is a perennial herb belonging to the dicotyledonous family of Leguminosae. It is slow growing but long lived, with an average life span of 80 – 200years. Today tamarind grows widely in most tropical and subtropical regions of the world. Tamarind is well adapted to semiarid tropical conditions and also grows well in many humid tropical areas with seasonally high rainfall. Tamarind is grown commercially in plantations and homestead gardens for its product, and along avenues as an ornamental plant. Of all the fruits trees of the tropics, none is more widely distributed nor more appreciated as an ornamental than the tamarind. The tree grows well in full sun in clay, loam, sandy, and acidic soil types, with a high drought and aerosol salt (wind-borne salt as found in coastal area) resistance (Rao*et al.,* 1999). The tamarind has also long been naturalized in Indonesia, Malaysia, the Philippines, and the Pacific Islands. Thailand has the largest plantations of the Asian nations, followed by Indonesia, Myanmar, and the Philippines. In India, extensive tamarind orchards produce 275,500 tons (250,000 MT) annually. The pulp is marketed in northern Malaya. It is cultivated all over India, especially in Andhra Pradesh and Tamil Nadu. Although native to Sudan and tropical Africa, Asia and Mexico are the largest consumers and producers of tamarind (Maiti *et al.,* 2004).

2.2.3 Propagation

The tree tolerates a great diversity of soil types. It withstands salt spray and can be planted fairly close to the seashore. Tamarind seeds remain viable for months; will germinate in a week

after planting. Seeds can be scarified or briefly boiled to enhance germination. They retain germination capability after several months if kept dry.In the past, propagation has been customarily by seed sown in position, with thorny branches protecting the young seedlings. However young trees are usually grown in nurseries. The tree can be grown easily from cuttings, or by shield-budding, side-veneer grafting, or air layering. Nursery-grown trees are usually transplanted during the rainy season. With sufficient water and regular weeding, the seedlings will reach 2ft (60cm) the first year and 4 ft (120cm) by the second year (Rao *et al.,* 1999).

Tamarind may be left on the tree for as long as 6 months after maturity so that moisture content will be reduced to20% or lower. Fruits for immediate processing are often harvested by pulling the pod away from the stalk which is left with the long, longitudinal fiber attached.Tamarind is harvested by pulling the pod from its stalk. A mature tree may be capable of producing up to 175 kg (350 lb) of fruit per year. Veneer grafting, shield (T or inverted T) budding, and air layering may be used to propagate desirable selections. Such trees will usually fruit within three to four years if provided optimum growing conditions.In Thailand, two species of tamarind occur, the so called sweet fruits and sour tamarind. A mature tree may annually produce 150 to 225 kg fruits, of which the pulp may constitute 30-55%, the shells and fiber 11-30% and the seeds 33-40%. To preserve tamarind for future use, they may be merely shelled, layered with sugar in boxes or pressed into tight balls and covered with cloth and kept in a cool dry place (Gunasena and Hughes, 2000).

2.2.4 **Chemical Composition**

The sticky pulp of tamarind is rich source of non-starch polysaccharides (NSP) or dietary fiber such as gums, hemicelluloses, mucilage, pectin and tannins. The fruit pulp 100g provides 5.1 or over 13% of dietary fiber (El-Siddig *et al.,* 1999). Dietary fiber in the food increases its bulk and augments bowel movements thereby help prevent constipation. The fiber also binds to toxins in the food thereby help protect the colon mucus membrane from cancer causing chemicals. Dietary fibers in the tamarind pulp bind to bile salts (produced from cholesterol) and decrease their re-absorption in the colon; thereby help excretion of "bad" or LDL cholesterol levels from the body. Essential amino acids of tamarind fruit pulp exceed those of the 'ideal' protein standard established by the world health organization, except for tryptophan (82%). Although tamarind fruit contains potentially useful amounts of protein, they have been shown to be poorly digested and utilized by rats, as compared with proteins in coconut met that are extensively digested and effectively utilized by mammals (Glew *et al.,*2005*)*.

The pulp constitutes 30-50% of the ripe fruits. Tamarind pulp typically contains 20.6% water, 3.1% protein, 0.4% fat, 70.8% carbohydrates, 3.0% fibre and 2.1% ash, thus the pulp has low water content and a high level of protein, carbohydrates and minerals (El-siddig *et al.,* 2006). The tamarind is best described as sweet and sour in taste, and is high in acid, sugar, B vitamins (thiamine, riboflavin and niacin) and, oddly for a fruit, calcium. *Tamarindus indica* fruit pulp is also a rich source of several macro and micro elements, including relatively high amounts of copper, manganese and zinc. The fruit is also a good source of calcium and phosphorous (El-Siddig *et al.,* 1999), but is unfortunately, extraordinarily low in iron (Glew *et al.,* 2005). Almeida *et al.,* (2009) indicated that tamarind is a rich source of all minerals available, especially magnesium, copper and potassium, in addition to being a good source of calcium,

phosphorous, iron and selenium. *Tamarindus indica* can also provide smaller amounts of iron and vitamin A. The consumption of 100g of tamarind fruit pulp by an adult will cover 10.6% of the recommended daily intake of calcium, 20.4% of magnesium, 14.21% of phosphorous, 12.0% of iron, 2.61% of manganese, 1.29% of zinc, 32.22% of copper and 9.21% of selenium. (Almeida *et al.,* 2009).

Figure 1: The Amino Acid Composition of *Tamarindus indica* Fruit Pulp.

Amino Acid	**Composition (mg/g dw)**
Crude protein (total protein)	116.00
Aspartic acid (ASP)	12.00
Glutamic acid (GLU)	16.70
Serine (SER)	6.88
Glycine (GLY)	5.15
Histidine (HIS)	3.37
Arginine (ARG)	8.74
Threonine (THR)	6.05
Alanine (ALA)	6.20
Proline (PRO)	7.61
Tyrosine (TYR)	4.34
Valine (VAL)	6.97
Methionine (MET)	2.48
Isoleucine (ILE)	5.20
Leucine (LEU)	8.89
Phenynanaline (PHE)	4.78
Lysine (LYS)	8.22
Cysteine (CYS)	1.35
Tryptophan (TRP)	1.04

Source: Glew *et al* (2005).

The ascorbic acid content in tamarind is very low and varies from 2-20mg/100g (Ishiola, 1990). *Tamarindus indica* contains high levels of crude protein with many essential amino acids, which help to build strong and efficient muscles (Garba *et al.,* 2003). Phytochemical investigation carried out on *Tamarindus indica* revealed the presence of many active constituents, such as phenolic compounds, cardiac glycosides, L-mallic acid, tartaric acid, the mucilage and pectin, arabinose, xylose, galactose, glucose, and uronic acid. The ethanolic extract of *Tamarindus indica* showed presence of fatty acids and various elements like arsenic, calcium, cadmium, copper, iron, sodium, magnesium, manganese, potassium, phosphorus, lead, and zinc (Samina *et al*., 2008).

The volatile constituents of the fruit pulp are furan derivatives (44.4%) and carboxylic acid (33.3%) of the total volatiles. The pulp contains organic acids, such as tartaric acid, acetic acid, citric acid, malic acids, and succinic acid; amino acids, invert sugar (25-30%); pectin protein; fat; some pyrazines (trans-2-heenal);and some thiazoles (2-ethylthiazole, 2-methylthiazole) as fragrant; and the seed polysaccharides are found with a main chain consisting of β-1,4- linked glucose molecules together with xylose (alpha-1,6) and galactose; total protein; lipids with fatty oils; and some keto acids (Imam *et al.,* 2007).

Tamarind fruit pulp is relatively poor in oil (25.3 g/kg of crude lipid), greenish yellow in colour and liquid at room temperature. Saponification values of the oil are high, indicating that it contains a high proportion of low molecular weight fatty acids (Ishiola *et al*., 1990). With regard to the two essential fatty acids, the fruit pulp contains very little linoleic acid (3.42mg/g dry weight) and even lower amounts of α-linolenic acid (0.21mg/g dry weight). Linoleic and linolenic acids are unsaturated essential fatty acids that humans require and cannot synthesize.

Replacing saturated fatty acids by either oleic or linoleic acid lowers serum cholesterol levels (Keys *et al.,* 1965).

2.2.5 Uses of Tamarindus Indica

- **Culinary Uses**

Tamarind is used for a wide variety of domestic and industrial purposes.The acidic pulp is used as a favorite ingredient in culinary preparations, such as curries, chutneys, sauces, ice cream, and sherbet in countries where the tree grows naturally (Little and Wadsworth, 1964). In India, the pulp is also eaten raw and sweetened with sugar. Tamarind pulp is also used to make sweet meats mixed with sugar called Tamarind balls. (Lotschert and Besse,1994). Tamarind pulp is used as a raw material for the manufacture of several industrial products, such as Tamarind juice concentrate, Tamarind pulp Powder, tartaric acid, pectin, tartarates, and alcohol. The ripened fruit is considered the more palatable, as it becomes sweeter and less sour (acidic) as it matures. It is used in desserts as a jam, blended into juices or sweetened drinks, sorbets, ice creams and all manner of snacks (Kulkarni *et al.,* 1993).

In India, the tamarind is used in preparing soup. In northern Nigeria, it is used with millet powder to prepare *kunun tsamiya*, a traditional pap mostly used as breakfast, and usually eaten with bean cake (Lockett and Grivetti, 2000). A tamarind-based sweet-and-sour sauce is served over deep-fried fish in central Thailand.

MEDICINAL AND PHARMACOLOGIC PROPERTIES

- **Antimicrobial activity**

Tamarindus indica has a broad spectrum of antibacterial activity. The menthanolic leaf extract of *Tamarindus indica* was assessed for antibacterial activity against *Burkholderia pseudomallei.* Methanol and acetone extracts *of Tamarindus indica* have showed significant antimicrobial acitivity against*Klebsiella pneumonia.* The activity was compared with standard antimicrobials *Amikacin* and *Pipercillin* (Vaghasiya and Chanda, 2009). The antimicrobial acitivity of the concentrated extracts (aqueous, ethonolic, acetone extract) were evaluated by determination of the diameter of zone of inhibition against both gram-positive and gram-negative bacteria and fungi using the paper disk diffusion method. These have potent antimicrobial activity against *Salmonella paratyphi, Bacillus subtilus, Salmonella typhi, and Staphylococcus aureus* (Doughari, 2006). Studies have suggested that *T. indica* has shown potential antimicrobial activity: and that petroleum ether, aqueous, ethanol extract of *T. indica* ripe fruit were evaluated for possible antibacterial activity against gram positive and gram negative species (Warda *et al*., 2007).Extracts from the fruit appear promising as a potential fungicidal agent against cultures of Aspergilus niger and Candida albicans (El-Siddig *et al.,* 2006). Extracts from tamarind fruit pulp have shown molluscidal activity against Bulinus trancatus snails. This may probably be due to the presence of saponins in the fruit (El-Siddig *et al.,* 2006).

- **Antioxidantproperties**

This activity of *T. indica* extract may be attributed to its free radical-scavenging ability. *T. indica* seed coat, a by product of the tamarind gum industry, could be used as a safe and low-

cost source of antioxidant, although other herbals may be more effective. All extracts of *T. indica* exhibited good antioxidant activity (64.5-71.7%) against the linoleic acid emulsion system and the values were lower and higher than the synthetic antioxidant, butylated hydroxyl anisole and ascorbic acid (Siddhuraju, 2007).

- **Laxative properties**

The fruit of *T. indica* is used traditionally as a laxative, due to the presence of high amounts of malic and tartaric acids (Irvine, 1961). Children in Madagascar are given whole Tamarind fruits for break fast to overcome constipation. In Burkina Faso, the fruits are crushed and soaked for a half a day in water with a little salt before consumption. Tamarind use as a laxative refers to the use of its fruit, and also the use of a macerate of its leaves with potash has been reported in northern Nigeria (Bhat *et al*., 1990).

- **Abdominal pain**

Soaked fruits are eaten by rural Fulani in Nigeria, to relieve constipation (Lockett and Grivetti, 2000). Roots prepared as an extract, are used in the treatment of stomach ache or painful abdomen, in east Africa. Also in Benin and Burkina Faso, the roots and fresh bark of young stems are used for abdominal pain and/or purgative (Bhat *et al.,*1990).

- **Wound Healing**

Tamarind bark is mostly sold for wound healing purposes. Occasionally other tamarind plant parts are found in wound healing medicine, such as the fruit, the pod husks, or the gum. A decoction of *T. indica* leaves is one of the most important agents to clean wounds caused by Guinea worm infections (Tignokpa *et al*., 1986).

- **Malaria and fever**

  Fruits of tamarind are known as a febrifuge in Madagascar; in Ghana, malaria is treated with tamarind leaves and the fruit pulp is used as a febrifuge and laxative (Asase *et al*., 2005)

- **Antidiadbetic activity**

  An aqueous extract from *T. indica* seeds had a potent antidiabetogenic activity in streptozotocin-induced diabetic male rats. The aqueous extract of *T. indica* seeds was given to mild diabetic and severe diabetic rats, and hyperglycemia was significantly reduced, measured by fasting blood glucose levels. Similarly, hyperlipedemia was found to be reduced, measured by different contents of cholesterol (Maiti *et al.,* 2005).

- **Effect on Cardiovascular System and Blood**

  In Bangladesh, fruits of *T. Indica* were evaluated for their effects on the lipid profile, systolic blood pressure, and the body weight of humans. In hypercholesterolic hamsters, the effect of the crude extract from the pulp was investigated on lipid serum levels and atherosclerotic lesions. Tamarind extract has a high potential in diminishing the risk of atherosclerosis in humans which is the main contributor for the pathogenesis of myocardial and cerebral infarction (Martinello *et al*., 2006).

- **Antivenom Activities**

  Tamarind seed extract inhibited phospholipase A, protease, hyaluronidase, l-amino acid oxidase, and 5'-nucleotidase enzyme activities of venom in a dose dependent manner. The extract of *T. indica* neutralized the degradation of the β-chain of the human fibrinogen and the indirect hemolysis caused by the venom. The extract prolonged the clotting time moderately,

and myotoxic effects, such as edema and hemorrhage, induced by the venom were neutralized significantly when different doses of the extract were administered, hence *T. indica* extract is an alternative for the serum therapy (Ushanandini *et al.,* 2006).

- **Effect on Cellular System**

The methanolic extract of *Tamarindus indica* fruit L-(-)-Di-n-butyl maleate was isolated and it exhibited a pronounced cytotoxicity against sea urchn embryo cells. In the descending colon of swiss albino mice, the fruit pulp caused a greater rate of cell proliferations than in the ascending part, when they were fed a diet of the pulp, compared with the negative control. A polysaccharide isolated and purified from *Tamarindus indica* showed immunomodulatory properties like phagocytic enhancement and inhibition of leukocyte migration during cell proliferation. Phenolic flavonoids from the seed coat extract showed inhibitory effect on nitric oxide production (Sreelekha *et al.,*1993).

- **Hepatoprotective and Antiasthmatic Activity**

Some experimental studies have predicted that *T. indica* shows antiasthmatic and hepatoprotective effect. The methanolic extract of leaves of *T. indica*, exhibited antihistamic, adaptogenic and mass cell stabilizing activity in laboratory animals. Protective effect of *T. indica* was evaluated by injecting rats with paracetamol. A significant hepatoregenerative effect was observed for the aqueous extracts of tamarind leaves, fruit, and unroasted seeds as judged from the parameters studied (Pimple *et al.,* 2007).

- **Anti-inflammatory and Analgesic Activity**

In northern Nigeria, fresh stem bark and fresh leaves are used as decoction mixed with potash for the treatment of stomach disorders, general body pain, jaundice, yellow fever and as blood tonic and skin cleanser (Doughari, 2006).

T. indica bark is used in the treatment of pain traditionally, possibly as a result of the presence of sterols and triterpenes. Leaf juice with ginger is used in the treatment of bronchitis, and the bark dried and pounded and added to water for the treatment of eye inflammation (Irvine, 1961).

- **Helminthes Infections (Parasitic Worms)**

Tamarind leaves are used in the extraction of Guinea worms, and afterward in the treatment of wounds, left by the parasite. Macerate of the seeds is used as vermifuge and the fruits are used for this purpose. An extract of the leaves and the root is used to treat ankylostomiasis (hook worm) in some parts of Tanzania (Keita *et al.,* 1993).

- **Diarrhea and Dysentery**

Tamarind is also used for treating diarrhea and dysentery. Dysentery is a type of diarrhea containing mucus or blood, usually caused by an infection of the intestine. When diarrhea is not treated properly, the patient has risks of dehydration and death. The tamarind pulp with lemon is used to treat diarrhea and the root is used to treat dysentery (Chharbra *et al*., 1987).

- **Other Medicinal Uses**

The fruit pulp is used as liniment to treat rheumatism (El-Siddig *et al.,* 2006), for purifying drinking water (El-Siddig *et al.,* 2006), enhance bioavailability of ibuprofen in humans (Garba *et al.,* 2003), applied on eye diseases and ulcers (El-Siddig *et al.,* 2006), for digestive, carminative, expectorant, remedy for billousness, bile disorders, febrile conditions, and blood tonic (El-Siddig *et al.,* 2006; Martinello *et al.,* 2006).

C. Carpentry Uses

In temples, especially in Buddhist Asian countries, the fruit pulp is used for polishing, cleaning and brightening of brass shrine furniture and copper vessel, which helps to remove dulling and the greenish patina that forms. The wood is a bold red color. Due to its density and durability, tamarind heartwood can be used in making furniture and wood flooring. The ash is used to remove hair from animal hides (Irvine, 1961)

2.3 Lipids

The term Lipids is sometimes used as a synonym for fats, which are a sub-group of lipids called triglycerides. Lipids also encompases molecules such as fatty acids and their derivatives (including tri-, di-, and monoglycerides and phospholipids), as well as other sterol-containing metabolites such as cholesterol. The lipids are generally classified into the following groups:

a. Simple Lipids:

1. Triglycerides or fats and oils, which are fatty acids esters of glycerol (Coleman and Lee, 2004). Examples are butter and cotton seed oil.

2. Waxes which are fatty acid esters of long chains alcohols.Example include bees wax, spermacet, and carnauba wax.

b. Derived Lipids:

1. Steriod which are lipids derived from partially or completely hydrogenated phenanthrena. Examples are cholesterol and ergosterol (Villinski *et al*., 2008).

c. Complex Lipids:

1. Phosphatides or phospholipids which are lipids containing phosphorous and in many instances, nitrogen (Farooqi *et al*.,2000). Examples are lecithin, and phosphatidylinositol.
2. Glycolipids are lipids which contain carbohydrate residues. Examples include sterol-glycosides, cerebrosides, and plant phyoglycolipid.
3. Sphingolipids are lipids containing the long-chain amino alcohol called sphingosine and its derivatives. Examples include sphingomyelins, ceramidesand cerebrosides.

Lipids are present in all living cells, but the proportion varies from tissue to tissue. The triacylglycerols accumulate in certain areas, such as adipose tissue in animals and in the seeds of plants, where they represent a form of energy storage (Stryer *et al*., 2007). The more complex lipids occur closely linked with proteins in the membranes of cells and subcellular particles, where they serve as signaling molecules or cellular messenger (Eyster, 2007) and transporting molecules (Helenius and Aebi, 2001).

2.3.1 Serum Lipids

Blood lipids (or blood fats) are lipids in the blood, either free or bound to other molecules. They are mostly transported in a protein capsule, and the density of the lipids and type of protein determines the fate of the particle and its influence on metabolism. The concentration of blood lipids depends on intake and excretion from the intestine, and uptake and secretion from cells. Blood lipids are mainly fatty acids and cholesterol. Hyperlipidemia is the presence of elevated or abnormal levels of lipids and/or lipoproteins in the blood, and is a major risk factor for cardiovascular disease (Chait and Brunzell, 1990). Hyperlipidemias may basically be

classified as either familial (also called primary) caused by specific genetic abnormalities, or acquired (also called secondary) when resulting from another underlying disorder that leads to alterations in plasma lipid and lipoprotein metabolism. Also, hyperlipidemia may be idiopathic, that is, without known cause. Hyperlipidemias are also classified according to which types of lipids are elevated, that is hypercholesterolemia, hypertriglyceridemia or both in combined hyperlipidemia. Elevated levels of Lipoprotein(a) may also be classified as a form of hyperlipidemia (Chait and Brunzell, 1990).

2.3.2 Fatty acids

Blood fatty acids are in different forms in different stages in the circulation. They are taken in through the intestine in chylomicrons, but also exist in very low density lipoproteins (VLDL) after processing in the liver. In addition, when released from adipocytes, fatty acids exist in the blood as free fatty acids. Short- and medium chain fatty acids are absorbed directly into the blood via intestine capillaries and travel through the portal vein. Long-chain fatty acids, on the other hand, are too large to be directly released into the tiny intestine capillaries. Instead they are coated with cholesterol and protein (protein coat of lipoproteins) into a compound called chylomicron. After a meal, when the blood concentration of fatty acids rises, there is an increase in uptake of fatty acids in different cells of the body, mainly liver cells, adipocytes and muscle cells. This uptake is stimulated by insulin from the pancreas. As a result, the blood concentration of fatty acid stabilizes again after a meal. After a meal, some of the fatty acids taken up by the liver are converted into very low density lipoproteins (VLDL) and again secreted into the blood (Harvey *et al.*, 2000)

The fate of cholesterol in the blood is highly determined by its constitution of lipoproteins, where some types favour transport towards body tissues and others towards the liver for excretion into the intestines.The 1987 report of National Cholesterol Education Program, Adult Treatment Panels suggest the total blood cholesterol level should be: <200 mg/dl normal blood cholesterol, 200–239 mg/dl borderline-high, >240 mg/dl high cholesterol (NCEP, 1988).

In lipid digestion, cholesterol is packed into chylomicrons in the small intestine, which are delivered to the portal vein and lymph. The chylomicrons are ultimately taken up by liver hepatocytes via interaction between apolipoproteinE and the LDL receptor or lipoprotein receptor-related proteins. The average amount of *blood cholesterol* varies with age, typically rising gradually until one is about 60 years old. There appear to be seasonal variations in cholesterol levels in humans, more, on average, in winter. These seasonal variations seem to be inversely linked to vitamin C intake (MacRury *et al*., 1992).

2.3.3 Lipoproteins

Cholesterol is minimally soluble in water; it cannot dissolve and travel in the water-based bloodstream. Instead, it is transported in the bloodstream by lipoproteins - protein "molecular-suitcases" that are water-soluble and carry cholesterol and triglycerides internally. The apolipoproteins forming the surface of the given lipoprotein particle determine from what cells cholesterol will be removed and to where it will be supplied.

Classes of Lipoproteins

Lipoproteins may be classified as follows, listed from larger and less dense to smaller and denser. Lipoproteins are larger and less dense when the fat to protein ratio is increased. They are classified on the basis of electrophoresis and ultracentrifugation.

- **Chylomicrons**

  Chylomicrons transport dietary lipids from the intestines to other locations in the body. Chylomicrons are one of the five major groups of lipoproteins that enable fats and cholesterol to move within the water-based solution of the bloodstream. Chylomicrons transport exogenous lipids to liver, adipose, cardiac, and skeletal muscle tissue, where their triglyceride components are unloaded by the activity of lipoprotein lipase. As a consequence, chylomicron remnants are left over and are taken up by the liver. Chylomicrons are lipoprotein particles that consist of triglycerides (85-92%), phospholipids (6-12%), cholesterol (1-3%) and proteins (1-2%)(Mahmood, 2000).

- **Very low-density lipoprotein (VLDL)**

  Very-low-density lipoprotein (VLDL) is a type of lipoprotein made by the liver. VLDL is one of the five major groups of lipoproteins. VLDL is assembled in the liver from triglycerides, cholesterol, and apolipoproteins. VLDL is converted in the bloodstream to low-density lipoprotein (LDL). VLDL particles have a diameter of 30-80 nm. VLDL transports endogenous products, whereas chylomicrons transport exogenous (dietary) products.

- **Intermediate density lipoprotein (IDL)**

  Intermediate-density lipoproteins belong to the lipoprotein particle family and are formed from the degradation of very low-density lipoproteins. Each native IDL particle consists of protein that encircles various fatty acids, enabling, as a water-soluble particle, these fatty acids to travel in the aqueous blood environment as part of the fat transport system within the body. Their size is, in general, 25 to 35 nm in diameter, and they contain primarily a range of triacylglycerols and cholesterolesters. They are cleared from the plasma into the liver by receptor-mediated endocytosis, or further degraded to form LDL particles. In general, IDL, somewhat similar to low-density lipoprotein (LDL), transports a variety of triglyceride fats and cholesterol and, like LDL, can also promote the growth of atheroma (Brown and Goldstein, 1986).

- **Low Density Lipoprotein (LDL)**

  Low-density lipoprotein (**LDL**) is one of the five major groups of lipoproteins, that enable transport of multiple different fat molecules, including cholesterol, within the water around cells and within the water-based bloodstream. There has also been noted a correspondence between higher triglyceride levels and higher levels of smaller, denser LDL particles and alternately lower triglyceride levels and higher levels of the larger, less dense LDL (Warnick *et al.,* 1990). Each native LDL particle contains a single apolipoprotein B-100 molecule (Apo B-100, a protein that has 4536 amino acid residues and a mass of 514 kDa), which circulates the fatty acids, keeping them soluble in the aqueous environment. In addition, LDL has a highly hydrophobic core consisting of polyunsaturated fatty acid known as *linoleate* and about 1500 esterified cholesterol molecules. This core is surrounded by a shell of phospholipids and unesterified cholesterol, as well as the single copy of Apo B-100. LDL particles are

approximately 22 nm (0.00000087 in.) in diameter and have a mass of about 3 million daltons, but since LDL particles contain a changing number of fatty acids, they actually have a distribution of mass and size (Segrest *et al.,200*1).

When a cell requires cholesterol, it synthesizes the necessary LDL receptors, and inserts them into the plasma membrane. The LDL receptors diffuse freely until they associate with clathrin-coated pits. LDL particles in the bloodstream bind to these extracellular LDL receptors. The clathrin-coated pits then form vesicles that are endocytosed into the cell. After the clathrin coat is shed, the vesicles deliver the LDL and their receptors to early endosomes, onto late endosomes to lysosomes. Here the cholesterol esters in the LDL are hydrolysed. The LDL receptors are recycled back to the plasma membrane. LDL particles are formed as VLDL lipoproteins lose triglyceride through the action of lipoprotein lipase (LPL) and they become smaller and denser (i.e. fewer fat molecules with same protein transport shell), containing a higher proportion of cholesterol esters (Warnick *et al.,*1990).

- **High Density Lipoprotein (HDL)**

High-density lipoprotein (HDL) is the smallest of the lipoprotein particles which enable lipids like cholesterol and triglycerides to be transported within the water-based bloodstream. In healthy individuals, about thirty percent of blood cholesterol is carried by HDL. Those with higher levels of HDL-C seem to have fewer problems with cardiovascular diseases, while those with low HDL-C cholesterol levels (less than 40 mg/dL or about 1 mmol/L) have increased rates for heart disease. While higher HDL levels are correlated with cardiovascular health, no incremental increase in HDL has been proven to improve health. In other words, while high HDL levels might correlate with better cardiovascular health, specifically increasing one's HDL

might not increase cardiovascular health. They are the densest because they contain the highest proportion of protein to cholesterol. HDL contains approximately 55% protein, 3-15% triglycerides, 26-46% phospholipids, 15-30% cholesteryl esters, and 2-10% cholesterol. Their most abundant apolipoproteins are apo A-I and apo A-II (Despres, 2009).

The liver synthesizes these lipoproteins as complexes of apolipoproteins and phospholipid. They are capable of picking up cholesterol, carried internally from cells. A plasma enzyme called lecithin-cholesterol acyltransferase (LCAT) converts the free cholesterol into cholesteryl ester (a more hydrophobic form of cholesterol), which is then sequestered into the core of the lipoprotein particle, eventually making the newly synthesized HDL spherical. They increase in size (HDL particles have a size of 6-12.5 nanometers) as they circulate through the bloodstream and incorporate more cholesterol and phospholipid molecules from cells and other lipoproteins. HDL transports cholesterol mostly to the liver or steroidogenic organs such as adrenals, ovary, and testes by direct and indirect pathways.HDL is removed by HDL receptors such as scavenger receptor BI (SR-BI), which mediate the selective uptake of cholesterol from HDL. In humans, probably the most relevant pathway is the indirect one, which is mediated by cholesteryl ester transfer protein (CETP). This protein exchanges triglycerides of VLDL against cholesteryl esters of HDL. As the result, VLDLs are processed to LDL, which are removed from the circulation by the LDL receptor pathway. Through the action of cholesterol ester transfer protein, TGs are transferred from VLDL to HDL, creating TG-rich HDL particles, which are hydrolyzed by hepatic lipase and rapidly cleared from plasma (Hopkins and Barter, 1986). A similar cholesterol ester protein-mediated transfer of TGs from VLDL to LDL contributes to the formation of small dense LDL particles (Berneis and Krauss, 2002). Other mechanisms, including impaired clearance of lipid and lipoproteins may also be involved. The

triglycerides are not stable in HDL, but degraded by hepatic lipase so that finally small HDL particles are left, which restart the uptake of cholesterol from cells. The cholesterol delivered to the liver is excreted into the bile and, hence, intestine either directly or indirectly after conversion into bile acids. Delivery of HDL cholesterol to adrenals, ovaries, and testes is important for the synthesis of steroid hormones. However, HDL carries many lipid and protein species, several of which have very low concentrations but are biologically very active. For example, HDL and their protein and lipid constituents help to inhibit oxidation, inflammation, activation of the endothelium, coagulation, and platelet aggregation. All these properties may contribute to the ability of HDL to protect from atherosclerosis (Kwiterovich, 2000).

2.3.4 Triglycerides

A triglyceride (TG, triacylglycerol, TAG) is an ester derived from glycerol and three fatty acids. Triglycerides are a blood lipid that help enable the bidirectional transference of adipose fat and blood glucose from the liver. Triglycerides, as major components of very-low-density lipoprotein (VLDL) and chylomicrons, play an important role in metabolism as energy sources and transporters of dietary fat. They contain more than twice as much energy (approximately 9 kcal/g or 38 kJ/g) as carbohydrates (approximately 4 kcal/g or 17 kJ/g). There are many triglycerides: depending on the oil source, some are highly unsaturated, some less so.Triglycerides are the main constituents of vegetable oil (typically more unsaturated) and animal fats (typically more saturated) (Nelson and Cox, 2000).

When the body requires fatty acids as an energy source, the hormone, glucagon, signals the breakdown of the triglycerides by hormone-sensitive lipase to release free fatty acids. As the

brain cannot utilize fatty acids as an energy source (unless converted to a ketone), the glycerol component of triglycerides can be converted into glucose, via glycolysis by conversion into dihydroxyacetone phosphate and then into glyceraldehyde 3-phosphate, for brain fuel when it is broken down. Fat cells may also be broken down for that reason, if the brain's needs ever outweigh the body's. The American Heart Association (AHA, 2012) recommends that a triglyceride level of 100 mg/dL (1.3 mmol/L) or lower is considered optimal.

In the human body, high levels of triglycerides in the bloodstream have been linked to atherosclerosis and, by extension, the risk of heart disease and stroke. However, the relative negative impact of raised levels of triglycerides compared to that of LDL:HDL ratios is as yet unknown. The risk can be partly accounted for by a strong inverse relationship between triglyceride level and HDL-cholesterol level. Diets high in refined carbohydrates, with carbohydrates accounting for more than 60% of the total energy intake, can increase triglyceride levels (AHA, 2012). Of note is how the correlation is stronger for those with higher BMI (28+) and insulin resistance (more common among overweight and obese) is a primary suspect cause of this phenomenon of carbohydrate-induced hypertriglyceridemia (Parks, 2002).

2.3.5 Lipid Disorders

Lipid disorders are the broad term for abnormalities of cholesterol and triglycerides. Lipid abnormalities are associated with an increased risk for vascular disease, and especially heart attacks and strokes. Abnormalities in lipid disorders are a combination of genetic predisposition as well as the nature of dietary intake. Many lipid disorders are associated with being overweight. Lipid disorders may also be associated with other diseases including diabetes, the metabolic syndrome (sometimes called the insulin resistance syndrome), underactive thyroid or

the result of certain medications (such as those used for anti-rejection regimens in people who have had transplants). There is accumulating evidence that management of cholesterol and triglyceride disorders is associated with the reduced risk for heart attacks and strokes. Elevated levels of plasma LDL, TG, accompanied by reduced HDL levels, are associated with an increased risk of coronary heart disease (Isley, 2006; Cutri *et al.,* 2006).

Lipid disorders, like hyperlipoproteinemia, occur when there is too much lipid (fat) in the blood. Shorter terms that mean the same thing are hyperlipidemia and hyperlipemia. Dyslipidemia refers to a redistribution of cholesterol from one place to another that increases the risk of vascular disease withoutincreasing the total amount of cholesterol. When more precise terms are needed, hypercholesterolemia and hypertriglycericemia are used.

a. Hyperlipidemia

Hyperlipidemia is the presence of elevated or abnormal levels of lipids and/or lipoproteins in the blood. Hyperlipidemias may basically be classified as either familial (also called primary) caused by specific genetic abnormalities, or acquired (also called secondary) when resulting from another underlying disorder that leads to alterations in plasma lipid and lipoprotein metabolism. Also, hyperlipidemia may be idiopathic, that is, without known cause (Chait and Brunzell, 1990).

Hyperlipidemias are also classified according to which types of lipids are elevated, that is hypercholesterolemia, hypertriglyceridemia or both in combined hyperlipidemia. Elevated levels of lipoprotein may also be classified as a form of hyperlipidemia.

- **Familial (Primary)**

  Familial hyperlipidemias are classified according to the Fredrickson classification which is based on the pattern of lipoproteins on electrophoresis or ultracentrifugation (Fredrickson and Lees, 1965). It was later adopted by the World Health Organization (WHO). It does not directly account for HDL.

- **Acquired (Secondary) Hyperlipidemia**

  Acquired hyperlipidemias (also called secondary dyslipoproteinemias) often mimic primary forms of hyperlipidemia and can have similar consequences (Chait and Brunzell, 1990). They may result in increased risk of premature atherosclerosis or, when associated with marked hypertriglyceridemia, may lead to pancreatitis and other complications of the chylomicronemia syndrome. The most common causes of acquired hyperlipidemia are diabetes mellitus, use of drugs such as diuretics, beta blockers, and estrogens (Chait and Brunzell, 1990).

  Other conditions leading to acquired hyperlipidemia include: Hypothyroidism, renal failure, nephrotic syndrome, alcohol usage, some rare endocrine disorders and metabolic disorders (Chait and Brunzell, 1990).

b. Hypercholesterolemia

Hypercholesterolemia is the presence of high levels of cholesterol in the blood (Durrington, 2003). It is not a disease but a metabolic derangement that can be secondary to many diseases and can contribute to many forms of disease, most notably cardiovascular disease. Familial hypercholesterolemia is a rare genetic disorder that can occur in families, where sufferers cannot properly metabolise cholesterol (Durington, 2003).

c. **Hyperlipoproteinemia:**Hyperlipoproteinemias is the elevated level of lipoproteins. It can be classified into five categories

- Type1 has a pure elevation of triglycerides in the chylomicron fraction. These people sometimes get pancreatitis and abdominal pains, but they do not seem to have an increase in vascular disease.Type I hyperlipoproteinemia exists in several forms:

  Chylomicronemia due to circulating inhibitor of lipoprotein lipase (Type Ic)

  Lipoprotein lipase deficiency (Type Ia), due to a deficiency of lipoprotein lipase (LPL) or altered apolipoprotein C2, resulting in elevated chylomicrons, the particles that transfer fatty acids from the digestive tract to the liver.

  Familial apoprotein CII deficiency (Type Ib) (Yamamura *et al.,* 1979). A condition caused by a lack of lipoprotein lipase activator (James *et al.,* 2006).

- Type 2 appears in two distinct genetic patterns and a third category, which is by far the most important kind, because everyone is at risk for it. All Type 2s have elevated cholesterol. Some have elevated triglycerides also. The familial (genetic) versions of Type 2 often develop xanthomas, which are yellow fatty deposits under the skin of the knuckles, elbows, buttocks or heels. They may also have xanthelasmas, smaller yellow patches on the eyelids (Yamamura *et al.,* 1979).
- Type 3 appears in one in 10,000 people and elevates both triglycerides and cholesterol with consequent vascular disease.
- Type 4 elevates only triglycerides and does not increase the risk of vascular disease (Boman *et al.,* 1975*)*.

Type 5 is similar to Type 1but with high VLDL in addition to chylomicrons. It is also associated with glucose intolerance and hyperuricemia (Yamamura *et al.,* 1979)

2.4 DIABETES MELLITUS

Diabetes mellitus is a common disorder associated with marked increase in morbidity and mortality rate. Diabetes mellitus can be defined as a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action or both, resulting in impaired function in carbohydrate, lipid and protein metabolism (Gillett, 2009). Diabetes mellitus is a complex chronic metabolic disorder that is a major source of ill health worldwide. It is characterized by hyperglycemia and disturbances of carbohydrate, protein and fat metabolisms, secondary to an absolute or relative lack of the hormone insulin (Alberti and Zimmet, 1998). The number of people in the world with diabetes has increased dramatically over recent years. Indeed, by 2010 it has been estimated that the diabetic population will increase to 221 million around the world (Carter, 2004).

Insulin deprivation in type 1 diabetic patients causes a profound increase in catabolism, especially in skeletal muscle. Moreover, this net muscle protein catabolism is due to a net increase in protein breakdown rather than a decline in protein synthesis. There is a reservoir of basic information that suggests the involvement of oxidative stress in the pathogenesis of diabetes mellitus. It is now recognized that sustained hyperglycemia in diabetic patient, causes protein glycation and generates free radicals through auto-oxidation and polyol pathways (Ramakrishna and Jailkhani, 2008). The insulin deficiency causes changes in glucose metabolism and biochemical processes, thereby increasing fasting glucose level, decreasing hepatic and skeletal glycogen content and decreasing glucose-6-phosphate dehydrogenase

(G6PD) activity. Reduction in G6PD activity lowers the intracellular NADPH level that causes oxidative stress, a critical underlying mechanism in diabetes (Khosla *et al.,* 2000).

High levels of free radicals with concurrent decline of antioxidant defense mechanism may lead to damage of cellular organelles and enzymes (Ottaviano *et al.,* 2008). This can culminate in increased lipid peroxidation and development of insulin resistance, which may consequently promote the development of complications of diabetes mellitus (Demozay *et al.,* 2008). Diabetes mellitus is characterized by recurrent or persistent hyperglycemia, and is diagnosed by demonstrating any one of the following: People with fasting glucose levels from 110 to 125 mg/dl (6.1 to 6.9 mmol/l) are considered to have impaired fasting glucose. Patients with plasma glucose at or above 140 mg/dL (7.8 mmol/L), but not over 200 mg/dL (11.1 mmol/L), two hours after a 75 g oral glucose load are considered to have impaired glucose tolerance. Of these two pre-diabetic states, the latter in particular is a major risk factor for progression to full-blown diabetes mellitus, as well as cardiovascular disease (Santaguida *et al.,* 2008)**.**

2.4.1 Types of Diabetes Mellitus

There are two types of diabetes known as Type 1, insulin-dependent diabetes mellitus (IDDM) and Type 2, noninsulin-dependent diabetes mellitus (NIDDM). Type 1 patients are not capable of producing insulin and should therefore take daily insulin injection to stay healthy. Most diabetic patients are in Type 2 category, in which the body does not produce enough insulin. In this case drugs and natural products that aid in the release of insulin alleviate the condition of the patient by lowering the level of blood glucose (WHO, 1999).

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 cell loss is a T-cell-mediated autoimmune attack (Rother, 2007). Most affected people are otherwise healthy and of a healthy weight when onset occurs. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type 1 diabetes can affect children or adults, but was traditionally termed "juvenile diabetes" because a majority of these diabetes cases were in children. The mechanism responsible for the development of hyperlipidemia in people with uncontrolled IDDM is due to the effect of insulin on adipose tissue lipoprotein lipase activity. Insulin has important regulatory effects on plasma lipid as well as glucose metabolism, which explains why IDDM is associated with significant abnormalities of lipoprotein metabolism. The importance of lipid and lipoprotein disorders in IDDM is due to the increased risk of atherosclerosis. Long-term studies from the Joslin Clinic demonstrate the excess cardiovascular mortality in patients with IDDM (Krolewski *et al.,* 1987).

In IDDM patients with CHD, increases in total and LDL cholesterol and decreases in HDL cholesterol are more common than in IDDM patients without CHD (Laakso *et al.,* 1986). Uncontrolled IDDM is often associated with elevated plasma lipid levels, and insulin treatment usually restores lipid levels to normal. In a study of diabetic patients with ketoacidosis, Weidman *et al* (1982) examined the effect of treatment on plasma lipid levels. Before insulin therapy, elevated cholesterol and triglyceride levels were due to accumulation of triglyceride-

rich lipoproteins, chylomicrons, and VLDL. Insulin therapy resulted in a decrease in triglycerides to normal levels in most patients within 24 hours. LDL cholesterol was low initially and did not change with therapy. HDL cholesterol levels were also low but increased significantly after 24 hours. Studies of LDL metabolism in conventionally treated IDDM patients demonstrated that LDL-apoB synthesis and clearance are similar to those in nondiabetic subjects (Rosenstock *et al.,* 1988). The mechanism for the low HDL in people with untreated IDDM is probably related to low lipoprotein lipase activity, which can lead to reduced formation of HDL during impaired lipolysis of VLDL (Laakso *et al.,* 1986). Studies in rabbits with alloxan-induced diabetes also suggest that low HDL levels are due to decreased HDL apo A-l synthetic rates (Golay *et al.,* 1987). Alterations in surface and core lipids of HDL in people with IDDM also were reported (Bagdade and Subbaiah, 1989).

VLDL metabolism in patients with treated IDDM depends on diabetic control. In patients with poorly controlled but nonketotic IDDM, both overproduction and decreased clearance of VLDL can occur. Several studies also confirmed the importance of diabetic control on plasma lipoprotein abnormalities in people with IDDM (Lopes-Virella *et al.,*1985). VLDL from normolipidemic IDDM patients are enriched with cholesterol and apoB and depleted in triglycerides compared with that of nondiabetic subjects (Rivellese *et al.,* 1988). There are several mechanisms postulated to be responsible for abnormal LDL metabolism in IDDM. *1)* Nonenzymatic glucosylation of LDL-apoB interferes with normal LDL catabolism mediated by the LDL receptor (Witztum *et al.,* 1982). Nonenzymatic glucosylation of LDL was shown to inhibit uptake and degradation of LDL by endothelial cell (Lorenzi *et al., 1984*). *2)* Binding of LDL to its receptor is impaired, independent of LDL glucosylation (Lopes-Virella *et al.,* 1985). LDL uptake and binding to fibroblasts isolated from patients with poorly controlled IDDM is

slower than from nondiabetic control subjects, and insulin therapy restores LDL binding to normal (Lopes-Virella *et al.,* 1982). Insulin stimulates LDL catabolism (Mazzone *et al.,* 1984), and this process may be related to stimulation of LDL receptor activity (Krone *et al.,* 1988).

Diabetes Mellitus Type 2

Type 2 diabetes mellitus is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion. The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor. However, the specific defects are not known. Diabetes mellitus cases due to a known defect are classified separately. Type 2 diabetes is the most common type. In the early stage of type 2, the predominant abnormality is reduced insulin sensitivity. At this stage, hyperglycemia can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce glucose production by the liver. Although diabetes is one of those diseases for which there is no cure, there is now ample scientific evidence that has established beyond doubt that some natural products can be used to control Type 2 diabetes mellitus just as well as the modern drugs. Impaired insulin-stimulated glucose disposal (DeFronzo *et al.,* 1985), muscle mitochondrial ATP production, and reduced mitochondrial protein synthesis also occur in type 2 diabetic patients (Stump *et al.,* 2003),

One group of drugs introduced in the management of Type 2 diabetes is represented by the inhibitors of glucosidase. The enzymes summarized as glucosidase are responsible for the breakdown of oligo- and/or disaccharides to monosaccharides. The inhibition of these enzymes leads to a decrease of blood glucose level, because the monosaccharides dare the form of carbohydrates which is absorbed through the mucosal border in the small intestine.

Gestational Diabetes

Gestational diabetes mellitus (GDM) resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2%–5% of all pregnancies and may improve or disappear after delivery. Gestational diabetes is fully treatable, but requires careful medical supervision throughout the pregnancy. About 20%–50% of affected women develop type 2 diabetes later in life. Though it may be transient, untreated gestational diabetes can damage the health of the fetus or mother. Risks to the baby include macrosomia (high birth weight), congenital cardiac and central nervous system anomalies, and skeletal muscle malformations. Increased fetal insulin may inhibit fetal surfactant production and cause respiratory distress syndrome.

2.4.2 Diabetes Complications

All forms of diabetes increase the risk of long-term complications. These typically develop after many years, but may be the first symptom in those who have otherwise not received diagnosis before that time. Oxidative stress is suggested as one of the mechanism underlying diabetes and diabetic complications. Oxidative stress is developed from an imbalance between radical generating and radical scavenging system (Carter, 2004). The major long term complication relates to damage blood vessels. Diabetes doubles the risk of cardiovascular disease (Boussageon *et al.,* 2011). The main macrovascular diseases are stroke, ischaemic heart disease. Diabetes also causes microvascular complication which causes damage to small blood vessels. The impact of diabetes on the kidneys, can lead to scarring changes in the kidney tissue, loss of small or progressively larger amounts of protein in the urine and eventually chronic kidney disease requiring dialysis. Long term complications of diabetes mellitus

includes retinopathy with potential loss of vision, nephropathy leading renal failure peripheral neuropathy with risk of foot ulcer, amputation and charcoal joints. However much of the clinical and economical toll of diabetes arise from complication of the disease such as capillary basement, membrane thickening, neuropathy, nephropathy, and accelerated atherosclerosis.

1. Microvascular Complication of Diabetes Mellitus

Microvascular complications are those affecting smaller blood vessels, and are further differentiated by whether they primarily affect the kidneys (nephropathy), eyes (retinopathy), peripheral circulation (smaller bloodvessels), or nerves (neuropathy).

- **Diabetic Retinopathy**

The risk of developing diabetic retinopathy or other microvascular complications of diabetes depends on both the duration and the severity of hyperglycemia. There are several proposed pathological mechanisms by which diabetes may lead to development of retinopathy. Aldose reductase may participate in the development of diabetes complications. Aldose reductase is the initial enzyme in the intracellular polyol pathway. This pathway involves the conversion of glucose into glucose alcohol (sorbitol). High glucose levels increase the flux of sugar molecules through the polyol pathway, which causes sorbitol accumulation in cells. Osmotic stress from sorbitol accumulation has been postulated as an underlying mechanism in the development of diabetic microvascular complications, including diabetic retinopathy. In animal models, sugar alcohol accumulation has been linked to microaneurysm formation, thickening of basement membranes, and loss of pericytes. Treatment studies with aldose reductase inhibitors, however, have been disappointing (Fong *et al.,* 2004).

Oxidative stress may also play an important role in cellular injury from hyperglycemia. High glucose levels can stimulate free radical production and reactive oxygen species formation. Animal studies have suggested that treatment with antioxidants, such as vitamin E, may attenuate some vascular dysfunction associated with diabetes, but treatment with antioxidants has not yet been shown to alter the development or progression of retinopathy or other microvascular complications of diabetes (Fong *et al.,*2004).

Diabetic retinopathy is generally classified as either background or proliferative; Background retinopathy includes such features as small hemorrhages in the middle layers of the retina. They clinically appear as "dots" and therefore are frequently referred to as "dot hemorrhages." Hard exudates are caused by lipid deposition that typically occurs at the margins of hemorrhages. Microaneurysms are small vascular dilatations that occur in the retina, often as the first sign of retinopathy. They clinically appear as red dots during retinal examination. Retinal edema may result from microvascular leakage and is indicative of compromise of the blood-retinal barrier. The appearance is one of grayish retinal areas. Retinal edema may require intervention because it is sometimes associated with visual deterioration (Watkins, 2003).

Proliferative retinopathy is characterized by the formation of new blood vessels on the surface of the retina and can lead to vitreous hemorrhage. White areas on the retina ("cotton wool spots") can be a sign of impending proliferative retinopathy. If proliferation continues, blindness can occur through vitreous hemorrhage and traction retinal detachment. With no intervention, visual loss may occur. Laser photocoagulation can often prevent proliferative retinopathy from progressing to blindness; therefore, close surveillance for the existence or progression of retinopathy in patients with diabetes is crucial (Watkins, 2003).

- **Diabetic Nephropathy**

  It is defined by proteinuria > 500 mg in 24 hours in the setting of diabetes, but this is preceded by lower degrees of proteinuria, or "microalbuminuria." Microalbuminuria is defined as albumin excretion of 30-299 mg/24 hours. Without intervention, diabetic patients with microalbuminuria typically progress to proteinuria and overt diabetic nephropathy. This progression occurs in both type 1 and type 2 diabetes. As many as 7% of patients with type 2 diabetes may already have microalbuminuria at the time they are diagnosed with diabetes (Gross *et al.,* 2005).

  The pathological changes to the kidney include increased glomerular basement membrane thickness, microaneurysm formation, mesangial nodule formation (Kimmelsteil-Wilson bodies), and other changes. The underlying mechanism of injury may also involve some or all of the same mechanisms as diabetic retinopathy. Initial treatment of diabetic nephropathy, as of other complications of diabetes, is prevention. Like other microvascular complications of diabetes, there are strong associations between glucose control (as measured by hemoglobin A_{1c} [A1C]) and the risk of developing diabetic nephropathy. Patients should be treated to the lowest safe glucose level that can be obtained to prevent or control diabetic nephropathy (Gross *et al.,* 2005). Treatment with angiotensin-converting enzyme (ACE) inhibitors has not been shown to prevent the development of microalbuminuria in patients with type 1 diabetes but has been shown to decrease the risk of developing nephropathy and cardiovascular events in patients with type 2 diabetes (Gross *et al.,* 2005).

- **Diabetic Neuropathy**

  Diabetic neuropathy is recognized by the American Diabetes Association (ADA) as "the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes" (ADA, 2007). As with other microvascular complications, risk of developing diabetic neuropathy is proportional to both the magnitude and duration of hyperglycemia, and some individuals may possess genetic attributes that affect their predisposition to developing such complications. The precise nature of injury to the peripheral nerves from hyperglycemia is not known but likely is related to mechanisms such as polyol accumulation, injury from AGEs, and oxidative stress. Peripheral neuropathy in diabetes may manifest in several different forms, including sensory, focal/multifocal, and autonomic neuropathies. More than 80% of amputations occur after foot ulceration or injury, which can result from diabetic neuropathy (Boulton *et al.,* 2005).

  Chronic sensorimotor distal symmetric polyneuropathy is the most common form of neuropathy in diabetes. Typically, patients experience burning, tingling, and "electrical" pain, but sometimes they may experience simple numbness. In patients who experience pain, it may be worse at night. Patients with simple numbness can present with painless foot ulceration, so it is important to realize that lack of symptoms does not rule out presence of neuropathy.

  Physical examination reveals sensory loss to light touch, vibration, and temperature. Abnormalities in more than one test of peripheral sensation are > 87% sensitive in detecting the presence of neuropathy. Patients also typically experience loss of ankle reflex (Boulton *et al.,* 2005). Pure sensory neuropathy is relatively rare and associated with periods of poor glycemic control or considerable fluctuation in diabetes control. It is characterized by isolated sensory

findings without signs of motor neuropathy. Symptoms are typically most prominent at night (Boulton *et al.,* 2005).

- **Diabetic Cardiomyopathy** is a disorder of the heart muscle in people with diabetes. It can lead to inability of the heart to circulate blood through the body effectively, a state known as heart failure, with accumulation of fluid in the lungs (pulmonary edema) or legs (peripheral edema). Most heart failure in people with diabetes results from coronary artery disease, and diabetic cardiomyopathy is only said to exist if there is *no* coronary artery disease to explain the heart muscle disorder. Etiologically, four main causes are responsible for the development of heart failure in DCM: microangiopathy and related endothelial dysfunction, autonomic neuropathy, metabolic alterations that include abnormal glucose use and increased fatty acid oxidation, generation and accumulation of free radicals, and alterations in ion homeostasis, especially calcium transients. Diabetic cardiomyopathy is characterized functionally by ventricular dilation, myocyte hypertrophy, prominent interstitial fibrosis and decreased or preserved systolic function in the presence of a diastolic dysfunction (Keenan *et al.,*2007).

2. Macrovascular Complications

The central pathological mechanism in macrovascular disease is the process of atherosclerosis, which leads to narrowing of arterial walls throughout the body. Atherosclerosis is thought to result from chronic inflammation and injury to the arterial wall in the peripheral or coronary vascular system

- **Coronary Artery Disease** (**CAD**; also **atherosclerotic heart disease**) is the most common type of heart disease and cause of heart attacks. The disease is caused by plaque building up along the inner walls of the arteries of the heart, which narrows the arteries and restricts blood

flow to the heart. It is the leading cause of death worldwide. While the symptoms and signs of coronary artery disease are noted in the advanced state of disease, most individuals with coronary artery disease show no evidence of disease for decades as the disease progresses before the first onset of symptoms, often a "sudden" heart attack, finally arises. After decades of progression, some of these atheromatous plaques may rupture and (along with the activation of the blood clotting system) start limiting blood flow to the heart muscle. The disease is the most common cause of sudden death (Thomas *et al.,*1988).

As the degree of coronary artery disease progresses, there may be near-complete obstruction of the lumen of the coronary artery, severely restricting the flow of oxygen-carrying blood to the myocardium. Individuals with this degree of coronary artery disease typically have suffered from one or more myocardial infarctions (heart attacks), and may have signs and symptoms of chronic coronary ischemia, including symptoms of angina at rest and flash pulmonary edema.

Ischemia means that the amount of blood supplied to the tissue is inadequate to supply the needs of the tissue. When the myocardium becomes ischemic, it does not function optimally. When a large area of the myocardium becomes ischemic, there can be impairment in the relaxation and contraction of the myocardium. If the blood flow to the tissue is improved, myocardial ischemia can be reversed. Infarction means that the tissue has undergone irreversible death due to lack of sufficient oxygen-rich blood. An individual may develop a rupture of an atheromatous plaque at *any* stage of the spectrum of coronary artery disease. The acute rupture of a plaque may lead to an acute myocardial infarction (heart attack).

CAD is associated with smoking, diabetes, and hypertension. A number of recent studies have shown that family history of early CAD is an important predictor of CAD. Most of the familial

association of coronary artery disease may be related to common dietary habits. Saturated fats increase LDL cholesterol by inhibiting LDL receptor activity and enhancing apolipoprotein (apo)B-containing lipoprotein production (Dietchy, 1998). This LDL cholesterol–raising effect of saturated fatty acids has been shown to depend on the level of dietary cholesterol, such that the greatest increases in plasma LDL concentrations were observed at the highest levels of dietary cholesterol (Dietchy, 1998). Screening for CAD includes evaluating high-density and low-density lipoprotein (cholesterol) levels and triglyceride levels. Recent studies, however, demonstrated that it may be the level of apoA-I, rather than the level of HDL cholesterol, that is important in reducing the risk of atherosclerosis (Van der Steeg *et al.,* 2008). Despite much press, most of the alternative risk factors including homocysteine, C-reactive protein (CRP), Lipoprotein (a), coronary calcium and more sophisticated lipid analysis have added little if any additional value to the conventional risk factors of smoking, diabetes and hypertension.Patients with CAD and those trying to prevent CAD are advised to avoid fats that are readily oxidized (e.g., trans-fats), limit carbohydrates and processed sugars to reduce production of low density lipoproteins (LDLs), triacylglycerol and apolipoprotein-B (Parks *et al.,*2008).

- **Diabetic Myonecrosis:**

Diabetic Myonecrosis is a rare complication of diabetes. It is caused by infarcted muscle tissue, usually in the thigh. Other diabetic complications such as nephropathy, neuropathy, retinopathy and hypertension are usually present. Its major symptom is the acute onset muscle pain, usually in the thigh, in the absence of trauma. Signs include exquisite muscle tenderness and swelling. The pathogenesis of this disease is unclear. Arteriosclerosis obliterans has been postulated as the cause, along with errors of the clotting and fibrinolytic pathways such as antiphospholipid syndrome (Wintz *et al.,* 2006).

- **Peripheral Vascular Disease** (PVD)

  Peripheral vascular disease (PVD), commonly referred to as peripheral artery disease (PAD) or peripheral artery occlusive disease (PAOD), refers to the obstruction of large arteries not within the coronary, aortic arch vasculature, or brain. PVD can result from atherosclerosis, inflammatory processes leading to stenosis, an embolism, or thrombus formation. It causes either acute or chronic ischemia (lack of blood supply). PVD also includes a subset of diseases classified as microvascular diseases resulting from episodal narrowing of the arteries (Raynaud's phenomenon), or widening thereof (erythromelalgia), i.e. vascular spasms. Diagnosis is critical, as people with PAD have a four to five times higher risk of heart attack or stroke. Diabetes mellitus - causes between two and four times increased risk of PVD by causing endothelial and smooth muscle cell dysfunction in peripheral arteries. Diabetics account for up to 70% of nontraumatic amputations performed, and a known diabetic who smokes runs an approximately 30% risk of amputation within 5 years. Often PVD is a term used to refer to atherosclerotic blockages found in the lower extremity (Wintz *et al.,* 2006).

- **Stroke**

  A stroke, or cerebrovascular accident (CVA), is the rapid loss of brain function due to disturbance in the blood supply to the brain. It is the second leading cause of death worldwide. This can be due to ischemia (lack of blood flow) caused by blockage (thrombosis, arterial embolism), or a hemorrhage (Sims and Muyderman, 2009). As a result, the affected area of the brain cannot function, which might result in an inability to move one or more limbs on one side of the body, inability to understand or formulate speech, or an inability to see one side of the visual field (Donnan *et al.,* 2008). A stroke is a medical emergency and can cause permanent

neurological damage and death. Risk factors for stroke include old age, high blood pressure, previous stroke or transient ischemic attack (TIA), diabetes, high cholesterol, tobacco smoking and atrial fibrillation. High blood pressure is the most important modifiable risk factor of stroke (Donnan *et al., 2008)*.

Strokes can be classified into two major categories: ischemic and hemorrhagic. Ischemic strokes are those that are caused by interruption of the blood supply, while hemorrhagic strokes are the ones which result from rupture of a blood vessel or an abnormal vascular structure. About 87% of strokes are caused by ischemia, and the remainder by hemorrhage. Some hemorrhages develop inside areas of ischemia ("hemorrhagic transformation"). It is unknown how many hemorrhages actually start as ischemic stroke. Diabetes mellitus increases the risk of stroke by 2 to 3 times. While intensive control of blood sugar has been shown to reduce microvascular complications such as nephropathy and retinopathy it has not been shown to reduce macrovascular complications such as stroke (Dormandy *et al.,* 2005).

2.4.3 Biochemical Alterations in Diabetes Mellitus

Insulin production is more or less constant within the beta cells. Its release is triggered by food, chiefly food containing absorbable glucose. If the amount of insulin available is insufficient, if cells respond poorly to the effects of insulin (insulin insensitivity or resistance), or if the insulin itself is defective, then glucose will not have its usual effect, so it will not be absorbed properly by those body cells that require it, nor will it be stored appropriately in the liver and muscles. The net effect is persistent high levels of blood glucose, poor protein synthesis, and other metabolic derangements, such as acidosis. It is likely due to the increased β-oxidation due to the hyperlipidemia and altered insulin signaling. The rate of uptake of lipids, unlike that of

glucose, is not regulated by a hormone. Therefore, increased circulating lipids will increase uptake and thereby fatty acid oxidation. This, in turn, increases the concentration of citrate in the cell, a very potent inhibitor of phosphofructokinase, the first rate-limiting step of glycolysis. When the rate of uptake is greater than the rate of oxidation, fatty acids are shuttled to the triglyceride synthesis pathway. Increasing triglyceride stores prevent lipotoxicity but decrease heart function (Zhou *et al.,* 2000).

Biochemical Alteration in Carbohydrate Metabolism in Diabetes Mellitus

Insulin is the principal hormone that regulates uptake of glucose from the blood into most cells (primarily muscle and fat cells, but not central nervous system cells). Therefore, deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of diabetes mellitus. Humans are capable of digesting some carbohydrates, in particular those most common in food; starch, and some disaccharides such as sucrose, are converted within a few hours to simpler forms, most notably the monosaccharide glucose, the principal carbohydrate energy source used by the body. The rest are passed on for processing by gut flora largely in the colon. Insulin is released into the blood by beta cells (β-cells), found in the islets of langerhans in the pancreas, in response to rising levels of blood glucose, typically after eating. Insulin is used by about two-thirds of the body's cells to absorb glucose from the blood for use as fuel, for conversion to other needed molecules, or for storage.

When the glucose concentration in the blood is raised beyond its renal threshold (about 10 mmol/L, although this may be altered in certain conditions, such as pregnancy), reabsorption of glucose in the proximal renal tubule is incomplete, and part of the glucose remains in the urine (glycosuria). This increases the osmotic pressure of the urine and inhibits reabsorption of

water by the kidney, resulting in increased urine production (polyuria) and increased fluid loss. Lost blood volume will be replaced osmotically from water held in body cells and other body compartments, causing dehydration and increased thirst. Hyperglycemia causes an increase in diacylglycerol, which is also an activator of the Protein Kinase C (PKC) signaling pathway. Induction of PKC causes multiple deleterious effects, including but not limited to blood flow abnormalities, capillary occlusion and pro-inflammatory gene expression (Adler *et al.,* 2000).

Insulin is also the principal control signal for conversion of glucose to glycogen for internal storage in liver and muscle cells. Lowered glucose levels result both in the reduced release of insulin from the β-cells and in the reverse conversion of glycogen to glucose when glucose levels fall. This is mainly controlled by the hormone glucagon, which acts in the opposite manner to insulin. Glucose thus forcibly produced from internal liver cell stores (as glycogen) re-enters the bloodstream; muscle cells lack the necessary export mechanism. Normally, liver cells do this when the level of insulin is low (which normally correlates with low levels of blood glucose). Higher insulin levels increase some anabolic processes, such as cell growth and duplication, protein synthesis, and fat storage. Insulin (or its lack) is the principal signal in converting many of the bidirectional processes of metabolism from a catabolic to an anabolic direction, and *vice versa*. In particular, a low insulin level is the trigger for entering or leaving ketosis (the fat-burning metabolic phase). The consequences of increased intracellular glucose concentration are fourfold, all resulting from increasing concentration of glycolytic intermediates upstream of the rate-limiting glyceraldehyde-3-phosphate reaction which is inhibited by mechanisms activated by increased free radical formation, common in diabetes (Gross *et al.,* 2005).

Glucose, as well as other intermediates such as fructose and glyceraldehyde-3-phosphate, when present in high concentrations, promote the formation of advanced glycation endproducts (AGEs). These, in turn, can irreversibly cross link to proteins and cause intracellular aggregates that cannot be degraded by proteases and thereby, alter intracellular signalling. Also, AGEs can be exported to the intercellular space where they can bind AGE receptors (RAGE). This AGE/RAGE interaction activates inflammatory pathways such as NF-κB, in the host cells in an autocrine fashion, or in macrophages in a paracrine fashion. Neutrophil activation can also lead to NAD(P) Hoxidase production of free radicals, further damaging the surrounding cells. Finally, exported glycation products bind extracellular proteins and alter the matrix, cell-matrix interactions and promote fibrosis (Rossing *et al.,* 2003). A major source of increased myocardial stiffness is crosslinking between AGEs and collagen. Oxidative stress in DM was thought to be a result of free radicals generated during oxidation of glucose (Miyata *et al.,* 1999). Increased levels of ROS in type 2 DM was implicated to contribute to a hypercoagulable state (Collier *et al.,* 1992), and, most recently, evidence was provided for the accumulation of oxidation products prior to the development of diabetes (Matteucci and Giampietro, 2002).

Biochemical Alterations in Lipid in Diabetes Mellitus

The most of biochemical pathways strictly associated with hyperglycaemia (non-enzymatic glycosylation, glucose oxidation, and polyol pathways) and their abnormalities are high level leads to tissue damage and enzyme dysfunctions (Siemianowicz, 2003). Liver is an important organ that plays a pivotal role in glycolysis, gluconeogenesis and glycogenolysis. A central metabolic role of the liver is to maintain plasma glucose within narrow physiological limits regardless of the nutritional state of the animal. In energy excess, glucose is converted to fatty

acids, which are further used to synthesize triglycerides. Triglycerides can be stored as lipid droplets within hepatocytes or incorporated into very-low-density lipoproteins (VLDL) and secreted into the blood. Once in the blood, triglyceride content of these particles is progressively reduced by the action of lipoprotein lipase (LPL), eventually resulting in intermediate-density lipoproteins (IDLs) and low-density lipoproteins (LDL) with relatively high cholesterol content (Tulenko and Sumner, 2002).

Patients with insulin resistance increase VLDL secretion as they attempt to maintain hepatic lipid homeostasis. Therefore, insulin resistance is associated with abnormal concentration of lipoproteins, elevated VLDL production, and increase in plasma LDL (Haidari *et al.,* 2002).

Association of fatty liver and small dense LDL (sdLDL) concentration is now well documented (Sugino *et al., 2011*). As the triglyceride-rich VLDL is entering plasma at an accelerated rate, small, dense LDL, the most atherogenic subclass of LDL, develop after triglycerides are gradually removed from LDL. Two enzymes are implicated in this process. First, cholesteryl ester transfer protein (CETP) facilitates the transfer of triglycerides from VLDL to LDL (and cholesteryl esters from LDL to VLDL), and, second, hepatic lipase increases lipolysis of triglyceride-rich LDL resulting in the formation of sdLDL (Vegges, 2005). Thus, CETP remodels VLDL in circulation, enriches it in cholesterol, and also favors, together with HL, the formation of sdLDL. CETP activity is increased in hepatic steatosis patients LDL receptor shows a lower affinity for smaller particles, therefore such particles stay longer in the circulation (Toyota *et al.,* 1999). Hyperlipidemia can be further exacerbated by low activity of lipoprotein lipase, or by high level of apolipoprotein C-3 (APOC-3), an inhibitor of lipoprotein lipase. Indeed, APOC-3 polymorphisms have been associated with fatty liver in humans

(Petersen *et al.,* 2010). However, this association was not found in recent Dallas Heart Study (Kozlitina *et al.,* 2011).

The blockade of hepatic VLDL secretion results in accumulation of triglycerides in the liver. Microsomal triglyceride transfer protein (MTTP) is essential for the formation of VLDL in the liver (Olofson *et al.,* 2000). Mice that cannot secrete VLDL due to the conditional knockout of *Mttp* in the liver exhibit markedly reduced levels of triglycerides in the plasma and develop hepatic steatosis (Raabe *et al.,* 1999), however, without insulin resistance and inflammation (Minehira *et al.,* 2008). In line with the rodent data, human MTTP polymorphisms lead to decreased MTTP activity and VLDL export and are associated with greater intracellular triglyceride accumulation. On the other hand, a high-fat diet was shown to induce the methylation of MTTP and consequently reduce its mRNA level (Chang *et al.,* 2010).

Hepatic fatty acids are derived from several sources, including adipose tissue lipolysis, chylomicron-TAG lipolysis, and de novo lipogenesis, and can be stored as TAG in lipid droplets located within the cytosol (Nagle *et al.,* 2009). Hepatic TAG stores are mobilized by several hepatic lipases. Adipose triacylglycerol lipase (*Atgl*) that selectively performs the first step in TAG in the liver is reduced in several rodent models of obesity, and *Atgl* ablation leads to steatosis, although increased TAG content in the hepatocytes from Atgl-deficient mice does not enhance insulin sensitivity (Stefan *et al.,* 2011). Similarly, inhibiting expression of ATGL coactivator (gene identification-58 (*Cgi-58*)) resulted in a large increase in hepatic TAG content, yet in a decrease in insulin resistance (Brown *et al., 2010*).

Intracellular free cholesterol is converted into cholesteryl ester by acyl-Coenzyme A: cholesterol acyltransferase (ACAT). The function of ACAT2 in the hepatocyte is to provide

esterified cholesterol for incorporation into very-low-density lipoprotein (VLDL), as well as to provide cholesteryl ester for cytoplasmic lipid droplets, a means for storage when liver cholesterol is abundant. Increased VLDL cholesteryl ester secretion occurs in livers of monkeys fed dietary cholesterol (Rudel *et al.,* 2005). Mice, genetically engineered to lack *Acat2* in both the intestine and the liver, were dramatically protected against hepatic neutral lipid (TG and cholesteryl ester) accumulation, in particular with elevated cholesterol diet.

Increased delivery of triglycerides or nonesterified fatty acids to the muscle interferes with the utilization of glucose, through the principles of Randle cycle (Randle,1998), and impairs insulin action. Some studies have identified an interesting link between the development of insulin resistance and deregulation of intestinal lipoprotein metabolism (Haidari *et al.,* 2002).

Biochemical Alteration in Protein Metabolism in Diabetes

Insulin deficiency produces profound changes in metabolism, including whole-body protein catabolism with emaciation. The changes in metabolism and body composition that occur in IDDM are readily reversed by insulin treatment. Insulin appears to exert its anabolic effects chiefly through inhibition of muscle protein breakdown. The circulating levels of glucose, ketoacids, fatty acids and amino acids are all increased in the diabetic patient during insulin deficiency. Insulin deprivation is associated with an increase in circulating amino acids, especially the branched-chain amino acids, glucose, fatty acids and ketones. The effects of amino acid (the primary substrate for protein synthesis) availability on protein metabolism have been extensively studied. Branched-chain amino acids, especially leucine, have been shown to increase both leucine oxidation and whole-body protein synthesis while inhibiting whole-body

protein breakdown (Louard *et al.,* 1990, Nair *et al.,* 1987a). Amino acid supply is likely to be critical in maintaining protein synthesis during insulin administration (Charlton *et al.,* 1996).

Glucagon is consistently elevated during short-term insulin deprivation. Glucagon is known to play a role in glucose homeostasis and is also important in protein metabolism. Studies in healthy subjects have shown that, during insulin deficiency, glucagon increases energy expenditure (Nair, 1987a), leucine oxidation, and protein breakdown (Nair *et al.,* 1987b) and is catabolic during a protein meal (Charlton *et al.,* 1996).

Treatment of the diabetic ketoacidosis with insulin rapidly corrects these metabolic abnormalities and clears the lipemia. With adequately treated conventional therapy, plasma triglyceride levels are usually normal or only slightly elevated, and these patients tend to have normal production and clearance of VLDL-TG. Insulin's effect on protein synthesis at the translational level involves the AKT–mammalian target of rapamycin–P70S6 kinase pathway (Kimball *et al.,* 1994), and amino acids also act via the mammalian target of rapamycin–P70S6 kinase pathway (Bennet *et al.,* 1990).

Interactions of protein and amino acids with carbohydrate metabolism have been recognized for years. Amino acids directly contribute to *de novo* synthesis of glucose via gluconeogenesis and participate in recycling of glucose carbon via the glucose-alanine cycle. Dietary protein and, specifically, the amino acids glycine (Gannon *et al.,* 2002) and leucine (Layman and Baum, 2004) stimulate insulin release from the pancreas, and leucine serves to modulate the intracellular insulin signal in skeletal muscle and adipose tissue (Layman and Baum, 2004).Insulin resistance therefore theoretically can affect protein synthesis. Reduced glucose disposal and fuel flux during hyperinsulinemic clamp in type 2 diabetic patients may affect mitochondrial ATP production, and ATP-dependent processes such as protein synthesis could

be curtailed by reduced ATP availability. Association of insulin resistance with reduced ATP production occurs in aging (Petersen *et al.,* 2003) in association with reduced muscle mitochondrial protein synthesis (Rooyackers *et al.,* 1996). Since proteins are functional molecules, it is possible that many complications related to insulin resistance are related to defective synthesis of certain proteins. Insulin has effects not only at the translational level but also at the transcription level of protein synthesis (DeFronzo*et al.,* 1985). The most notable metabolic change associated with a reduction of muscle mass is a decrease in energy requirements. The most metabolically active body compartment is protein tissue and when the protein compartment is reduced in mass, then basal energy requirements needed to maintain the protein tissue decreases (Young, 1990).

2.4.4 Management

Diabetes mellitus (DM) is possibly the world's fastest growing metabolic disorder. It is one of the most important health problems worldwide, indicating high prevalence and mortality. The number of people suffering from diabetes has soared to 246 million and the disease now kills more people than AIDS (Edwin *et al.,* 2008). Management of diabetes without any side effects is still a challenge to medical communities, therefore herbal and natural products with anti-diabetic activity and fewer side effects are strongly needed (Vetrichelvan *et al., 2002)*. Diabetes mellitus is a chronic disease, which cannot be cured except in very specific situations. Management concentrates on keeping blood sugar levels as close to normal ("euglycemia") as possible, without causing hypoglycemia. This can usually be accomplished with diet, exercise, and use of appropriate medications (insulin in the case of type 1 diabetes, oral medications, as well as possibly insulin, in type 2 diabetes).Amylase inhibitors like tamarind have potential for

reducing diabetes because the carb-blocking effect helps lower post-meal blood glucose levels. Tamarind already has a history of traditional use as a diabetes aid. The insulin therapy often used to treat diabetes has numerous drawbacks associated with long-term treatment. These include insulin resistance, fatty liver, anorexia nervosa and brain atrophy. Tamarind, on the other hand, may protect against oxidative damage in the pancreas associated with diabetes, which have antioxidant properties.Tamarind has carb-blocking potential because it inhibits alpha-amylase, an enzyme the body needs to absorb carbohydrates. It is among numerous plants that inhibit this enzyme, including blueberry, lemon balm, rosemary and green tea extract (Bhutkar and Bhise, 2012). White kidney bean extract is the most-studied amylase inhibitor, with both human and animal studies confirming that it works as a carb blocker.Patient education, understanding, and participation is vital, since the complications of diabetes are far less common and less severe in people who have well-managed blood sugar levels (Nathan *et al*., 2005). When blood glucose levels are high, glucose molecules attach to the hemoglobin in RBCs. The longer hyperglycemia occurs in blood, the more glucose binds to red blood cell and the higher the glycosylated hemoglobin. Glycated haemoglobin (HbA1c) is the result of one-way non-enzymatic chemical binding of glucose to the protein chains of haemoglobin in erythrocytes. It reflects the average plasma glucose concentration and serves as a marker in determining the normality of blood sugar levels for the period between 4 weeks and 3 months. According to general consensus, normal blood sugar is indicated by levels of HbA1c under 7.0% (Harrison and Kasper, 2005).

Once a hemoglobin molecule is glycated, it remains that way. A buildup of glycated hemoglobin within the red cell, therefore, reflects the average level of glucose to which the cell has been exposed during its life-cycle. Measuring glycated hemoglobin assesses the

effectiveness of therapy by monitoring long-term serum glucose regulation. The HbA1c level is proportional to average blood glucose concentration over the previous four weeks to three months. The major proportion of its value is weighted toward the most recent 2 to 4 weeks (ADA, 2007)

The goal of treatment is an HbA1C level of 6.5%, but should not be lower than that, and may be set higher (NIHCE, 2008).Attention is also paid to other health problems that may accelerate the deleterious effects of diabetes. These include smoking, elevated cholesterol levels, obesity, high blood pressure, and lack of regular exercise (NIHCE, 2008).

Insulin therapy and oral hypoglycaemic agents offer effective glycaemic control, but insulin therapy has shortcomings such as ineffectiveness following oral administration, short shelf life, the need for constant refrigeration, and fatal hypoglycaemia, in the event of excess dosage (Anuradha and Ravikumar, 2001). As a result, there is a need to search for compounds with effective antidiabetic activity when taken orally. The oral hypoglycemic agents that are capable of reducing blood sugar level belong to two chemical classes; sulfonylureas and biguanides (Trejo-Gonzalez *et al.,* 1996).

However, the use of oral antidiabetics is limited due to their adverse side effects including hematological, cutaneous and gastrointestinal reactions, hypoglycaemic coma and disturbances of liver and kidney functions(Alarcon-Aguilara *et al.,*1998).Currently treatments of diabetes, in addition to insulin supplement includes many oral hypoglycemic agents like sulfonylureas, biguanides, thiazolidines, D-phenylalanine derivatives meglitinides and α-glucosidase inhibitors along with appropriate diet and exercise. However, none can be termed as an ideal one, due to their toxic side effects and sometimes diminution in response after prolonged

use.There are manyclinical and experimental evidences indicating involvement of oxidative stress in pathogenesis of diabetes mellitus and its complications namely diabetic neuropathy, retinopathy and nephropathy(Al-Azzawie, 2006). Increased oxidative stress is due to excessive reactive oxygen species and inadequate antioxidant defenses (Murugan, 2006). Thus, it is necessary to reduce the oxidative stress in diabetics to reduce the severity of the disease.

One group of drugs introduced in the management of Type 2 diabetes is represented by the inhibitors of glucosidase. The enzymes summarized as glucosidases are responsible for the breakdown of oligo- and/or disaccharides to monosaccharides. The inhibition of these enzymes leads to a decrease of blood glucose level, because the monosaccharides are the forms of carbohydrates which are absorbed through the mucosal border in the small intestine. The discovery and subsequent application of insulin to the treatment of diabetes not only improved control of glucose levels but also had a profound effect on protein metabolism. It appears that insulin exerts its overall anticatabolic effect in insulin-dependent diabetes mainly through the inhibition of muscle protein breakdown. Acute hyperglyceamia can be treated by direct administration of insulin in most cases. Severe hyperglyceamia can be treated by oral hypoglycemic therapy and life style modification (Ron, *et al.,* 2009).

Commonly used oral antiglycemic drugs in the treatment of diabetes include the older sulfonylurea, which act by stimulating production of insulin, and the newer ones that act by increasing sensitivity of cells to insulin and prevents additional release of glucose by the liver and intestines. Chlorpropamide (1-{p-chlorophenyl-sulfonyl-3-propylurea, MW; 276.74) commercially sold as Diabenese is a sulfonamide derivative that occurs as a white crystalline solid insoluble in water but soluble in alcohol. It is rapidly absorbed and metabolized and

excreted unchanged with a half life of 36 hours (Ferner and Chaplin, 1987). Its metabolism contributes to its high antidiabetic activity and minimal side effects compared to other antidiabetic drugs. However its pronounced accumulation due to its long elimination period could be a serious problem. The sulfonylurea moiety is responsible for itsdistribution and binding to beta-cell surface and insulin producing actions (Melander *et al.,* 1989).

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Equipment

These include Centrifuge Machine(Techmel Techmel, USA 800D), Spectrophotometer (Newlife, England 752s), Test tubes, Syringe, test tube racks, Hand gloves, Sample Tube, Weighing Balance (Gallenhamp, EnglandEL00000096), Freezer (Thermocool-Superdeluxe), Micropipettes (Switzerland, SOCOREX) Glucometer (Accu-Chek, Roche; (800) 858.8072).

3.1.2 Chemicaland Reagents

The chemical and reagents used for this study includes the following;

Alloxan Monohydrate (Explicit Chemicals, PGT India)

Glucose (Gluc-pap) Kit-Randox Laboratories Ltd United Kingdom.

Triglycerides (Trigs) Kit-Randox Laboratories Ltd United Kingdom.

Total protein (TP) Kit-Randox Laboratories Ltd United Kingdom.

Cholesterol (Chol) Kit-Randox laboratories Ltd United Kingdom.

HDL-Cholesterol (HDL) Kit-Randox Laboratories Ltd United Kingdom.

LDL-Cholesterol (HDL) Kit-Randox Laboratories Ltd United Kingdom.

LDH Kit-Agappe diagnostics Switzerland GmbH

Creatine Kinase Kit-Agappe Diagnostics Switzerland GmbH

Chlorpropamide (Diabenese) Neimeth International Pharmacy-Oregon, Lagos, Nigeria.

Cholesterol powder BDH Chemicals LTD. England

Others include Chemical reagents (such as normal saline, biuret reagent) and distilled water e.t.c.

3.2 Sample Collection and Preparation of Extract

Fresh matured Tamarind fruits were obtained from Rimi Market in Kano city, Kano State, and was kept away from direct sunlinght and allowed to dry. The dried fruits were peeled and the pulps were separated from the seed. The aqueous fruit pulp extract was prepared by soaking 100g of the pulp in onelitre of distilled water and mixed thoroughly (0.1g/mg ; 100mg/ml).After mixing, the extract was refrigerated without evaporation.The volume (cm^3)of the pulp aqueous extract given to each rat was determined by its weight and required dose as follows:

$$\text{Volume administered } (cm^3) = \frac{\text{weight of rat (kg) X required dose (mg/kg)}}{\text{Concentration of the extract } (mg/cm^3)}$$

3.3 Experimental Animals and Induction of Diabetes

Albino rats were obtained from the Animal House of the Biological Sciences Department, Bayero University, Kano. The rats were maintained under standard laboratory conditions and were allowed free access to both food and water throughout the period of the experiment.

Alloxansolution was prepared by dissolving alloxan monohydrate (0.9g) in distilled water ($6cm^3$) and diabetes was induced by single intraperitoneal injection of alloxan monohydrate (150mg/kg). The volume of the solution containing 150mg/kg given to each rat was determined by its weight according to the following relation:

$$\text{Volume administered } (cm^3) = \frac{\text{weight of rat (kg)} \times 150(mg/kg)}{\text{Concentration of the solution } (150mg/cm^3)}.$$

The group of rats to be induced was divided into six groups; the rats in Groups II, III, IV, V, and VI were intraperitoneally administered with alloxan solution respectively as determined by their body weight.

3.4 Preparation of Cholesterol-Rich Diet

The cholesterol-rich diet was formulated according to model described by Vesselinvitch *et al.* (1980) which is as follows:

Pure cholesterol 2% and 20% palm-oil were thoroughly mixed with the grower mash feeds.

3.5 Experimental Design

A total of 64 rats were used in this study. Forty eight of the rats were used for testing the antidiabetic effect and the remaining 16 rats were used for testing the anti-hypercholesterolemic effect of aqueous extract of *Tamarindus indica.*

Forty eight rats were divided into six (6) (I, II, III, IV, V, VI)groups of eight rats each; group Iwas used as normal control, groups II - VI wasinduced with diabetes by intraperitoneal administration of alloxan monohydrate (150mg/kg) and was used as indicated below.

Group 1: Normal control rats. (Non-diabetic rats and was not administered with aqueous extract (0.0mg/kg) *Tamarindus indica* fruit pulp.

Group 2: Diabetic control rats. (Diabetic rats not administered with aqueous extract (0.0mg/kg) *Tamarindus indica*fruit pulp.

Group 3: Diabetic rats orally administered with aqueous extract (200 mg/kg) of *Tamarindus indica* fruit pulp.

Group 4: Diabetic rats orally administered with aquoues extract (400 mg/kg) of *Tamarindus indica* fruit plup.

Group 5: Diabetic rats orally administered with aqueous extract (600 mg/kg) of *Tamarindus indica* fruit pulp.

Group 6: Diabetic rats orally administered with chlorpropamide (84 mg/kg).

Blood glucose level of the rats was determined before and after the induction of alloxan diabetes by using glucometer. Rats with blood glucose level above 180mg/dl after induction were considered diabetic and were selected for this study. After the 7^{th} and 14^{th} dose of treatment with 200, 400, and 600mg/kg of aqueous extract of *Tamarindus indica* fruit pulp and 84mg/kg of chlorpropamide, four of the rats were sacrificed from each group. Blood samples werecollected for estimation of serum glucose; total lipids (i.e. total cholesterol, triglycerides, LDL, HDL) and total protein.

For testing anti-hypercholesterolemic effect of aqueous extract of *Tamarindus indica* fruit pulp, 16 were divided into four groups of four rats each as follows;

Group 1: Normal control (non-hypercholesterolemic rats and was not administered with aqueous extract (0.0mg/kg) *Tamarindus indica* fruit pulp.

Group 2: Hypercholesterolemic control (non-hypercholesterolemic rats not administered with aqueous extract of (0.0mg/kg) *Tamarindus indica* fruit pulp.

Group 3: Hypercholesterolemic rats orally administered with 200mg/kg of aqueous extract of T*amarindus indica* fruit pulp.

Group 4: Hypercholesterolemic rats orally administered with 400mg/kg of aqueous extract of T*amarindus indica* fruit pulp.

After the five weeks of treatment with *Tamarindus indica* fruit pulp, the rats were sacrificed and blood samples was collected for the estimation of serum total lipids (i.e. total cholesterol, triglycerides, LDL, HDL) and cardiac enzymes (serum LDH and CK).

3.6 Methods

3.6.1 Determination of Serum Glucose

This was carried out using the method of Barham and Trinder (1972).

Principle:

Glucose is determined after enzymatic oxidation in the presence of glucose oxidase (GOD). The hydrogen peroxide formed reacts under catalysis of peroxidase with phenol and 4-aminophenazone to form a red-violet quinonamine dye as indicator. The intensity of the red violet colour is proportional to the amount of glucose.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{Phenol} \xrightarrow{\text{POD}} \text{Quinonamine} + 4H_2O$$

Procedure for serum glucose (GOD-PAP assay without deproteinization) measurement against reagent blank.

Three test tubes labeled Reagent Blank, Standard and Test were set. To the Reagent Blank, enzyme preparation (1.0cm^3) was added. To the Standard, enzyme preparation (1.0cm^3) and standard glucose solution (0.01cm^3) were added. To the test, enzyme preparation (1.0cm^3), and serum (0.01cm^3) were added. The test tubes were mixed and incubated at 37^{0}C for 5 minutes. The absorbance of the Standard and that of the Test was read against the Reagent Blank within 60 minutes at 520nm, in a colorimeter.

Calculation: Glucose concentration (mmol/L) = $\dfrac{\text{Absorbance of the Test x 5.56}}{\text{Absorbance of Standard}}$

Where 5.56 is the concentration of glucose standard

3.6.2 Determination of Serum Total Protein

This was determined by Biuret method (Tietz, 1995).

Principle: Cupric ions, in alkaline medium, interact with protein peptide bonds resulting in the formation of a coloured complex.

Procedure: Measurement against sample blank. Four test tubes labeled Reagent Blank, Standard, Sample and Sample blank were set. To the Reagent Blank, distilled water (0.02cm^3)

and Biuret reagent (1.0cm^3) were added. To the Standard, standard solution (0.02cm^3) and biuret reagent (1.0cm^3) were added. To the Sample, serum (0.02cm^3) and biuret reagent (1.0cm^3) were added. To the Sample Blank, serum (0.02cm^3) and blank reagent (1.0cm^3) were added. The tubes were mixed and incubated for 30 minutes at room temperature. The absorbance of standard and that of the Samples was read at 530nm.

Calculation:

$$\text{Total protein Concentration (g/dl)} = \frac{\text{absorbance of the Sample} \times \text{Standard concentration}}{\text{absorbance of Standard}}$$

3.6.3 Determination of Serum Level of HDL and LDL-Cholesterol

This was carried out using the method of Friedwald *et al* (1972).

Principle: Low density lipoproteins (LDL and VLDL) and chylomicrons fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL (high density lipoprotein) fraction, which remains in the supernatant, was determined.

- ApoB containing lipoproteins + α-cyclodextrin + Mg^{+2} + dextran SO_4 soluble non-reactive $\longrightarrow$ complexes with apoB-containing lipoproteins.
- HDL-cholesteryl esters $\xrightarrow{\text{PEG-Cholesteryl Esterase}}$ HDL-unesterified cholesterol + fatty acid.
- Unesterified chol. + O_2 $\xrightarrow{\text{PEG-cholesteryl Oxidase}}$ Cholestenone + H_2O_2

Procedure: Measurement against reagent blank. Three test tubes labeled Reagent Blank, Standard and Sample were set. To the Reagent Blank, distilled H_2O (0.1cm^3) and cholesterol

reagent ($1cm^3$) were added. To the Standard, supernatant ($0.1cm^3$) and cholesterol reagent ($1cm^3$) were added. To the Sample, sample supernatant ($0.01cm^3$) and cholesterol reagent ($1cm^3$) were added. The test tubes were mixed and incubated for ten minutes at room temperature. The absorbance of Standard and that of the Samples was read against the Reagent Blank at 500nm.

Calculation:

$$\text{Concentration of HDLCholesterol inthe supernatant(mmol/L)} = \frac{\text{Absorbance of the Sample X Concentration of Standard}}{\text{Absorbance of Standard}}$$

LDL Cholesterol (mmol/L) = Total cholesterol – HDL Cholesterol + 3/2

3.6.4Determination of Serum Triglycerides

This was determined by the method of Trinder (1969).

Principle: Triglycerides are measured enzymatically in serum or plasma using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol. Glycerol is then oxidized using glycerol oxidase, and H_2O_2, one of the reaction products, is measured as described above for cholesterol. Absorbance was measured at 500nm. The reaction sequence was as follows:

$$\text{Triglycerides} + 3H_2O \xrightarrow{\text{Lipase}} \text{Glycerol + fatty acids}$$

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

$$\text{Glycerol-3-phosphate} \xrightarrow{\text{Glycerophcosphate oxidase}} \text{Dihydroxyacetone} + O_2 + H_2O_2$$

H_2O_2 + 4-aminophenazone $\xrightarrow{\text{Peroxidase}}$ 4-(p-benzoquinone-monoimino)-+ 4-chlorophenol -phenazone + $2H_2O$ + HCl.

3.6.5 Determination of Serum Total Cholesterol

This was carried out using the method of Trinder (1969).

Principle: The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.

Cholesterol ester + H_2O $\xrightarrow{\text{Cholesterol esterase}}$ Cholesterol + Fatty acids

Cholesterol + O_2 $\xrightarrow{\text{Cholesterol oxidase}}$ cholestene-3-one + H_2O_2.

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

Procedure:

Three test tubes labeled Reagent Blank, Standard and sample were set. To the Reagent Blank, distilled water ($0.01cm^3$) and cholesterol reagent ($1cm^3$) were added. To the Standard, standard solution ($0.01cm^3$) and cholesterol reagent ($1cm^3$) were added. To the Sample, serum ($0.01cm^3$) and cholesterol reagent ($1.00cm^3$) were added. The test tubes were mixed and incubated for 10minutes at room temperature. The absorbance of the Standard and that of the Sample were read against the reagent blank at 500nm.

3.6.6 Determination of Serum Creatine Kinase Activity: The serum creatine kinase activity was determined using CKNAC_activiated colorimetric method (Gerhardt and Wulff, 1983).

Principle: Creatine Kinase (CK) catalyzes the reversible phosphoryulation of creatine by adenosine triphhosphate (ATP). The reverse reaction was used, in which ATP produced was coupled with hexokinase and glucose-6-phosphate dehydrogenase reactions to produce NADPH. N-acetyl-L-cysteine (NAC) was included to activate Creatine Kinase (CK), EDTA to bind Ca^{2+} and to increase the stability of the reaction mixtures, and diadenosine pentaphosphate (Ap_5A) together with adenosine-5'-monophosphate (AMP) were added to inhibit myokinase activity interference. The reaction was initiated by adding phosphocreatine and the absorbance was recorded continuously, after allowing for 2 minutes lag phase.

A narrow-band spectrophotometer equipped with a device to maintain the temperature of the cuvette compartment at 37^oC was used and wavelength was set at 340nm.

$$\text{Creatine} + \text{ATP} \underset{\text{pH 6.7}}{\overset{\text{pH 9.0}}{\underset{\text{Creatine Kinase}}{\rightleftharpoons}}} \text{Creatine Phosphate} + \text{ADP}$$

$$\text{Creatine Phosphate} + \text{ADP} \xrightarrow{\text{Creatine Kinase}} \text{Creatine} + \text{ATP}$$

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{Glucose-6-Phosphate} + \text{ADP}$$

$$\text{Glucose-6-phosphate} + NADP^+ \xrightarrow{\text{Glucose-6-phosphate dehydrogenase}} \text{glucose-6-phosphate} + NADPH + H^+$$

Procedure: To each of the test tubes labeled Blank and Test, $1.0cm^3$ of Creatine Kinase Working Reagent was introduced. Serum ($0.04cm^3$) was added to the test tubes labeled Test while $0.01cm^3$ distilled water to the Blank. The content of each test tube was thoroughly mixed and incubated at 37^0C for 100 seconds, after which the absorbance of the Test was read against Blank at 340nm wavelength and timer was started simultaneously and the absorbance was read again after 1, 2, and 3 minutes.

Calculation: The activity of Creatine Kinase (CK) in each test was calculated by using the formula given below,

Creatine Kinase Activity (U/L) = 4127 X Δ A

Δ A Means change in absorbance at 340nm per minute.

4127 was the factor given in diagnostics kit.

3.6.7 Determination of Serum Lactate Dehydrogenase Activity:

The serum lactate dehydrogenase activity was determined using colorimetric method (McComb, 1983).

Principle: Lactate Dehydrogenase (LDH) is a hydrogen transfer enzyme that catalyzes the oxidation of L-Lactate to pyruvate with the mediation of NAD^+ as hydrogen acceptor, which is reversible and the equilibrium strongly favors the reverse reaction.

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{Lactate Dehyrogenase}} \text{L-Lactate} + NAD^+$$

The reaction was initiated by addition of the coenzyme and the absorbance was read continuously, after allowing for 30 seconds lag phase. A narrow-band pass spectrophotometer equipped with a device to maintain the temperature of cuvette compartment at 37^0C was used and wavelength was set at 340nm.

Procedure: To each of the test tubes labeled Blank and Test, 1.0 cm^3 LDH Working Reagent was introduced. Serum (0.01cm^3) was added to the Test, while 1 cm^3 distilled water to the Blank. The content of the test tube was thoroughly mixed and incubated at 37^0C for 30 seconds, after which the absorbance of the Test was read against the reagent Blank at 340nm wavelength

and timer was started simultaneously and the absorbance was read again after 1, 2 and 3 minutes.

Calculation: The activity of Lactate Dehydrogenase (LDH) in each test samples was calculated by using the formula given below,

Lactate Dehydrogenase Activity (U/L) = 16030 X Δ A

Δ A mean change in absorbance at 340nm per minute.

Where 16030 was the factor given in diagnostic kit.

3.6.8 Statistical Analysis

Results were expressed as Mean±Standard deviation and analysed by ANOVA followed by Dunnett test $p<0.05$ considered as statistically significant.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Anti-Diabetic Effect

The effect of *Tamrindus indica* aqueous fruit pulp extract on blood glucose and protein on alloxan induced diabetic rats after 7 days is shown on Table 1. There was significantincrease in the serum glucose level ($p<0.05$) in the diabetic control rats compared to the normal control rats. No significant difference was observed in the serum glucose level of diabetic control rats and diabetic rats administered with AFPETI after 7 days. However there was a significant decrease in the serum glucose level of diabetic control rats administered with chlorpropamide when compared to diabetic control rats after 7 days. On the other hand, significant decrease in serum total protein ($P<0.05$) was observed in diabetic rats compared to normal control rats. No significant difference was observed in the AFPETI treated groups (200, 400 and 600mg/kg body weight) compared to diabetic control groups. There was significant elevation in the serum total protein in the group treated with chlorpropamide compared to the diabetic control group after 7 days.

Table 1: Effect of aqueous fruit pulp extract of *Tamarindus indica* on blood glucose concentration and serum total protein levels of alloxan induced diabetic rats after 7 days.

Groups	Serum Glucose (mmol/l)	Serum Protein (g/dl)
Normal Control	4.92±0.61*	7.63±0.20*
Diabetic Control	12.42±1.14*	5.00±0.01*
Diabetic Rats + 200mg/kg *AFPETI*	12.11±0.26	5.07±0.09
Diabetic Rats + 400mg/kg *AFPETI*	11.82±0.24	5.15±0.06
Diabetic Rats + 600mg/kg *AFPETI*	11.38±0.30	5.28±0.23
Diabetic Rats + 84mg/kg Chlorpropamide	9.97±0.05[a]	6.25±0.23[a]

Results are mean ± Standard Deviation. Values with the same superscripts* in a column are significantly different with respect to each other (P<0.05). Values with different alphabetical superscript [a] in a column are significantly different with respect to the diabetic control (P<0.05).
Abbreviations: AFPETI, Aqueous Fruit Pulp Extract of *Tamarindus indica*

The effect of aqueous fruit pulp extract of *Tamrindus indica*onserum glucose and protein of alloxan induced diabetic rats after 14 days is shown in Table 2. A significant increase was observed in serum glucose level of diabetic control rats compared to normal control rats. No significant difference between serum glucose in diabetic control rats and diabetic rats administered with 200mg/kg of AFPETI, 400mg/kg, 600mg/kg of AFPETI. Chlorpropamide (84mg/kg) caused a significant reduction in glucose concentration of diabetic rats compared to diabetic control rats after 14 days. Serum total protein was significantly decreasedin diabetic control rats compared to normal control. There was no significant difference in serum total protein concentration of diabetic rats treated with 200mg/kg body weight of AFPETI. Serum total protein concentration was significantly increased in diabetic rats that were treated with 400mg/kg, 600mg/kg AFPETI and chlorpropamide compared to diabetic control rats after the 14^{th} day.

Table 2: Effect of *Tamarindus indica* aqueous pulp extract on blood glucose concentration and serum total protein levels of alloxan induced diabetic rats after 14 days.

Groups	**Serum Glucose ((mmol/l)**	**Serum Protein (g/dl)**
Normal Control	4.77±0.42*	8.07±0.26*
Diabetic Control	12.11±0.55*	5.05±0.13*
Diabetic Rats + 200mg/kg *AFPETI*	11.69±0.22	5.29±0.15
Diabetic Rats + 400mg/kg *AFPETI*	11.18±0.27[a]	5.56±0.35[a]
Diabetic Rats + 600mg/kg *AFPETI*	10.89±0.08[b]	5.69±0.36[b]
Diabetic Rats + 84mg/kg Chlorpropamide	7.46±0.35[c]	7.00±0.03[c]

Results are mean ± SD; n= 4. Values with same superscripts in a column are significantly different with respect to each other ($P<0.05$). Values with the different alphabetical superscript [a, b, c] in a column are significantly different with respect to diabetic control ($P<0.05$).

Abbreviations: AFPETI, Aqueous Fruit Pulp Extract of *Tamarindus indica*

4.1.2 Anti-Hyperlipedemic Effect

The effect of *Tamarindus indica* aqueous fruit pulp extract on body weight of hypercholesterolemic rats after 5 weeks is shown in Table 3. There was no significant difference in the weight of all the different groups before the rats became hypercholesterolemic. However, the weight of hypercholesterolemic control rats was significantly increased compared to normal control. Also the weight of AFPETI treated group was significantly decreased compared to hypercholesterolemic control group

Table 3: Effect of *Tamarindus indica* aqueous pulp extract on body weight of hypercholesterolemic rats after 5 weeks.

Groups	Weight Before (g)	Weight After (g)	Daily Weight gain (g)
Normal Control	115±4.08	133.75±2.50*	0.54
Hypercholesterolemic Control	115±4.08	192.5±5.00*	2.21
Hypercholesterolemia + 200mg/kg of *AFPETI*	115±4.08	167.5±5.00[a]	1.50
Hypercholesterolemia + 400mg/kg of *AFPETI*	118.5±4.78	145±5.70[b]	0.77

Results are mean ± SD, n= 4. Values with the superscript *in a column are significantly different with respect to each other (P<0.05). Values with different alphabetical superscripts[a, b,]in a column are significantly different with respect to the hypercholesterolemic control (P<0.05).
Abbreviations: AFPETI, Aqueous Fruit Pulp Extract of *Tamarindus indica*

Table 4 presents the effect of *Tamarindus indica* aqueous fruit pulp extract (AFPETI) onlipid profile (HDL-Ch., LDL., TC and Triglycerides) of hypercholesterolemic rats after 5 weeks.The result showed a significant increase in hypercholesterolemic control group compared to normal controland a significant decrease in AFPETI treated groups (200mg/kg and 400mg/kg of body weight) compared to hypercholesterolemic control of serum TC ($p<0.05$).From Table 4, the result also showed no significant difference in serum HDL-Ch. Level of normal control rats andhypercholesterolemic control.A significant increase was observed in HDL-Ch., of hypercholesterolemic control rats and rats treated with 200mg/kg and 400mg/kg AFPETI ($p<0.05$). Serum LDL-Ch., was significantly increased in hypercholesterolemic control rats compared to normal control rats. There is also a significant decrease of serum LDL-Ch., in the groups treated with 200mg/kg and 400mg/kg AFPETI compared to hypercholesterolemic rats ($p<0.05$). As shown in table 4 there was significant increase in serum triglycerides in hypercholesterolemic control compared to normal control and a significant decrease ($p<0.05$)in AFPETI treated hypercholesterolemic rats compared to hypercholesterolemic control rats.

Table 4: Effect of *Tamarindus indica* aqueous pulp extract on lipid profile (HDL-Ch., LDL., TC and Triglycerides) of hypercholesterolemic rats after 5 weeks.

Groups	Total Ch. (mmol/L)	HDL-Ch. (mmol/L)	LDL-Ch. (mmol/L)	Triglycerides (mmol/L)
Normal Control	4.42±0.50*	1.08±0.06	1.99±0.54*	1.60±0.08*
Hypercholesterolemic Control	7.89±0.34*	1.18±0.06	5.35±0.30*	4.24±0.44*
HC + 200mg/kg of *AFPETI*	6.02±0.20[a]	1.49±0.04[a]	3.18±0.20[a]	3.07±0.11[a]
HC + 400mg/kg of *AFPETI*	4.77±0.19[b]	1.88±0.18[b]	1.54±0.08[b]	2.19±0.17[b]

Results are mean ± SD, n= 4. Values with the same superscripts *in a column are significantly different with respect to each other (P<0.05). Values with the different alphabetical superscript [a, b] in a column are significantly different with respect to the hypercholesterolemic control (P<0.05).

Abbreviations: AFPETI, aqueous fruit pulp extract of *Tamarindus indica,* HDL-Ch., high density lipoprotein-cholesterol.LDL-Ch., Low Density Lipoprotein Cholesterol. TC, Total Cholesterol.

Table 5 presents the effect of *Tamarindus indica* aqueous fruit pulp extract on cardiac enzymes of hypercholesterolemic rats after 5 weeks. Serum LDH and CK activity was significantly high in hypercholesterolemic control group compared to normal control group ($p<0.05$). The result also showed that there is a significant decrease in LDH and CK activities in AFPETI treated group compared to hypercholesterolemic control group.

Table 5: Effect of aqueous fruit pulp extract of*Tamarindus indica*on Creatine Kinase and Lactate dehydrogenase of hypercholesterolemic rats after 5 weeks.

Groups	Lactate Dehydrogenase (U/L)	Creatine Kinase (U/L)
Normal Control	352.66 ±29.26*	149.34±44.28*
Hypercholesterolemic Control	1318.46±72.13*	343.72±59.51*
HC + 200mg/kg of *AFPETI*	989.85±83.16[a]	262.49±14.48[a]
HC + 400mg/kg of*AFPETI*	557.04±40.07[b]	221.21±18.18[b]

Results are mean ± SD, n= 4. Values with the same superscripts* in a column are significantly different with respect to each other ($P<0.05$). Values with different alphabetical superscript[aa, bb] in a column are significantly different with respect to the hypercholesterolemic control ($P<0.05$).

Abbreviations: AFPETI, aqueous fruit pulp extract of *Tamarindus indica,* CK: Creatine Kinase, LDH: Lactate Dehydrogenase.

4.2 DISCUSSION

4.2.1 Anti-Diabetic Effect

Diabetes mellitus is a chronic disease characterized by hyperglycemia and by disturbances of carbohydrate, fat, and protein metabolism (Staten *et al.,* 1986). Intraperitoneal administration of alloxan to rats in this study lead to significant increase in blood glucose concentration, a result that is consistent with several studies in rats (Milagro and Martinez, 2000). The alloxan-induced diabetic rats in this experiment were all clearly hyperglycemic; an indication of a successful induction of diabetes. The diabetic control group exhibited an increase in Fasting Blood Glucose (FBG) during the experiment. The alloxan induced diabetic rats elicited significant rise in blood glucose to 12.42 mmol/L ($P<0.05$) compared to normal control (4.92). Alloxan, a β-cytotoxin, induces diabetes mellitus by damaging the insulin secreting β-cells of the pancreas, resulting in decreased endogenous insulin release. Intraperitoneal administration of alloxan (150 mg/kg body weight) effectively induced diabetes mellitus in normal rats as polydypsia, polyuria and polyphagia compared with normal rats was observed during the experiment. The major clinical significance of diabetes is often indicated by the presence of symptoms such as polydipsia, polyuria and unexplained weight loss and confirmed by measurement of abnormal hyperglyceamia (WHO, 1999).

The pancreatic β cells were destroyed using alloxan, a toxic glucose analogue that accumulate in pancreatic beta cells via GLUT 2 glucose transporter. In the presence of thiols, especially glutathione (GSH), alloxan generates reactive oxygen species (ROS) in cyclic redox reactions.

Diabetogenic effect of alloxan is due to excess production of reactive oxygen species (ROS) leading to cytotoxicity in the pancreatic β cells which reduces the synthesis and release of insulin (Anne *et al.,* 2004).

The effect of oral administration of varying doses of aqueous fruit pulp extract of *Tamarindus indica* fruit on alloxan induced diabetic rats is shown in Table 1. A dose-dependent hypoglycemic effect of the fruit extract was observed; however, the results were significant only after the second week at higher doses of 400 and 600 mg extract/kg body weight. Chlorpropamide at a dose of 84mg/kg body weight demonstrated greater potency on decreasing glucose levels in alloxan induced diabetic rats. It was observed that after 7 days aqueous fruit pulp extract of *Tamarindus indica* slightly reduced serum glucose level in the rats but the reduction was not significant. It was evident from the results that aqueous fruit pulp extract of *Tamarindus indica* (AFPETI) reduced the fasting blood glucose level in alloxan-induced diabetic rats. The aqueous extracts-treated diabetic groups at 200, 400 and 600 mg/kgrecorded a dose-depended drop in Fasting Blood Glucose after 14 days. Although the glucose lowering activity was not comparable to the standard drug chlorpropamide even at the highest dose of extracts of fruit tested, nevertheless both extracts at the higher doses of 400 mg extract/kg body weight and 600mg extract/kg body weight significantly lowered blood glucose levels in diabetic rats when compared to diabetic controls after 14days. Fruits of the plant are known to contain limonene and has been shown to possess hypoglycemic activity (Conforti, 2010). Additionally, it possesses anti-oxidant properties, which could be beneficial during diabetes. The antihyperglycaemic effect of AFPETI could be linked to more than one mechanism. The possible mechanism includes the stimulation of β-cells and subsequent release of insulin and activation of the insulin receptors. The plant's antihyperglyacemic action may be by potentiation of pancreatic secretion of insulin.

In light of the results from Table 1, it indicates that the aqueous extract of *Tamarindus indica* exhibited significant anti-hyperglyceamic activity in alloxan-induced diabetic rats after 14days. The anti-hyperglycemic effects of *Tamarindus indica* are possibly linked to their antioxidant properties, which could counteract the toxic and pro-oxidant effects of alloxan. Flavonoids, sterols/triterpenoids, alkaloids and phenolics are known to be bioactive anti-diabetic principles (Atta-Ur-Rahman and Zaman, 1989). Flavonoids are known to regenerate the damaged ß cells in the alloxan diabetic rats (Chakavarthy *et al.,* 1980). Phenolics were also found to be effective anti-hyperglycemic agents (Kamalakkannan and Prince, 2006).

A decrease in serum total protein was observed in diabetic control group and this was reported to be due to net increase in protein breakdown rather than a decline in protein synthesis (Moller and Nair, 2008). After oral administration of the *Tamarindus indica* pulp extracts, the alteration in protein metabolism was slightly restored. Poorly controlled diabetes is associated with altered body protein metabolism (Gougeon *et al.,* 1997). Insulin-mediated net protein anabolism occurs largely in skeletal muscle; therefore, the catabolic state resulting from insulin deficiency or resistance may lead to muscle wasting (Anderson and Geil, 1994). Similarly, decreased synthesis of hepatic plasma proteins (eg, albumin) has been shown with diabetes (Sinagra *et al.,* 1997). Thus, the goal of diabetes management is to improve glycemic control and to provide adequate nutrient intake to help reduce muscle wasting, morbidity, and the risk of mortality (ADA, 1995).

Glucose itself and hyperglycemia increase protein glycosylation which are important source of free radicals. Elevated glucose causes significant non-enzymatic glycosylation of proteins in diabetes (Wild*et al.,* 2004). In diabetes, oxidative stress has been found to be mainly due to an increased production of oxygen free radicals and a sharp reduction of antioxidant defenses (Al-

Azzawie, 2006). Cell utilizes oxygen in the making of energy, but that process produces free radicals, toxic products that damage DNA and protein. Under physiological condition, a wide spread antioxidant defense system protects the body against the adverse effects of free radical production (Murugan, 2006).

The result from Table 1 and 2 shows that the diabetic control rats had low serum total protein levels compared with the untreated control rats, while the diabetic rats showed significant ($P<0.05$) increase in total protein level after the treatment. After 7 days of AFPETI administration to diabetic rats, there was an increase in the serum total protein level although the increase was not significant. After 14 days of administration of AFPETI to diabetic rats, there was a significant increase in the serum total protein level compared to the diabetic rats. Although the administration of AFPETI caused significant increase of serum total protein in diabetic rats, Chlorpropamide caused a more significant increase. In diabetes mellitus, a variety of proteins are subjected to non-enzymatic glycation and this is thought to contribute to the long-term complications of the disease (Vlassara *et al.,* 1981). The level of serum total proteins was found to be decreased in this study. This decrease in diabetic rats may be ascribed to (i) decreased amino acid uptake; (ii) greatly decreased concentration of variety of essential amino acids; (iii) increased conversion rate of glucogenic amino acids to carbon dioxide and water; and (iv) reduction in protein synthesis secondary to a decreased amount and availability of mRNA(Ahmed, 2005). Oral administration of aqueous fruit pulp extract of *Tamarindus indica* improved total protein concentration in the serum after the 14days at a dose of 600mg/kg body weight.

Insulin deficiency leads to various metabolic aberrations in the animals, namely increase blood glucose, decreased protein content and increased levels of cholesterol and triglyceride (Ghosh, and Suryawanshi, 2001).

4.2.2 Anti-Hyperlipidemic Effect

Body weight gain in hypercholesterolemic control (hyperlipidemic) rats (Table 3) was significantly (P>0.05) higher than weight gain in normal control rats fed with normal diet. The treatment of the hypercholesterlemic groups with different concentrations of the AFPETI showed significant (P>0.05) reduced weight gain after 5th week in this studywhen compared to the test control group. In this study, the treatment of hyperlipidemic rats with AFPETI reduced weight gain which indicates that AFPETI can be used to control weightgain by obese people hence confirming earlier report of the use of *Tamarindus indica* fruit pulp extract to regulate body weight in hypercholesterolemia (Martinello *et al.,* 2006).

Cholesterol may be synthesised, the majority in the liver and about 25 % in the intestine, may be absorbed from the diet or reabsorbed from bile. The lipoproteins are the major transporters of cholesterol and fatty acids. Total fat intakes are related to obesity, which affects many of the major risk factors for atherosclerosis. Dietary cholesterol raises total cholesterol and LDL-C and the intake of cholesterol has been positively related to the risk of coronary heart disease after adjusting for other risk factors such as age, blood pressure, serum cholesterol and cigarette smoking. There is a great deal of experimental, epidemiological, and clinical evidence suggesting that disorders of lipid metabolism play an important role in the pathogenesis of atherosclerosis (Stober, 1983).

In this research, experimental animals were fed with cholesterol rich diet which led to a significant rise in the blood cholesterol of the animals. From Table 4, it shows that the serum

total cholesterol of the normal control rats increased steadily from 4.42mmol/L to 7.89Mmol/L in hypercholesterolemic rats and LDL-C of the normal rats also increased from 1.86Mmol/L to 5.53Mmol/L in hypercholesterolemic rats. Administration of AFPETI (200 and 400mg/kg body weight) led to a significant decrease in serum total cholesterol of the rats compared to hypercholesterolemic control rats. There was also a significant decrease in low density lipoproteins (LDL-C) level of rats administered with 200 and 400mg/kg body weight of AFPETI. Epidemiologic surveys have observed that elevated levels of total cholesterol and low-density lipoprotein cholesterol (LDL-C) are associated with increased risk of coronary heart disease (CHD) (LaRosa, 2003), and therapeutic strategies that lead to a statistically significant reduction in LDL-C lower CHD event rates (Baigent *et al.,* 2005). Subsequently, studies have elucidated a role of LDLs in cardiovascular disease development, particularly the role of oxidized LDL **(**ox-LDL; LDL particles that contain oxidized fatty acids) in infiltrating and damaging arterial walls, and leading to development of lesions and arterial plaques. Lowering serum cholesterol to an "optimal" range (total cholesterol 160 – 180; LDL-C 50-99) is one of the most frequently used strategies for reducing heart disease risk in persons without CHD.

The magnitude of CHD risk reduction as a consequence of LDL-C lowering often ranges between 25% and 35% (LaRosa *et al.,* 1999). One potential impediment limiting further reduction in CHD events despite low on-treatment LDL-C is residual elevation in serum triglyceride (TG) levels (Miller, 2000). Elevated TG has predicted CHD events in univariate analysis, only to weaken after adjustment for other covariates, including plasma glucose and high-density lipoprotein cholesterol (HDL-C), to which it is strongly and inversely correlated (Criqui *et al.,* 1993). Yet, even after adjustment for HDL-C, detailed evaluation of population-

based prospective studies has disclosed an independent effect of TG on CHD events (Sarwar *et al.,* 2007). Coupled with the knowledge that combined hyperlipidemia (i.e., elevated LDL-C and TG) promotes CHD to a significantly greater extent than either high LDL-C or TG alone (Maninnen*et al.,* 1992),

High-density cholesterol (HDL) levels are reported to correlate closely and inversely with the risk of CHD (Stensvold *et al.,* 1992). In this study, the HDL-C level rose from 1.08mmol/L in the normal control rats to 1.78mmol/L in the hypercholesterolemic rats. However hypercholesterolemic rats that received various doses of the extract have significantly reduced triglycerides, total cholesterol, and LDL cholesterol and a significantly increased HDL cholesterol. The crucial risk factor for cardiovascular diseases (CVD) includes a low level of HDL-cholesterol. The association between a low level of HDL-cholesterol and an increased risk of CVD has been well established through epidemiology and clinical studies (Assmann and Gotto, 2004).Since low level HDL-cholesterol plays a direct role in the atherogenic process, therapeutic intervention to raise HDL-cholesterol together with other risk factor is widely encouraged. In this study, aqueous fruit pulp extract of *Tamarindus indica* led to significant elevation of serum HDL-cholesterol indicating its promising role against CVD.This role has been suggested to occur in various ways (Noffer *et al.,* 2002). It exerts part of its anti-atherogenic effect by counteracting LDL-cholesterol and recent studies also showed that it promotes the reverse cholesterol transport pathway, by inducing an efflux of excess accumulated cellular cholesterol and prevent the generation of an oxidatively modified LDL (Yokozawa *et al.,*2006).

In this research, aqueous fruit pulp extract of *Tamarindus indica* significantly lowered LDL level of hypercholesterolemic rats. Excess LDL can be deposited in the blood vessel walls and becomes a major component of atherosclerotic plaque lesions. Therefore, serum LDL-cholesterol is used for monitoring the treatment of patients with elevated blood cholesterol levels. The LDL particle is a cholesterol-rich, triglyceride-poor particle. Under normal circumstances, LDL catabolism depends on the particle uptake by the LDL receptor which is present in almost all the cells of the body (Goldstein and Brown, 2008). Thus, LDL is a vehicle to supply cholesterol all over the body in order to maintain cell viability and to provide cholesterol for the synthesis of the steroid hormones. Reduction in endogenous cholesterol synthesis up-regulates the LDL receptor and stimulates LDL clearance (Brown *et al.,* 1974). However it also stimulates cellular cholesterol absorption from the intestine (Tremblay *et al.,* 2011). The core of the particle includes esterified cholesterol and triglyceride together with the fatty acid tails of the phospholipid. As with most proteins in the circulation, the particle may act as acarrier for other insoluble particles such as free fatty acids, which may be loosely attached (Phillips *et al.,* 2005). Perhaps more importantly, lipoprotein lipase also attaches to the particle and this facilitates attachment of the particle to the endothelial surface. This hypolipemic effect may have its origins in elevation of serum HDL which transfers cholesterol from tissue to liver for metabolism. Also, TG-lowering effect of *Tamarindus indica* fruit pulp extract may contribute to inhibition of VLDL secretion by liver and increase VLDL clearance via lipoprotein lipase pathway.

Hypercholesterolemia is generally caused by an increase in LDL and HDL and does not produce a visible hyperlipidemia. Myocardial infarction (MI) and ischemic heart disease (IHD)

often result from complications of metabolic disorders that include hyperlipidemia and insulin resistance. In hypertriglyceridemic states, remnants accumulate, resulting in a proinflammatory and oxidative milieu that may enhance adhesion molecule expression, foam cell formation, and smooth muscle cell toxicity (Yu and Cooper, 2002). Indirectly, high levels of TG may also be associated with hypertriglyceridemic HDL particles, which are thought to be less efficient in reverse cholesterol transport (Skeggs and Morton, 2002), as well as an increased proportion of small, dense LDL particles which may be more susceptible to oxidative modification (Austin, *et al.,*1990). This study showed a significant decrease and increase in the serum TG and HDL-C levels respectively after treatment of the hypercholesterolemic rats with various doses of AFPETI. The HDL particles induce the removal of cholesterol from cells, including those in atherosclerotic plaques, and carry them to the liver, but the mechanisms by which HDL confer protection from atherosclerosis include more than just reverse cholesterol transport. HDL particles seem to have anti-inflammatory and antioxidant properties, inhibiting the oxidation of LDL cholesterol and the expression of cellular adhesion molecules and monocyte recruitment. The HDL may also reduce the risk of thrombosis by inhibiting platelet activation and aggregation. HDL plays a part in reverse cholesterol transport and also protects LDL from oxidation (Khera *et al.,* 2011). There is a strong inverse correlation between triglycerides and HDL which has been known for many years. Low HDL is known to be an independent and powerful predictor of atherosclerosis and the functions of HDL have been intensively investigated. HDL's major function may be reverse cholesterol transport but an important function relates to its ability to protect LDL from oxidation. Delivery of cholesterol to the atherosclerotic plaque occurs following binding of the LDL particle to the endothelial surface

via the lipase receptors. Thus, low HDL and, perhaps more importantly dysfunctional HDL, have a bearing on the atherogenicity of LDL (Kontush and Chapman, 2010).

Table 5 showed that the enzymes CK and LDH were significantly elevated ($p<0.05$) in the hypercholesterolemic rats compared to the control rats. This is so because the metabolism of hypercholesterolemia caused infarcted myocardium and this may be studied by assessing the level of marker enzymes (CK and LDH) in the serum. It is interesting to know that as myocardial diseases are rich sources of CKMB (isoenzyme of creatine kinase found predominantly in cardiac muscle), so are skeletal muscular diseases are good sources of creatine kinase isoenzyme (Hamm *et al.,* 1992). Therefore the significant reduction in CK and LDL enzymes at the dose of 200 and 400mg/kg body weight of aqueous fruit pulp extract of *Tamarindus indica* may be due to some physiological effects on muscular activity. This fact may be associated with the efficacy of aqueous fruit pulp extract of *Tamarindus indica* in the management of muscular pains, atherosclerosis and inflammation.

Tamarind contains many health benefiting essential volatile chemical compounds, minerals, vitamins and dietary fiber (El-siddig *et al.,* 1999; Ajayi *et al.,* 2006). Tamarind fruit pulp contains many volatile phytochemicals such as limonene, geraniol, safrole, cinnamic acid, methyl salicylate pyrazine and alkylthiazoles. Together these compounds account for the medicinal properties of tamarind. Tamarind is a good source of minerals like copper, potassium, calcium, iron, selenium, zinc and magnesium (Almeida *et al.,* 2009). It is also a rich source of many vital vitamins including thiamine, vitamin A, folic acid, riboflavin, niacin, carotene and vitamin-C that are essential anti-oxidants (El-siddig *et al.,* 1999,). On the basis of the results in this experiment it may be stated that aqueous fruit pulp extract of *Tamarindus*

indica has a beneficial effect in preventing diabetes and it complications as well as improving lipid metabolism in hypercholesterolemia.

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATION

5.1 SUMMARY

Diabetes can lead to complications including damage to large and small blood vessels, which can lead to heart attack, stroke and problems with the kidneys, eyes, teeth, feet and nerves. However, the risk of most diabetes-related complications can be reduced. Smoking, poor diet, alcohol and obesity can increase the risk of complications. Result from this study showed that *Tamarindus indica* has a significant effect in decreasing serum glucose level at higher dosage and for a prolonged period of time (14 days).*Tamarindus indica* is very rich in tartaric acid which gives a sour taste to food. It is a very powerful antioxidant which helps to protect the body from harmful free radicals generated by hyperglyceamia. From such information it may be stated primarily that the aqueous extract of fruit of *Tamarindus indica* may contain some phytochemicals that may stimulate the beta stem cell of islets of pancreas in alloxan-induced diabetic rat that may restore plasma level of insulin when consumed at higher doses and possibly for a prolonged period of time. This study shows that consumption of high cholesterol rich diet causes significant rise in serum cholesterol levels, making the study subjects to become hypercholesterolemic.*Tamarindus indica* pulp caused a significant decrease in serum TC, LDL-C, TG and a significant increase in serum HDL-C levels. Tamarind fruit pulp is a rich source of non-sticky polysaccharides or dietary fiber such as gums, hemicelluloses, mucilage, pectin and tannins. Dietary fiber in the fruit pulp of *Tamarindus indica* bind to bile salts (produced from cholesterol) and decrease their re-absorption in the colon; thereby help in the excretion of “bad” or LDL cholesterol levels from the body.

5.2 CONCLUSION

This research concludes that firstly, the aqueous fruit pulp extract of *Tamarindus indica* posseses some anti-diabetic properties, which may be due to the presence of some biologically active components. The anti-diabetic effect can only be achieved when *Tamarindus indica* fruit pulp is used for a long period of time and at a high dose of 600mg/kg body weight.The results in diabetic animals in this experiment showed a protective effect of aqueous fruit pulp extract of *Tamarindus indica* at a dose level of 600 mg/kg body weight for a period of at least two weeks. From these results, it may be stated that the aqueous fruit pulp extractof *Tamarindus indica* can be used in the management of diabetes mellitus and may likely prevent diabetes mellitus associated complications.Secondly, *Tamarindus indica* fruit pulp extract can be used to manage hypercholesterolemia based on the fact that it contains some volatile phytochemicals and essential chemical compounds, vitamins and dietary fiber.Thirdly, the fact that *Tamarindus indica* fruit pulp extract significantly lowered activities of CK and LDH at 400 mg/kg body weight scientifically suggest that the aqueous fruit pulp extract of *Tamarindus indica* may have the potential of cardiac protection. Finally, the effect of oral administration of *Tamarindus indica* fruit pulp extract is dose dependent and the effect is most significant ata dose of 600mg/kg body weight for at least two weeks.

5.3.1 RECOMMENDATIONS

At the end of this study, the following recommendations can be made: firstly, *Tamarindus indica* fruit pulp can be recommended for clinical trials such that it can be used as an alternate or additional therapy for the management of diabetes mellitus and hyperlipideamia. Secondly, further studies should be carried out to ascertain and to purify the bioactive compounds that are responsible for the anti-diabetic and hyperlipidemic activities of *Tamarindus indica* fruit pulp. Thirdly, studies should be carried out on other parts of *Tamarindus indica* such as the seed, leaves, stem bark and possibly roots to ascertain any anti-diabetic and anti-hyperlipidemic properties so that a combined therapy might be used in the treatment of diabetes in the future. Finaly, LD_{50} and the chronic toxicity of *Tamarindus indica* Pulp should be studied.

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APPENDICES

SERUM GLUCOSE LEVELS OF DIABETIC RATS AFTER 7 DAYS

NORMAL CONTROL	DIABETIC CONTROL	DIABETIC RATS + 200MG/KG *AFPETI*	DIABETIC RATS + 400MG/KG *AFPETI*	DIABETIC RATS + 600MG/KG *AFPETI*	DIABETIC RATS + 84MG/KG CHLORPROPAMIDE
5.36	11.86	11.80	12.10	11.65	9.96
4.98	14.08	11.99	11.80	11.32	9.94
5.32	12.22	12.36	11.52	11.58	10.05
4.04	11.53	12.30	11.89	10.98	9.94

SERUM PROTEIN LEVELS OF DIABETIC RATS AFTER 7 DAYS

NORMAL CONTROL	DIABETIC CONTROL	DIABETIC RATS + 200MG/KG *AFPETI*	DIABETIC RATS + 400MG/KG *AFPETI*	DIABETIC RATS + 600MG/KG *AFPETI*	DIABETIC RATS + 84MG/KG CHLORPROPAMIDE
7.82	5.02	5.0	5.19	5.10	6.00
7.73	5.00	5.2	5.08	5.22	6.12
7.35	5.00	5.1	5.22	5.20	6.50
7.64	4.98	5.0	5.12	5.63	6.40

SERUM PROTEIN LEVELS OF DIABETIC RATS AFTER 7 DAYS

NORMAL CONTROL	DIABETIC CONTROL	DIABETIC RATS + 200MG/KG *AFPETI*	DIABETIC RATS + 400MG/KG *AFPETI*	DIABETIC RATS + 600MG/KG *AFPETI*	DIABETIC RATS + 84MG/KG CHLORPROPAMIDE
7.82	5.02	5.0	5.19	5.10	6.00
7.73	5.00	5.2	5.08	5.22	6.12
7.35	5.00	5.1	5.22	5.20	6.50
7.64	4.98	5.0	5.12	5.63	6.40

SERUM GLUCOSE LEVELS OF DIABETIC RATS AFTER 14 DAYS

NORMAL CONTROL	DIABETIC CONTROL	DIABETIC RATS + 200MG/KG *AFPETI*	DIABETIC RATS + 400MG/KG *AFPETI*	DIABETIC RATS + + 600MG/KG *AFPETI*	DIABETIC RATS + 84MG/KG CHLORPROPAMIDE
4.97	11.72	11.75	10.89	10.89	7.32
5.03	12.51	11.98	11.38	11.00	7.24
4.97		11.45	11.00	10.90	7.30
4.14		11.60	11.45	10.80	8.00

SERUM PROTEIN LEVELS OF THE DIABETIC RATS AFTER 14 DAYS

NORMAL CONTROL	DIABETIC CONTROL	DIABETIC RATS + 200MG/KG *AFPETI*	DIABETIC RATS + 400MG/KG *AFPETI*	DIABETIC RATS + + 600MG/KG *AFPETI*	DIABETIC RATS + 84MG/KG CHLORPROPAMIDE
8.37	4.89	5.12	5.90	5.70	7.00
8.12	5.00	5.47	5.75	5.17	7.05
7.72	5.12	5.22	5.08	6.00	7.00
8.07	5.20	5.38	5.54	5.89	6.97

WEIGHTS OF HYPERCHOLESTEROLEMIC RATS BEFORE HYPERCHOLESTEROLEMIA WAS INDUCED

NORMAL CONTROL	HYPERCHOLESTEROLEMIC CONTROL	HC + 200MG/KG OF *AFPETI*	HC + 400MG/KG OF *AFPETI*
110	110	115	115
120	115	110	120
115	120	120	115
115	115	115	125

WEIGHTS OF HYPERCHOLESTEROLEMIC RATS AFTER HYPERCHOLESTEROLEMIA WAS INDUCED

NORMAL CONTROL	HYPERCHOLESTEROLEMIC CONTROL	HC + 200MG/KG OF *AFPETI*	HC + 400MG/KG OF *AFPETI*
130	200	170	150
135	190	160	140
135	190	170	140
135	190	170	150

SERUM TRIGLYCERIDES LEVELS OF HYPERCHOLESTEROLEMIC RATS AFTER WEEKS

NORMAL CONTROL	HYPERCHOLESTEROLEMIC CONTROL	HC + 200MG/KG OF *AFPETI*	HC + 400MG/KG OF *AFPETI*
1.747	4.715	3.226	2.130
1.558	3.807	3.123	2.461
1.601	4.530	2.960	2.075
1.626	3.943	3.008	2.130

SERUM HDL-CHOLESTEROL LEVELS OF HYPERCHOLESTEROLEMIC RATS AFTER 5 WEEKS

NORMAL CONTROL	HYPERCHOLESTEROLEMIC CONTROL	HC + 200MG/KG OF *AFPETI*	HC + 400MG/KG OF *AFPETI*
1.15	1.20	1.49	1.85
1.11	1.13	1.55	1.70
1.00	1.12	1.44	2.14
1.05	1.25	1.47	1.82

SERUM LDL-CHOLESTEROL LEVELS OFHYPERCHOLESTEROLEMIC RATS AFTER 5 WEEKS

NORMAL CONTROL	HYPERCHOLESTEROLEMIC CONTROL	HC + 200MG/KG OF *AFPETI*	HC + 400MG/KG OF *AFPETI*
1.71	5.790	2.90	1.45
1.91	5.200	3.32	1.65
2.77	5.096	3.33	1.56
1.55	5.326	3.15	1.48

SERUM TOTAL CHOLESTEROL LEVELS OF HYPERCHOLESTEROLEMIC RATS AFTER WEEKS

NORMAL CONTROL	HYPERCHOLESTEROLEMIC CONTROL	HC + 200MG/KG OF *AFPETI*	HC + 400MG/KG OF *AFPETI*
4.22	8.35	5.75	4.66
4.38	7.69	6.23	4.71
5.13	7.58	6.13	5.06
3.96	7.94	5.98	4.66

SERUM LDH LEVELS OF HYPERCHOLESTEROLEMIC RATS AFTER 5 WEEKS

NORMAL CONTROL	HYPERCHOLESTEROLEMIC CONTROL	HC + 200MG/KG OF *AFPETI*	HC + 400MG/KG OF *AFPETI*
384.72	1378.58	993.86	609.14
336.63	1282.40	1106.07	561.05
320.60	1378.58	929.74	545.02
368.69	1234.31	929.74	512.96

SERUM CK LEVELS OF HYPERCHOLESTEROLEMIC RATS AFTER 5 WEEKS

NORMAL CONTROL	HYPERCHOLESTEROLEMIC CONTROL	HC + 200MG/KG OF *AFPETI*	HC + 400MG/KG OF *AFPETI*
192.36	379.43	283.73	230.83
182.74	408.77	259.69	229.23
111.68	298.16	253.27	193.96
110.61	288.54	253.27	230.83