

THE EFFECT OF N-HEXANE EXTRACT OF KOLA *NITIDA* BARK ON  
LIVER FUNCTION TEST OF  
ALBINO WISTAR RATS FED WITH HIGH FAT FROM COW'S BRAIN

BY

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**APPROVAL PAGE**

This is to certify that the research work entitled ‘the effect of n-hexane extract of kola *nitida* bark on liver function test on albino wistar rat fed with high fat from cow’s brain was carried out by Odo Oluchukwu BC/2009/279

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## **DEDICATION**

This work is dedicated to Almighty God for his guidance and protection. And to my beloved parents; Mr. and Mrs. Simon Odo, who out of their financial, moral, and spiritual support have contributed to my life. May God continue to strengthen their life with sound health so that they can be able to harvest the fruit of their labour.

## **ACKNOWLEDGMENT**

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## ABSTRACT

Kola bark extract have been widely used in tradition medicine for thousand of year, it improves liver functions and provides protection against high fat fed metabolic rats. Present investigations were carried out on the hepatoprotective role of Kola bark extract meal treatment to high fat fed wistar rat. Healthy adult male wistar rats were divided into four groups Group I: rats were fed a standard Laboratory diet (groups match) (20g/rat/day), Group II: rats were fed a high-fat diet alone (3.6g/rat/day), Group III: rats were fed with combined mixture of 70% of high-fat diet with 30% Kola bark meal (9.8g/rat /day), Group/ V: rats were fed with high fat diet and was administered with Lipitor (Atovastatin) (10mg/rat/day) . The rats were sacrificed at the end of the experiment (two weeks) period. The high fat fed rat substantially elevated its serum and liver tissue AST, ALT, ALP, bilirubin with decreased in total protein levels. Where as the levels of all parameters significantly restored towards normalization by the kola bark husk meal treatment. The results obtained suggest that the Kola bark meal have potent hepatoprotective action on high fat fed rats. A companion of the performance in both Kola bark meal and Lipitor treatment on high fat fed rat in respect of hepatoprotective role is clearly indicator that the Kola bark meal treatment was more and related to the result of Lipitor as well as to the normal level.

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## **CHAPTER ONE**

### **1.0 INTRODUCTION**

#### **BACKGROUND OF THE STUDY**

Liver is the largest and most complex internal organ in the body. It plays an important role in the maintenance of internal environment through its multiple and diverse functions. Liver is involved in several vital functions such as metabolism, secretion and storage. Hepatitis or inflammatory disorder involves inflammation and damage to the hepatocytes. Hepatitis is one of the most prevalent diseases in the world. Every year 18,000 people had been reported to die due to liver cirrhosis caused by viral hepatitis [L] Ectopic fat storage occurs in obesity particularly in the liver leading to a condition termed nonalcoholic fatty liver disease [NAFLD] characterized by varying degree of liver injury that progresses from steatosis to tratrohepatitis, fibrosis and necrosis Due to its prominent association with IR (insulin resistance). Obesity, NAFLD is regarded as the hepatic manifestation of metabolite [3] syndrome. Liver has great capacity to detoxicate toxic substances.

Kola pod is the traditional health food around the world. Kola pod Husk meal is the residue after grinding the pod of kola and filtration, and it is an excellent source in slightly high in fiber (16.74%), low in crude protein (9.98%), moderate NFE (42.391) and metabolizable energy 2497 kcal/kg, and

ash content is relatively high. (13.26%). when compared with the values reported by (Oluokun, 1999) ;(Hamzat and Babatunde, 2001). These differences might be due to varieties differenced and shelf life of the test ingredient before analysis. However, the crude protein content fairly put kola pod husk meal (KPHM) with feed ingredients like Sorghum while the fibre content put it in same proximate composition with Bremer's grains. Kola pod being one of the important pods for medical treatment in the world. The kola pod husk provides some essential materials for both drying. The pod bark, when mixed with some ingredients is used in traditional medicine to reduce Labour pains. The bark is used in treating swellings and fresh wounds. The roots provide excellent chewing sticks for cleaning the teeth and the wood is used in local carvings coachwork and boatbuilding. Lipid refers to the different kind of fats present in the bodies of human plasma include triglyceride (TG), phospholipids (PL), cholesterol esters (CE), and free cholesterol (FC) and non-esterified fatty acid (NEFA). Each lipid plays a different role in our bodies yet all of them except free cholesterol (FC) contain esters of long chain fatty acid in their structures. Lipids are insoluble in plasma due to their non- polar structures thus; they need some carriers that are soluble in polar and non- polar environment. Lipids are packaged and carried

in the form of lipoprotein, which is a molecule that contains both protein (i.e. apolipoprotein) and lipids.

Statins (lipitor) are group of drugs approved for their Cholesterol reduction properties and are commonly used to treat atherosclerosis and coronary artery disease. These drugs inhibit 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) reductase. The rate limiting enzyme in cholesterol biosynthesis; which converts HMG.CoA to mevalonate. Apart from cholesterol, mevalonate is also the substrate for the synthesis of nonsteroid, isoprenoids such as coenzyme Q. Depletion of these isoprenoids results in the so called pleiotropic effects of statins which are independent of cholesterol.

Lowering effect. These pleiotropic effects of anti-inflammatory, anti-apoptotic and ant-proliferative effect. In addition, data from experimental and observational studies indicated that statins have neuroprotective effects and be a treatment option for neurodegenerative diseases of the central nervous system and the ocular structures particularly the retina (Schemer, 2007). Statins (Atorvastatin) is widely used in the treatment of hepatic diseases. They have been widely used in chinning proactive as cholesterol lowering agents to reduce morbidity and mortality from coronary artery diseases. It also has an

effect in inflammatory diseases such as rheumatoid arthritis, and multiple sclerosis.

However, limited studies are available on the effect of kola pod husk meal treatment on hepatoprotective effect in high fat diet fed animal in this regard, the present study was aimed to investigate the effect of kola pod meal on high-fat fed rats related to different hepatoprotective effect by comparing Lipitor Treatment.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Origin of kola

The genus Kola of the family *sterculiaceae* (Russel, 1955), is indigenous to tropical Africa and has its centre of greatest diversity in west Africa in the forest areas of west Africa, kola is perhaps second only to palm oil in importance in the list of indigenous cash crops. About 40 kola species have been described in West Africa. However, in Nigeria, the kola species of real importance are kola *acuminata* and kola *nitida* (Quarcoo,1973;(Daramola,1978) Kola *acuminata* and Kola *nitida* are important economic crops in the forest areas of west and central Africa, caribbean islands, mauritius, Sri Lanka and Malaysia (Eijnatten,1969);(Oladokun, 1985). The cultivation of kola nitida in Nigeria began sometime in the 19<sup>th</sup> century. The “goro nut” (kola *nitida*) was observed to be growing abundantly in the otta bush in 1854 while its cultivation was noted in Egba Division in 1902 and in Labochi and environs in 1901. From Agege, kola *nitida* cultivation presumably spread to the forest areas following first the course of the railway line into Abeokuta, Ibadan and Offa replacing the local kola *acuminata* and penetrating later along streams and river banks into the Guinea savannah and at present, south and Eastern state (Eijnatte, 1969).

Kola nuts have for hundreds of years served as an important article of internal trade in Nigeria and other parts of Africa (Nzekwu, 1961). It has been an item of trade in West Africa and in the Trans-saharan trade routes for many countries (Egbe and Sobamiwa, 1989).

Kola nut is used as a masticatory stimulant by Africans and has numerous uses in social, religious, ritual and ceremonial functions by the natives in the forest region of Africa it is used during ceremonies related to marriage, child naming, installation of chiefs, funeral and sacrifices made to the various gods of Africa Mythology (Nzekwu, 1967) ;( Daramola, 1978) ;(Opeke, 1992). There is also increasing demand for its usage in pharmaceuticals industries and for production of soft drinks, wines, and candles (Beattie, 1970); Ogotuga, 1975). Its uses have inevitably created a high demand in excess of its production (Oladokun, 1985).

Kola nut has been used as abuse for a new brand of chocolate and wine (Kola chocolate and kola wine) which were developed by CRIN researchers (famuyima, 1987). Eka, (1971), has also reported the possible use of pulverized kola nuts for the preparation of non-alcoholic beverages.

Kola pod husk has also been utilized for the production of liquid soap. The most recent and remarkable advancement is kola by-product utilization is the use of kola pod husk in the replacement of up to 60% of the maize used in

pottery feed formulations (yahaya *et al*, 2001); (Hamzat, 2001);( Hamzat and Babatunde, 2001) ;( Hamzat and longe, 2002) ;( Hamzat *et al*; 2000) ;( Olubamima *et al*, 2002). Other uses of kola according to Nzekwu (1961) are as follows: - (1) It provides some essential materials for doth dying. (2) The pod bark, when mixed with some ingredients is used in traditional medicine to induce labour pains. (3) The bark is used in treating swellings and fresh wounds. Research on the improvement of productivity of kola is carried out at the cocoa research institute of Nigeria (CRIN) with the mandate in Nigeria for research into production and extension aspects of cocoa, kola, coffee, cashew and tea.

## **2.1 Strategies for enhancing investment opportunities in kola production.**

1. The first step is to improve the quality and quantity of kola nut production in the country. This can be achieved by two approaches.
2. Rehabilitation of kola orchards: a more efficient utilization of land can be achieved by the cutting down of unproductive trees and replacing them with proven materials.
3. New planting and suitable soils for kola: soils of inferior moisture retention and Nutrient properties which cannot sustain cocoa, or coffee can be utilized for kola production provided that they are deep and well drained.

## **2.2 LIPIDS MATABOLISM**

### **2.2.1 Lipids and lipoproteins:- definition and classification**

The term 'Lipid' refers to the different kind of fats present in the bodies of humans and others animals. The major Lipids found in human places include triglyceride (TG), phospholipids (PL), cholesterol esters (CE) and free cholesterol (FC) and non-esterified fatty acids (NEFA) each Lipid class plays a different role in bodies yet all of them except FC, contain esters of long-chain fatty acids in their structure. Thus, the final fatty acid composition of the lipids is influenced and determined by dietary patterns (i.e. By the consumption of a saturated/monounsaturated/polyunsaturated fatty acid diet) (16) also, each lipid class has a different physiological role for example TG is a storage form of energy for various tissues (e.g. major building blocks for cell membranes as well as maintaining the cell structure CE, on the other hand, is a major component in the synthesis of some steroid hormones (e.g. estrogen) whereas NEFA is the energy form released from TG.

Lipids are insoluble in plasma due to their non-polar nature thus they need some carriers that are soluble in both polar and non-polar environments. To solve this dilemma, all lipids are packaged and carried in for lipoprotein, which is a molecule that contains both protein (i.e. apolipoprotein) and lipid. On the other hand free fatty acids which is the product released after hydrolysis

of TG and is also insoluble in plasma, is transported within the plasma compartment by binding to a major plasma protein called albumin

There are five Lipoprotein classes, chylomicrons, very low-density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL), and high density lipoprotein (HDL). Even though the type of apolipoprotein and the proportion of each lipid carried by each lipoprotein are different from each other, all of them contain FC and PL on the surface of the lipoprotein particles whereas CE and TG reside in the core of the particle

#### 2.2.2 .The function and role of each lipoprotein class in lipid metabolism

Chylomicrons are mainly secreted in the small intestine and are the carriers for dietary TG and cholesterol. They appear in the bloodstream after consumption of a meal and reach their peak concentrations between 3-6 hours after that meal (i). They are usually cleared from circulation within 10 to 12 hours after the meal (ii) thus, chylomicrons do not usually appear in a fasting (i.e. 10-12 hours) plasma sample unless the person suffers from type 1 dyslipoproteinaemia. chylomicrons are carried in the lymphatic from the intestine and entered into the blood circulation, they interact with lipoprotein lipase (LPL) that hydrolyses the TG content in the core of the molecule. As these particles lose their core lipids as the hydrolysis proceeds, these particles become smaller in size and are now called remnants are carried to the liver

where they are both taken up by hepatic cells and TG is further hydrolyzed and stored in the liver or the TG is packaged and carried by another lipoprotein, VLDL (II). VLDL, Like chylomicrons, is the main carrier of TG but it carries endogenous (i.e. hepatic) TG (II) VLDL is synthesized in the liver and it carries TG from the liver to other tissues for storage and /or immediate energy use. As the VLDL particles exit from liver and join the circulation, like chylomicrons, they interact with LPL where the TG content is subsequently hydrolyses by the enzyme. At the same time, there particle-size also reduces in size as they lose TG from the core. Thus, during hydrolysis, VLDL is subsequently metabolized to IDL and some IDL may be further metabolized to LDL. As this VLDL to IDL and LDL metabolism continues, the Lipoprotein becomes both more cholesterol rich and smaller in size due to the subsequent lose of TG in ester transfer protein (CETP) other than being produced by the metabolic cascade, a small portion of IDL and LDL are also synthesized directly by the liver and secreted into the bloodstream (22)

HDL, on the other hand, is secreted from the liver and intestine in its nascent form that is HDL particles without any CE in the core which appear like a PL-bilayered disc under the microscope. As they travel in the circulation, the particles mature as they pick up FC from tissue and/or other lipoproteins and the FC is esterifies to CE under the catalysis of lecithin cholesterol

acyltransferase. Furthermore HDL also removes TG from other Lipoproteins in exchange for CE and this process is mediated by CETP.

### **2.2.3 Plasma lipoprotein**

Composition structure and Biochemistry Lipoproteins are complex aggregates of lipids and proteins that render the lipids compatible with the aqueous environment of body fluids and enable their transport throughout the body of all vertebrates and insects to tissues where they are required. Because of their crucial importance, a very high proportion of research on lipoprotein deals with their functions in humans in relation to health, and the discussion that follows has a human bias.

Lipoproteins are synthesized mainly in the liver and intestine within the circulation these aggregates are in a state of constant flux, changing in composition and physical structure as the peripheral tissues take up the various components before the remnants return to the liver. The most abundant lipid constituents are triacylglycerols, free cholesterol, cholesterol esters and phospholipids (phosphatidylcholine and sphingomyelin especially), through fat-soluble vitamins and anti-oxidants are also transported in this way free (unspecified) fatty acids and lysophosphatidylcholine are bound to the protein albumin by hydrophobic forces in plasma and in effect

Detoxified

#### **2.2.4 Cholesterol metabolism**

Cholesterol is the essential precursor of steroid hormones (progesterone, estrogen, testosterone, glucocorticoids, and mineral corticoids), bile acids, and vitamin D. It is also a vital constituent of cell membrane, which modulates the fluidity and permeability of the membrane. Cholesterol can be derived from the diet as well as from endogenous biosynthesis. The latter being the major source in humans. Homeostasis of cholesterol involves the movement of cholesterol between peripheral tissues and liver. The liver regulates de novo biosynthesis of cholesterol. The excretion of cholesterol into bile (directly or after conversion to bile acids) the secretion of cholesterol into blood as very low-density lipoproteins (VLDL), the modulations of receptor-mediated cellular cholesterol uptake, the formation, of cholesterol esters, which are more hydrophobic than cholesterol itself and the storage of cholesterol. The intestine regulates cholesterol absorption and excretion into feces.

#### **2.3 LIVER X RECEPTORS**

The liver x receptor (LXRs) LXR $\alpha$  (NRIH3) and LXR $\beta$  (NRIH2) belong to the nuclear receptor super family of ligand activated transcription factors. LXR $\alpha$  was initially isolated from rat liver DNA library as a novel orphan nuclear receptor, i.e. receptor with no known physiological ligands, hence the name

LXR several groups identified the LXR isoform by screening of different DNA libraries. The human LXR $\alpha$  gene is located on chromosome 11q12, which the human LXR $\beta$  gene is located on chromosome 19q13. LXR $\alpha$  expression predominates in metabolically active tissues such as the liver, small intestine, kidney, macrophages, and adipose tissue, whereas LXR $\beta$  is more ubiquitously expressed with particularly high levels in the developing brain (fan *et al*, 2008). Suggesting regulation of different physiological functions for the two receptors. Human LXR $\alpha$  and LXR $\beta$  share almost 80% amino acid identity in their DNA-binding domain and ligand-binding domain. The LXR paralogs are highly conserved between rodents and humans. Human LXR $\alpha$  and rat LXR $\alpha$  show close to 100% homology in amino acid sequence in their DNA-binding domain and ligand binding domain (Kruit, J.K. 2008).

**2.3.1 Liver X Receptors (LXRs) as cholesterol sensors:** Liver X receptors act as cholesterol sensors when cellular oxysterols accumulate as a result of increasing concentration of cholesterol, LXR induces the transcription of genes that protect cells from cholesterol overload. LXR activation regulates bile acid synthesis and metabolism/excretion, reverse cholesterol Transport/excretion in the intestine (see Fig.1).

Figure I

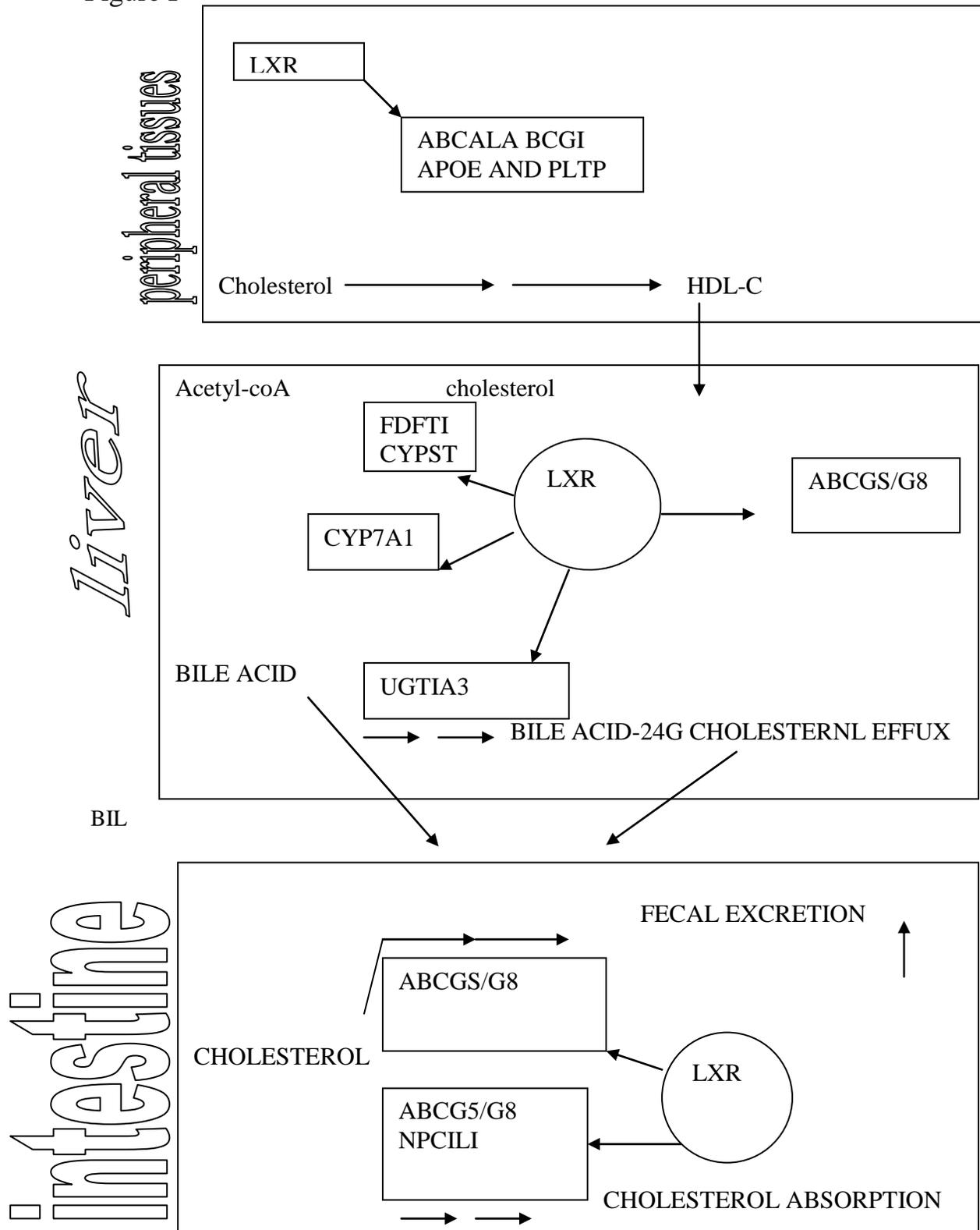


Figure 1: Role of Liver x receptor in Cholesterol metabolism. In the liver, Cholesterol biosynthesis efflux and bile acid metabolism /excretion are all regulated by LxR. LxR increases efflux in the peripheral fecal excretion. See text for details. Boxes represent LxR target genes. HDL—C, high density lipoprotein cholesterol, ABC, ATP-binding cassette transporters; APOE, apolipoprotein E; PLTP phospholipids transfer protein; UGT1A1, UDP glucuronosyl transferases/ family, polypeptide A.3; CYP7A1, cholesterol 7 $\alpha$ -hydroxylase; FGF19, fibroblast growth factor 19; CYP27A1, cytochrome P450, family 27, subfamily A, polypeptide; NPC1L1, Niemann-Pick C1-Like 1.

### **2.3.2 Liver x receptor and bile acid synthesis, metabolism and excretion.**

Bile acid synthesis and excretion constitutes the major route for elimination of cholesterol from the body. Oxysterols, natural ligands for LxRs, are generated when cholesterol levels are high. The classical pathway of bile acid synthesis is initiated by 7 $\alpha$ -hydroxylation of cholesterol catalyzed by the cytochrome p450 cholesterol 7 $\alpha$ -hydroxylase (cyp7A1), which encodes the rate-limiting enzyme of this pathway. In rodents, LXR $\alpha$  stimulates the expression of cyp7A1 via binding to an LXRE present in the cyp7A1 promoter. Thus, rats and mice

have the capacity to convert dietary cholesterol into bile acids (Plat, J and Nicholas, J.A. 2005). As a consequence, these species quickly adapt to a diet rich in cholesterol by increasing its conversion to bile acids. The importance of LXR $\alpha$  activated cyp741 in regulating cholesterol balance in the rodent liver becomes evident from studies of LXR knockout mice. LXR $\alpha$  but not LXR $\beta$ . Knockout mice accumulates large amount of cholesterol esters in the liver after being fed with a high-fat cholesterol diet due to failure of inducing expression of the cyp741 gene. In contrast to observation in rats and mice, LXR $\alpha$  agonist treatment suppresses the expression of cyp7A1 in primary human hepatocytes. This repression is at least in part, due to the direct induction of small heterodimer partner, a gene that has a repressive effect on cyp741 via liver receptor homolog 1 (LRHI); also called FIF in rat and CPF in humans. This result suggests that different species may employ distinct molecular strategies to regulate cholesterol homeostasis, emphasizing the importance of valid experimental models for the development of pharmaceuticals for human use.

In addition to its role in controlling bile acid anabolism, LXR also plays a role in regulating bile acid catabolism. Recent reports indicate that ligand – activated LXR  $\gamma$  up regulate human, UGT1A5 gene expression through binding to an LXR- like sequence in the promoter (Barbier *et al*, 2009). UGT1A5 is one the most active enzyme for glucuronide conjugation of bile

acid. Bile acid glucuronidation allows their conversion into urinary excretable metabolites. Based on these observations, it was proposed that LXR activation may facilitate definitive cholesterol elimination in the form of urinary bile glucuronides.

Most bile acids are N-acylamidates with glycine or taurine to decrease toxicity and increase solubility for secretion into bile (Hofman, 1999). Taurine occurs naturally in many foods and is known to lower cholesterol profile. Additionally, taurine has been shown to induce Cyp741 activity thereby increasing bile acid synthesis. Interestingly, it has been shown that taurohydroxycholic acid can activate the LXRE in the CYP741 promoter via LXR, suggesting their activation of LXR signaling is one mechanism by which taurine activates CYP741 activity. Excretion of free cholesterol into the bile is another major route for eliminating excess cholesterol from the liver. In the liver, ABCG5 and ABCG8 have been proposed to transport cholesterol from hepatocytes to the bile canaliculi. ABCG5 and ABCG8 are half transporters that form obligate heterodimers, and are both regulated by LXR activation (Berge *et al*, 2000); (Repa *et al*, 2002). ABCG5 and ABCG8 are expressed in the apical membrane of enterocytes and at the canalicular membrane of hepatocytes. These transport proteins promote secretion of hepatic cholesterol into bile. Mice lacking ABCG5 and ABCG8 exhibit

profound reduction in biliary cholesterol level and an accumulation of cholesterol in the liver after cholesterol feeding (yu et al, 2002). Mutation in the genes encoding either ABCG5 or ABCG8 result in B-sitosterolemia, an autosomal recessive disorder characterized by an increased risk of atherosclerosis and elevation of plasma levels of phytosterols (Lee *et al*, 2001);(Lu *et al*, 2001) the human ABCG5 and ABCG8 genes are oriented in a head-to-head configuration separated by a 374-6p intergenic region. No LXREs have the intergenic region was found to act as a bidirectional promoter and be partially responsive to treatment with LXR agonists (ramaley *et al*, 2002).

### **2.3.3 Liver x receptor biosynthesis**

Recently, wang *et al* (2008), demonstrated that LXR $\alpha$  negatively regulated two genes squalenesynthase (FDFTI) and lanosterol 14 $\alpha$  - demethylase (CYP5141), which encode they enzymes in the cholesterol biosynthesis pathway. LXRES that confer LXR-mediated repression were identified in these two genes. Based on these observations it was proposed that LXR $\alpha$  plays an important role in suppression of cholesterol biosynthesis.

### **2.3.4 Liver x receptor and cholesterol uptake**

The major part of cholesterol in human blood is transported within LDL.C. The LDLR mediates the removal of LDL and remnant lipoproteins from circulation by binding to apolipoprotein B-100 (APOB-100) and APOE. It also plays 0 major roles in regulation of plasma cholesterol level in human. Recently, zelceret *al*, demonstrated that LXR decreases LDLR- dependent cholesterol uptake through a LXR- idol (inducible degrader of the LDLR) pathway. LXR induce the expression of idol. Which in term catalyze the ubiquitination of the LDLR, thereby targeting it for degradation. On the contrary, induction of LDLR repression via an LXRE by LXR agonist has been reported by Repaet *al* (2002). The use of different cell lines and different LXR agonists in the two studies may account for the contradictory results. Clearly the exact role of LXR in regulation of LOLR expression and subsequent uptake needs to be further exploited

### **2.3.5 Liver x receptor and intestinal cholesterol absorption**

Intestinal cholesterol absorption has been shown to be a major determinant of plasma cholesterol levels LXR activation results in a reduced absorption of intestinal cholesterol by regulating express of several genes such as heterodimers ABCG5/ABCG8 and Niemann-pick C1 like 1 (NPC1L1) involved in this process LXR activation increases the expression of both ABCG5 and ABCG8 which transport absorbed cholesterol back to the humans of the

intestine. Consistent with this finding, administration of LXR agonists substantially decreases intestinal net cholesterol absorption in mice. NPC1L1 is expressed in the small intestine, most likely in the brush border membrane of enterocytes, and it is required for intestinal cholesterol absorption (Altmann *et al*, 2004). It was recently reported that LXR activation down-regulates NPC1L1 expression in both mouse and in a human enterocyte cell line.

### **2.3.6 Liver x receptor as therapeutic targets**

As described above, LXRs function as cholesterol sensors with important roles in regulating cholesterol homeostasis, and thus there is a widespread interest in the development of synthetic LXR ligands as therapeutic agents. Indeed the abundant expression of the LXR < protein in macrophages present in human atherosclerotic lesions supports the hypothesis that LXR< agonists could have a beneficial effect against development of atherosclerosis. Recently, synthetic LXR ligands have been characterized in several animal models for the treatment of atherosclerosis. In a study by Wang *et al* (2008), the influence of a non steroidal LXR agonist GW3965 on the development of atherosclerosis was investigated in both Ldlr-1- and Apoe-1- mice. The results showed that GW3965 inhibits the development of atherosclerotic lesions in both murine models providing direct evidence for an atheroprotective effect of LXR agonists. In the study by Haefliger *et al* (1999). To901317, a synthetic LXR Ligand, was administered to

LDLR-1- mice T0901317 significantly reduced the atherosclerotic Lesions inLdlr-1- mice without affecting total plasma cholesterol levels moreover an agonist for RXR, the obligate heterodimers partner of Lxr has shown to be effective in reducing atherosclerosis (yulet *al*, 2002). these results suggests that LXR ligands may be useful therapeutic agents for the treatment of atherolerosis however, this therapeutic strategy needs to address that LXR activation is associated with lipogenesis resulting in increased plasma TG level and hepatic steatosis. Several in vivo studies massive TG accumulation in the lever and increased plasma TG level (Repeat *al*, 2002);( schultet *al*, 2000);(Grephostet *al*, 2002). The LXR agonist Gui3965 also increases hepatic TG levels in mice (Grephostet *al*, 2002.) Interestingly, a potent synthetic steroidal LXR activator, N.N- dimethyl-3b- hydroxyl-cholenamide(DMHCA) has recently been demonstrated to reduce atherosclerosis in APOE- deficient mice, without inducing hepatic and plasma TG levels based on these observation, DMHCA could be a candidate for further development as a therapeutic agent for treatment of atherosclerosis ( kruit, J.K., 2009).

## CHAPTER THREE

### MATERIALS AND METHOD

#### 3.1 Identification of plant material

Healthy pod kola *nitida* were obtained from a village in UmuezeAwkunanaw town in Nkanu-west local government of Enugu state in the month of November 2012. The pod was identified and authenticated by a plant taxonomist: Prof J.C. Okafor of the Enugu State University of Science and Technology, Agbani, Enugu state.

The pods were dried at a room temperature for eighteen (18) days the dried pod were later ground into fine powder with the aid of a clean dry electric grinder. A 130g portion of the ground pod was soaked in 13ml of distilled water for twelve (12) hours, filtered and then extracted with double distilled water by hot-continuous percolation method in a soxlet apparatus. The water solvent in the extract was then distilled off in a distillatory and evaporated to dryness at 40<sup>0</sup>c.the solid extract weighing 18.3g was placed in a sterile container labeled and stored at 40c in a refrigerator. The 18.3g was later divided into two containers (6.3g and 12g). The first container of 6.3g was used for experimental animal model while the other of 12g was used for phytochemical analysis.

### **3.2 Phytochemical Analysis**

The photochemical test on the solid extract was carried out based on procedures outlined by Harbourne (1973) and later modified by Trease and Evans (1996).

The phytochemical tests carried out and their procedures are as follows:

#### **1) Steroid determination**

I weighed 1g of kola *nitida* pod and macerated it in 20mls of ethanol and then was filtered after which I pipette 2mls of the filtrate, I then added 2mls of colour reagent and left it to stand for 30 minutes and finally measured the absorbance at 550nm.

#### **2) Saponin determination**

I weighed 1g of kola *nitida* pod then macerated it in 10mls of petroleum ether which I decanted it into a beaker then another 10mls of petroleum ether was added after which I poured it back into a beaker, then I combined the filtrates and evaporate it to dryness after which I added 6mls of ethanol and pipette 2mls of the sample into a test tube and left to stand for 30 minutes, finally I measured absorbance at 550n.

#### **3) Flavonoids determination**

I weighed 1g of *kolanitida* pod and macerated in 20mls of ethyl acetate and filtered it after which I pipette 5mls of the filtrate and added 5mls of ammonia slake thereafter I collected the upper layer and measure absorbance at 490nm.

#### **4) Reducing sugar determination**

I weighed 1g of kola *nitida* pod then macerated it in 20mls of distilled water. after which I pipette 1ml of the filtrate and added 1ml of copper reagent and boil for 5 minutes, then I left it to cool and later added 1ml of phosphomolybdic acid reagent after which I added 7mls of distilled water and finally measured the absorbance at 420nm.

#### **5) Alkaloid Determination**

I weighed 1g of kola *nitida* pod and macerated it in 20mls of 20% H<sub>2</sub>SO<sub>4</sub> in ethanol (1:1). I filtered it and pipette 1ml of the filtrate and then added 5mls of 60% H<sub>2</sub>SO<sub>4</sub> and 5mls of 0.5% formaldehyde in 60% H<sub>2</sub>SO<sub>4</sub>. After which I mixed them and allowed it to stand for 3 hours and finally measured absorbance at 565nm.

#### **6) Terpenoid Determination**

I weighed 1g of kola *nitida* pod and macerated it in 50mls of ethanol then I filtered it, there after I pipette

2.5mls of the filtrate and added 2.5mls of 5% aqueous phosphomolybdic acid solution gradually I added 2.5mls of conc. H<sub>2</sub>SO<sub>4</sub> and mixed it and I left to

stand for 30 minutes, after which I added up to 12.5mls with ethanol and measured absorbance at 700nm.

#### **7) Glycoside determination**

I weighed 1g of kola *nitida* pod, I added 2.5mls of 15% lead acetate and filtered after which I added 2.5mls of chloroform and shake vigorously, later I collected the lower layer and evaporated. Later I collected the lower layer and evaporated it to dryness, after which I added 3mls of glacial acetic acid, 0.1ml of 5% of ferric chloride and 0.25mls conc. H<sub>2</sub>SO<sub>4</sub> and shaken it and I placed it in a dark place for 2 hours. Absorbance was finally measured at 530nm.

#### **8) Tannin determination**

I weighed 1g of kola *nitida* pod then macerated it in 50mls of methanol, filtered it and pipette in 5mls of filtrate. I added 0.3mls of 0.1ml of ferric chloride in 0.1ml HCL and finally measured absorbance at 720nm.

#### **(9) Cyanide determination**

I weighed 1g of kola *nitida* pod macerated it in 50mls of distilled water and left to stand for 24 hours, after which I filtered it and pipette 1ml of filtrate and then added 4mls of alkaline pirates solution. I boiled it for 5 minutes and left to cool. Absorbance was finally measured at 490nm.

#### **(10) Soluble Carbohydrate Determination**

I weighed 1g of the kola *nitida* pod and macerated it in 50mls of distilled water, then filtered it. After which, I pipette 1ml of the filtrate and added 2mls of saturated picric acid. Finally I measured absorbance at 530nm

### **3.3 Experimental Animal Model**

Twelve (12) health male albino wistar rats with mean weight of  $1.50 \pm 0.60$ kg were obtained locally from Nsukka, Enugu State. The rats were randomly distributed into four (4) groups (I-IV) of three (3) rats each. They were housed separately and fed with water and grower's mash (Guinea feed Nigeria) and allowed for 3 days to acclimatize. A high lipoprotein food (cow's brain) was prepared by dissolving 300g of cow's brain in 500ml of distilled water forming a semi-solid mixture. A known antilipidemic drug; atorvastatin (brand name-lipitor, 10mg) was prepared by dissolving 5mg (half of one tablet) in 2ml of distilled water. Also kola pod water extract weighing 93.6g was dissolved in 17ml of distilled water forming a liquid drug extract. Group I rats were fed orally with 6ml of the lipoprotein food mixture which contains 3.6g of the cow's brain twice a day (morning and evening) for seven (7) days.

Group II rats were also fed orally with 6ml of the lipoprotein food mixture for seven (7) days (morning and evening) and later were administered orally with the 2ml dissolved atorvastatin drug for the following three (3) days (once each day) during which grower's mash and water was their food. Each group of rat was

also fed orally with 6ml of the lipoprotein food mixture for seven (7) days morning and evening ) and later were administered orally with 0.5ml of the liquid drug later were kola pod water extract mixture) for the following three (3) days (once a days ) during which grower's mash water was their food.

### **3.4 Collection of blood Sample**

The collection of blood samples from the rats in each group was simple done by dissecting of the rats, followed by cardiac puncture after a mild anesthesia with chloroform. About 5-9mls of blood samples was collected in an EDTA tube from each group using a medical syringe. Serum was separated from the blood after clotting by centrifugation and then used for lipid analysis.

Blood samples were collected from group I rats and group ii rats on the following day of after the 7<sup>th</sup> day orally feeding the rats with lipoprotein food mixture and normal feed (grower's mash and water) respectively.

Blood samples were collected from group iii and iv rats on the following day of after the 3<sup>rd</sup> day of orally administering a known drug (atorvastatin; Lipitor) and the liquid drug extract kola pod water extract mixture) respectively.

### **3.5 LIPIDS PROFILE ANALYSIS**

In the lipid profile analysis, test is conducted for serum total cholesterol (TC) high-density lipoprotein cholesterol (HDL-C), and triacylglycerol. Low-

density lipoprotein cholesterol! (LDL-C) is then calculated using a standard formula.

**1) TEST FOR TOTAL CHOLESTEROL(TC)**

**a) Material/ reagent involved**

- a) serum
- b) ferric chloride reagent (2.5g of ferric chloride mixed with 100ml of 85% phosphoric acid )
- c) standard cholesterol (25mg of pure cholesterol mixed with 100ml of glacial acetic acid)

**b) Procedure**

I diluted the serum at about 1:20 with distilled water acetic acid

Using three glass test tubes labeled- test, standard and blank respectively, following procedures listed below.

Sample	test	standard	Blank
Ferric chloride Reagent	5.0ml	5.0ml	5.0ml
Diluted serum	0.5ml	-	-
Diluted Standard Cholesterol	-	0.5ml	-
Distilled water	-	-	0.5ml

I shake the tubes very well for about 10 seconds in order to mix-up the contents .immediately drop the test tube in a boiling water bath for about 90 seconds cools for about 5 minutes with running tap water. after wards, I read the absorbance at 560nM against the blank with a dry cuvette. Meanwhile, the colour should be stable for 15 minutes. Finally, calculate using the formula absorbance standard the unit is mg/dl. Then to convert the result from mg/dl to mmol/l. simply multiply the mg/dl result by 0.0259 (constant value).

## **2) TEST FOR HDL-CHOLESTEROL (HDL-C)**

### **a) Materials/ reagents involved**

- Serum
- Cholesterol standard (100mg of pure cholesterol mixed with 100ml of glacial acetic acid)
- Colour reagent (5.6g of 2, 5-dimethyl Benzene sulphuric acid mixed with 200ml of glacial acetic acid and 300ml of acetic anhydride)
- Phosphotungstic acid reagent (4.5g of phosphotungstic acid mixed with 50ml of water, add 16ml of 1N NaOH and make up to 100ml with water)
- Magnesium chloride (40.6g of magnesium chloride mixed with 100ml with water)
- Tris buffer (1.21g of tris mixed with 90ml of water, then the PH was reduced to about 7.6 with 1N HCl and diluted up to 100ml with water)

#### **d) Procedure**

I pipette about 1ml of serum in a test tube, drop 0.1ml of phosphotungstic acid reagent and mix very well, add 0.05ml of magnesium chloride and mix very well. Then I Centrifuged at 2500rpm or 1500g for 30 minutes, which after I carefully remove the clear supernatant with a [Pasteur pipette add 2 drops of the colour reagent and allow to stable for 15 minutes. I then read the absorbance at 560Nm. Finally i calculated using the formula

$$\text{HDL (mg/dl)} = \frac{\text{absorbance of test}}{\text{Absorbance of standard}} * 115 \text{ (constant valve)}$$

Absorbance of standard

#### **3) TEST FOR LDL-CHOLESTEROL (LDL-C)**

The LDL-Cholesterol is calculated using the standard formula: total cholesterol-HDL-Cholesterol+0.46 (constant valve)\*

#### **4) TEST FOR TRIGLYCERIDES (TG)**

##### **a) Material/ reagents involved**

- Serum
- Heptane
- Isopropanol
- Sodium methyrate (50mg of sodium methyrate diluted in 100mi of isopropanol)

- Sulphuric acid (0.08N) –prepared by mixing 2.25ml conc. H<sub>2</sub>SO<sub>4</sub> of about 36.0N with 500ml of distilled water. The dilution should be up to 1 liter
- Periodate reagent (1.23g) of NaO<sub>4</sub> was mixed with 100ml of 0.88N, about 5% v/v acetic acid. Then stored in a brown bottle)
- Acetylacetonner reagent (0.75ml of acetyl acetone was dissolved with 2.5ml of dissolved with 2.5ml of isopropanol, then 2N of ammonium acetate (15.4%) was added to make the volume up to 100ml. then stored in a brown at 4<sup>0</sup>c
- Triglyceride standard (200mg of pure triolein was mixed with 100ml of isopropanol)

**b)Procedure**

I used three glass test tubes labeled –Test, Standard and Blank respectively, the following procedures was conducted

Sample	test	standard	Blank
Serum	0.5ml	-	-
Triglyceride Standard	-	0.5ml	0.5ml
Distilled water	-	0.5ml	0.5ml
Isopropanol	3.5ml	3.0ml	3.5ml
H <sub>2</sub> SO <sub>4</sub> (0.08N)	1.0ml	1.0ml	1.0ml
Heptane	2.0ml	2.0ml	2.0ml

## CHAPTER FOUR

### Results

#### Quantities analysis table 1: phytochemical composition of samples

(mg/100g)

Parameters

samples

Soluble sugar	1.59 +-e 0.003
Cyanide	0.49 +-c 0.003
Reducing sugar	826.485 +- f 0.004
Saponin	1.16+-g 0.004
Tannin	2.54+-b 0.004
Flavonoid	3.76+-a 0.004
Alkaloid	3.96+-c 0.004
Steroid	0.66+-a 0.0004
Terpenoid	0.31+-f 0.004

## Qualitative analysis table 2: quantitative phytochemistry

Secondary metabolites	sample (kola pod extract)
Tannin	+++
Alkaloid	+++
Carbohydrate	+
Saponin	+
Steroid	+
Hydrogen cyanide	+
Flavonoid	++
Reducing sugar	++
Terpenoid	+

**Table 3: Liver Function Test of rats fed with various samples**

Parameter	N-Hexane extract of pod c	Normal feed D	Cow's Brain e	Lipitor F
Total Bilirubin (mg/dl)	0.50+-0.00	0.71+-d 0.01	0.90+-0.01	0.40+-f 0.00
Direct Bilirubin (mg/dl)	0.20+-c 0.00	0.10+-a 0.00 4.85+-d 0.07	0.18+-d 0.00	0.20+-c 0.00
Indirect Bilirubin (mg/dl)	0.30+-c 0.00	2.51+-d 0.01	0.90+-d 0.00	1.55+-e 0.07
Total serum/plasma protein(g/dl)	3.01+-c 0.01	2.40+-d 0.00	3.35+-b 0.07	0.50+-f 0.21
Albumin (mg/dl)	2.11+-c 0.01	3.50+-d 0.00	3.71+-e 0.01	0.70+-f 1.14
Globulin (g/dl)	0.91+-c 0.01	0.51+-d 0.01	1.85+-c 0.07	0.81+-f 0.01
Serum plasma Alkaline phosphate (ALP) (KAunits/100ml)	2.45+-0.01	30.00+-d 0.00	5.01+-e 0.01	1.51+-f 0.01
Serum plasma phosphate Acid Phosphate (unis/ml)	0.05+-c 0.00	11.00+-d 0.00	1.20+-e 0.00	1.20+-e 0.00
Creatinine phosphate kinase (units/ml)	8.75+-0.07	13.91+-c 0.01	35.00+-e 0.00	0.40+-f 0.01
Aspartate Amino Transferase (AST) U/1)	5.55+-c 0.07	6.91+-d 0.01	13.00+-e 0.00	8.75+-f 0.07
Alanin Amino Transferase (ALT (U/1)	9.61+-d 0.01	2.50+-d 0.00	15.00+-d 0.00	
y-GlutamyTransferase (GGT) u/1)	3.91+-c 0.01	2.40+-d 0.00	8.01+-e 0.00	2.71+-f 0.01
Lactate dehydrogenase (LDH) (u/ml)	0.71+-c 0.01	2.50+-d 0.01	3.34+-e 0.00	1.4+-f 0.01

## **4.2 Discussion**

Consumption of a calorie-rich diet results in lipid accumulation excess production of inflammatory cytokines, and macrophage infiltration that favour the progression of liver disease. High fat diet could induce the hyperlipidemia in rats and hyperlipidemia could alter the related marker enzyme profile in serum and liver tissue and progress to liver cirrhosis. The write up have revealed that high fat diet promote hyperglycemia and its effect on muscle and liver physiology as well as endothelial functions. Liver is the key organ in the metabolism, detoxification and secretory function in the body and its disorders are numerous with no still ongoing. Many folk remedies from plant origin have been long used for treatment of liver diseases. Management of liver diseases is still challenge to the modern medicine. In Ayurveda various herbal and herbomineral preparations are extensively use for the treatment of various live disorders. From the above statement it is generally accepted that high -fat diets can be used to generate a valid rat model for the analysis of pathophysiology of hyperlipidemia. Therefore ,in this study high-fat diet fed to rat model for the induced alterations on examine the comparative effect of hexane extract of kola pod meal and lipitor combination with dietary (high-fat diet) administration for their hepatoprotective role.

Assessment of liver function can be made by estimating the activities of serum AST, ALT and ALP which are enzymes originally present in higher concentration in cytoplasm, when there is hepatopathy, these enzymes leak into blood stream in conformity with the extent of liver damage indication of hepatic cellular integrity most commonly measured in clinical toxicology bilirubin levels. ALT is frequently included in biochemical profile for the purpose of assessing hepatic injury.

❖ In the present investigation the increased levels of AST, ALT and ALP have been observed in both liver and serum of high-fat fed rat compared to control groups (table 3) indicating the hepatotoxic role of high-fat diet. An elevation in the levels of serum marker enzymes is generally regarded as one of the most sensitive index of the hepatic damage. ALP originates in the liver mainly from bone and its elevation in serum occurs through biliary excretion during hepatobiliary diseases. The elevation of ALP in liver tissue of high-fat fed rat indicates the disturbed excretory function of liver, which is in accordance with the finding of previous study.

❖ As indicated in literature, the enzyme ALT and AST, are present in the hepatic and biliary cells. Observed elevated level of these enzymes in serum and liver tissue of high fat diet fed rat indicates elevation might be due to hepatocellular damage caused by high-fat diet. These elevated enzymes in

hepatocytes usually released into circulation causing increase in their serum level under hepatocellular injury or inflammatory of the biliary tract cells. Similarly (recknagel, reported that the hepatic damage induced in high-fat diet fed animal which could be rise in the level of AST and ALT in liver and blood.

- ❖ In the present study serum as well as liver protein depletion in the protein levels might be due to liberate energy of their metabolism during the toxicity of high-fat fed rats. The reduction of protein in high –fat fed rat determination of serum bilirubin represents an index for the assessment of hepatic function and any abnormal increases in the level of bilirubin in the serum indicate hepatobiliary disease and server disturbance of hepatocellular function.
- ❖ Findings on the rat fed with Lipitor (atorvastatin) and n-hexane extract of kola pod meal in separately mixing with high fat diet should the appreciable normal level of enzyme profile (ALT, AST, ALP, GGT) and total protein in serum and liver tissue as well as bilirubin in serum of wistar rat. The results on lipitor group indicated that Lipitor was effective in the treatment of high-fat-fed rats possibly through the mechanism of regulating the enzyme metabolism. Research in Lipitor for the treatment of liver disease induces in rats by either high-fat diet administration or bile ligation

indicates that Lipitor inhibit both hepatic inflammation and hepatic stellate cell activation were, the Lipitor induced the liver disease after prolonged period in high-fat-fed rat. “Galli reported that inflammation of liver and elevated liver enzymes have surfaced in patients given Lipitor. In the groups of pod meal mixing with high –fat diet component (enzymatic level) in both serum and liver tissue compared with control groups, indicating the anti-hpatotoxic role of it N-hexane extract of the kola pod diet or meal. The pod extract treatment excreted beneficial effect on ameliorating the enzyme resistance in high fat diet fed rat.

- ❖ The present investigation showed that the kola resistance in high-fat diet induced obesity and diabetes through its ability to decrease the elevated activity of ALT, ALP and AST at the cellular level treatment indicate that the extract more effective from the treatment of high-fat diet toxicity than the treatment of lipitor.

## **CHAPTER FIVE**

### **Conclusion**

This study conclusively stated that N-Hexane extract of kola pod has anti-hepatotoxic effect on serum, hepatic marker enzyme activities and blood bilirubin level as well as improving protein level from high fat diet induced hepatotoxicity. The presence of phytochemical constituents like flavonoids possesses antioxidant and hepatoprotective properties. N-hexane extract of kola pod was more effective in hepatoprotective role by improving biochemical components in high-fat diet fed rat towards normal tendency without any side effect after longer period in comparison to Lipitor treatment.

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