TITLE

THE EFFECTS OF ALCOHOL ADMINISTRATION ON SERUM LIPID PROFILE, TOTAL PROTEIN AND LIVER ENZYMES IN RATS

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR AWARD OF DEGREE OF MASTER OF SCIENCE (M.Sc) IN PHARMACOGICAL BIOCHEMISTRY, UNIVERSITY OF NIGERIA, NSUKKA

BY

NRABALU, CECILIA CHINENYE
(PG/M.Sc/08/48611)

DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF NIGERIA
NSUKKA

SUPERVISOR: PROF. E.O. ALUMANAH

NOVEMBER, 2011

CERTIFICATION

Nrabalu, Cecilia Chinenye, a postgraduate student of the Department of Biochemistry with the Reg. No PG/M.Sc/08/48611 has satisfactorily completed her requirements for research work for the degree of Master of Science (M.Sc) in Biochemistry (Pharmacology). The work embodied in this project (dissertation) is original and has not been submitted in part or full for any other diploma or degree of this or any other University.

.....

PROF. E.O. ALUMANAH
(Supervisor)

.....

PROF. L.U.S. EZEANYIKA
(Head of Department)

.....

EXTERNAL EXAMINER

DEDICATION

To my late beloved husband, Barrister Nnaemeka Nrabalu. May your gentle soul rest in perfect peace, AMEN.

ACKNOWLEDGEMENT

I am immensely grateful to Almighty God for His Grace, Mercy, and Favour upon me throughout the course of this project. My profound gratitude goes to my supervisor Prof. E. O. Alumanah whose assistance, fatherly advice, encouragements and moral support has been the foundation towards the achievement and success of my work during this period of my bereavement. My appreciation also goes to Dr. Vitalis Ikpe, the Head of Chemical Pathology, National Orthopaedic Hospital Enugu and Mrs. Onwe for their assistance during the course of my practical work.

My appreciation also goes to all my lecturers who made my dreams come to reality especially Prof. P.N. Uzoegwu and the Head, Department of Biochemistry, Prof. L.U.S Ezeanyika, for their encouragements. I am grateful to my lovely children, Favour, Chisom and Ifechukwu for their prayers, and I pray that the Good Lord will bless and protect them all. I also wish to express my sincere gratitude to my only and beloved Mum Mrs. B.I Ofora, for her moral support and prayers. I am also grateful to my sisters Ebele, Kate, Dr Uche, Dr. Chiko and my only brother Emeka for their contribution towards the success of this work.

ABSTRACT

Alcohol has many biological actions, and adverse effects on lipid metabolism. The main objective of this present study was designed to study the effects of alcohol administration on serum lipid profile, total protein, liver enzymes and histopathological effect in rats. A total twenty five male wistar rats (190-200g) were divided into five groups of five animals each. The animals were grouped so that the mean difference in the various groups would not vary significantly. Group one served as the control whereas groups two, three, four and five were made up of ethanol treated rats. The ethanol was administered intraperitoneally (I.P) and the treatment was carried out for three months and analysis of the parameters done on a four week basis. After .the last dose of every four weeks, blood was collected, centrifuged to form the serum. Ethanol administration on rats produced a marked and significant ($p<0.05$) increase in lipid profile when compared to the normal. It caused an initial increase in total cholesterol in first four weeks of the treatment and decline. Ethanol administration made a significant increase in high density lipoprotein cholesterol, low density lipoprotein cholesterol and triacylglycerol. Furthermore ethanol administration enhanced the liver enzymes by increasing their activities. The activities of alanine aminotransferase, alkaline phosphatase and aspartate aminotransferase increased significantly ($p<0.05$) when compared to control. Serum total protein levels indicated a significant increase ($p<0.05$) with ethanol administration.

Alcoholism is a progressive disease. Liver disease is the most common complication from ethanol abuse. Alcoholic fatty liver may progress to alcoholic hepatitis and finally to cirrhosis and liver failure

TABLE OF CONTENTS

CHAPTER TWO: MATERIALS AND METHODS

CHAPTER THREE: RESULTS

LIST OF FIGURES

LIST OF PLATE

LIST OF TABLES

LIST OF ABBREVIATIONS

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ALP	Alkaline phosphatase
ABCA1	ATP-bind cassette transporter
CETP	Cholestery ester transfer protein
EtOH	Ethanol
EDTA	Ethylene diamine tetracetic acid
FAT	Fatty acid transporter
GGT	Gamma glutamyl transferase
HDL-C	High density lipoprotein cholesterol
I .P	Intraperitoneal
LCAT	Lecithin: cholesterol acyltransferase.
LDL-C	Low density lipoprotein cholesterol
SR-B1	Scavenger receptor B1
TAG	Triacylglycerol
TNF-Ŭ	Tumour necrosis factor alpha
VLDL	Very low density lipoprotein
SR-B1	Scavenger receptor B1

CHAPTER ONE

INTRODUCTION

Alcoholism leads to fat accumulation in the liver, hyperlipidemia, and ultimately cirrhosis (Murray et al., 2006). The fatty liver is caused by a combination of impaired fatty acid oxidation and increased lipogenesis, which is thought to be due to changes in the [NADH]/ [NAD^+] redox balance in the liver. Lipid homeostasis is altered by chronic ethanol consumption leading to the development of a fatty liver as well as lipid alterations in other organs (Carrasco *et al.*, 2002). Liver disease is the most common complication from ethanol abuse (Mello *et al*., 2007). It is estimated that 15 to 30% of chronic heavy drinkers eventually develop severe liver diseases. Alcoholic fatty liver may progress to alcoholic hepatitis and finally to cirrhosis and liver failure (Reuben, 2008). In the USA, chronic alcohol abuse is the leading cause of liver cirrhosis and the need for liver transplantation (Masters, 2001). On the other hand, it has been shown that alcohol consumption may protect against severe coronary atherosclerosis, but the mechanism through which alcohol might exert its protective effect remains unclear (Dai and Miller, 1997). High-density lipoprotein-cholesterol (HDL-C) like other lipids shows a dose-dependent relationship to alcohol intake. Because HDL-C is thought to play an important role in preventing atherosclerosis (Seppa *et al*., 1992), it has been proposed that alcohol protection occurs via increasing HDL-C. Sillanaukee *et al*., (2000) showed that all lipid values, except low density lipoprotein cholesterol (LDL-C), positively correlated with reported alcohol consumption.

1.1. Alcohols

Alcohols are compounds that have hydroxyl groups bonded to saturated carbon atoms. Alcohols can be thought of as organic derivative of water in which one of the water hydrogen is replaced by an organic group (Fig. 1).

H-O-H versus R-O-H.

Fig. 1: Formular of alcohol.

Alcohols occur widely in nature and have a great many industrial and pharmaceutical applications. Ethanol, for instance is one of the simplest yet best known of all organic substances, usually used as a fuel additive, an industrial solvent and a beverage.

Alcohols are classified as primary (1), secondary (2) or tertiary (3) depending on the number of the organic groups bonded to the hydroxyl bearing carbon and ethanol is a primary alcohol (Garrett and Grisham, 2005).

Ethanol was one of the first organic chemicals to be prepared and purified. Its production by fermentation of grains and sugar has been carried out for millennia and its purification by distillation. Only about five percent (5%) of the ethanol produced industrially comes from fermentation.

Most ethanol is currently obtained by acid-catalyzed hydration of ethylene (Fig. 2).

$H_2C=CH_2 + H_2O \text{ --------} \rightarrow CH_3CH_2OH$

Fig. 2: Equation for ethanol formation.

1.2. Lipids

Fats absorbed from the diet and lipids synthesized by the liver and adipose tissue must be transported between the various tissues and organs for utilization and storage. Since lipids are insoluble in water, the problem of how to transport them in the aqueous blood plasma is solved by associating non-polar lipids with amphipathic lipids and proteins to make water miscible lipoproteins. Lipids are transported in the plasma as lipoproteins.

1.2.1. Plasma Lipids

Plasma lipids consist of triacylglycerols, phospholipids, cholesterol and cholesteryl esters and a much smaller fraction of unesterified long chain fatty acids. These are metabolically the most active of the plasma acids. Because fat is less dense than water, the density of a lipoprotein decreases as the proportion of lipid to protein increases (Murray *et al*., 2006). Four major groups of lipoproteins have been identified that are physiologically important in clinical diagnosis. These are:

(a) Chylomicrons, derived from intestinal absorption of triacylglycerol and other lipids.

(b) Very low density lipoproteins (VLDL) derived from the liver for the export of triacylglycerols.

(c) Low density lipoproteins representing a final stage in the catabolism of VLDL.

(d) High density lipoproteins, involved in cholesterol transport and also in VLDL metabolism. Triacyglycerols are the predominant lipids in chylomicrons and VLDL, whereas cholesterol and phospholipids are the predominant lipids in LDL and HDL.

1.3. Liver's central role in lipid transport and metabolism

The liver carries out the following major functions in lipid metabolism.

(a) It facilitates the digestion and absorption of lipids by the production of bile, which contains cholesterol and bile salts synthesized within the liver *de novo* or from uptake of lipoprotein cholesterol.

(b) It actively synthesizes and oxidizes fatty acids and also synthesizes triacylglycerol and phospholipids.

(c) It converts fatty acids to ketone bodies.

(d) It plays an integral part in the synthesis and metabolism of plasma lipoprotein (Murray et al., 2006).

1.4. The effects of alcohol on the liver

Alcohol is metabolized by alcohol dehydrogenase (ADH) into acetaldehyde, then further metabolized by aldehyde dehydrogenase (ALDH) into acetic acid, which is finally oxidized into carbon dioxide (CO_2) and water (Inaba and Cohen 2004). This process generates NADH, and increases the NADPH/NADP+ ratio. A higher NADH concentration induces fatty acid synthesis while a decreased NAD level results in decreased fatty acid oxidation (Murray et al., 2006).

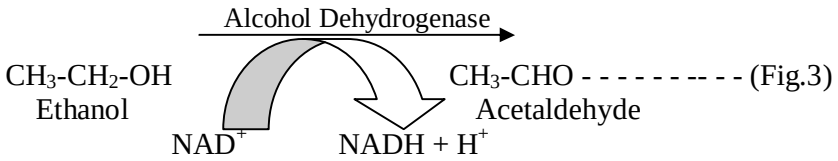


Fig. 3: Enzymic oxidation of ethanol.

Fatty liver or steatosis is the accumulation of fatty acids in liver cells. Alcoholism causes development of large fatty globules (macro vesicular steatosis) throughout the liver and can begin to occur after a few days of heavy drinking. Subsequently, the higher levels of fatty acids signal the liver cells to compound it to glycerol to form triacylglycerols. These triacylglycerols accumulate, resulting in fatty liver.

(a) Fatty liver

Alcoholic liver disease is a term that encompasses the hepatic manifestations of alcohol overconsumption, including fatty liver, alcoholic hepatitis, and chronic hepatitis with hepatic

fibrosis or cirrhosis (O shea et al.,2010). It is the major cause of liver disease in Western countries. Although steatosis (fatty liver) will develop in any individual who consumes a large quantity of alcoholic beverages over a long period of time, this process is transient and reversible (Menon et al.,2001). Of all chronic heavy drinkers, only 15ï 20% develops hepatitis or cirrhosis, which occurs in succession (Menon et al.,2001).

How alcohol damages the liver is not completely understood. 80% of alcohol passes through the liver to be detoxified. Chronic consumption of alcohol results in the secretion of pro-inflammatory cytokines, oxidative stress, lipid peroxidation, and acetaldehyde toxicity. These factors cause inflammation, apoptosis and eventually fibrosis of liver cells. Additionally, the liver has tremendous capacity to regenerate and even when 75% of hepatocytes are dead, it continues to function as normal (Longstreth et al.2009).

Pathophysiology

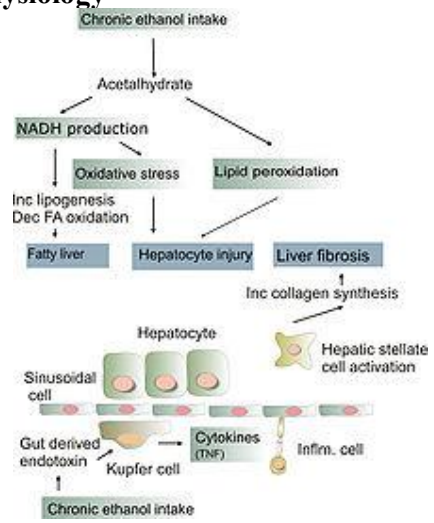


Fig. 4: Pathogenesis of alcohol induced liver injury [htt: //www Wikipedia, 2010].

(b) Alcoholic hepatitis

Alcoholic hepatitis is characterized by the inflammation of hepatocytes. Between 10% and 35% of heavy drinkers develop alcoholic hepatitis. While development of hepatitis is not directly related to the dose of alcohol, some people seem more prone to this reaction than others. This is called alcoholic steato necrosis and the inflammation appears to predispose to liver fibrosis. Inflammatory cytokines are thought to be essential in the initiation and perpetuation of liver injury by inducing apoptosis and necrosis. One possible mechanism for the increased activity of TNF-Ŭ is the increased intestinal permeability due to liver disease. This facilitates the absorption of the gut-produced endotoxin into the portal circulation. The

Kupffer cells of the liver then phagocytose endotoxin, stimulates the release of TNF-Ŭ. TNF-Ŭthen triggers apoptotic pathways through the activation of caspases, resulting in cell death (Menon et al., 2001).

(c) Cirrhosis

Cirrhosis is a late stage of liver disease marked by inflammation (swelling), fibrosis (cellular hardening) and damaged membranes preventing detoxification of chemicals in the body, ending in scarring and necrosis (cell death). Between 10% to 20% of heavy drinkers will develop cirrhosis of the liver. Acetaldehyde may be responsible for alcohol-induced fibrosis by stimulating collagen deposition by hepatic stellate cells The production of oxidants derived from NADPH oxidase and/or cytochrome P-450 2E1 and the formation of acetaldehyde-protein adducts damage the cell membrane (Menon et al.,2001).

Symptoms include jaundice (yellowing), liver enlargement, pain and tenderness from the structural changes in damaged liver architecture. Continued alcohol use, will eventually lead to liver failure. Late complications of cirrhosis or liver failure include portal hypertension (high blood pressure in the portal vein due to the increased flow resistance through the damaged liver), coagulation disorders (due to impaired production of coagulation factors), ascites (heavy abdominal swelling due to build up of fluids in the tissues) and other complications, including hepatic encephalopathy and the hepatorenal syndrome.

Cirrhosis can also result from other causes than alcohol abuse, such as viral hepatitis and heavy exposure to toxins other than alcohol. This phenomenon is termed the "final common pathway" for the disease.

Fatty change and alcoholic hepatitis with abstinence can be reversible. The later stages of fibrosis and cirrhosis tend to be irreversible, but can usually be contained with abstinence for long periods of time.

Lipids, mainly as triacylglycerol accumulate in the liver. Extensive accumulation is regarded as a pathological condition. When accumulation of lipids in the liver becomes chronic, fibrotic changes occur in the cells that progress to cirrhosis and impaired liver function (Plate 1).

Fatty liver falls into two main categories. The first type is associated with raised levels of plasma free fatty acids resulting from hydrolysis of lipoprotein triacyglycerols by lipoprotein lipase in extra hepatic tissues. The production of very low density lipoprotein (VLDL) does not keep pace with the increasing influx and esterification of fatty acids, allowing triacylglycerol to accumulate, causing fatty liver (Murray *et al*., 2006).

The second type of fatty liver is usually due to metabolic block in the production of plasma lipoproteins thus allowing triacylglycerol to accumulate. This may be due to one of the following;

(a) A block in apolipoprotein synthesis.

(b) A block in the synthesis of the lipoprotein from lipid and apolipoprotein.

(c) A failure in provision of phospholipids that are found in lipoprotein.

(d) A failure in the secretory mechanism itself.

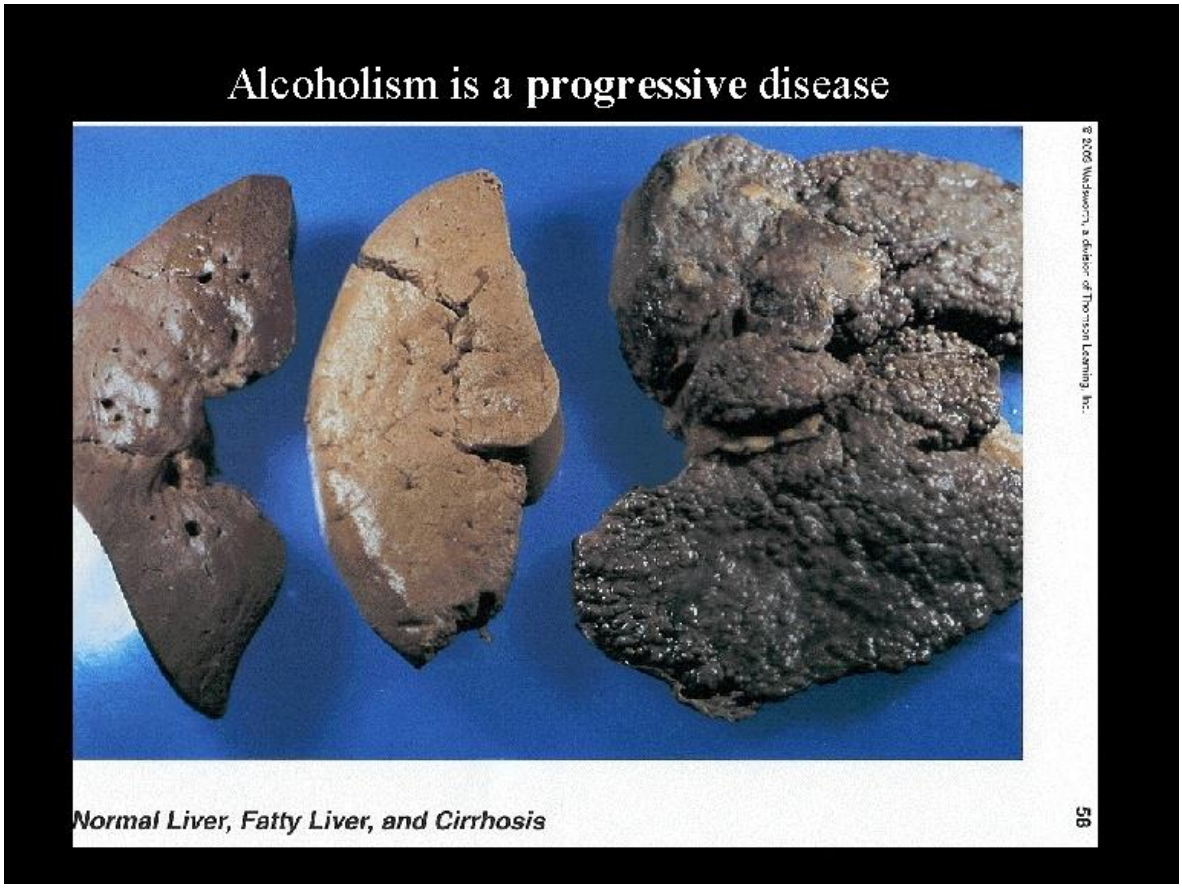


Plate 1: A cross section of the Normal Liver, Fatty Liver and Cirrhosis; [htt://www Wikipedia, 2010].

1.5 Cholesterol

Cholesterol is an amphipathic lipid and essential structural component of membranes and of the outer layer of plasma lipoproteins. Cholesterol is present in plasma either as free cholesterol or as a storage form, combined with a long-chain fatty acid as cholestery ester. In plasma, both forms are transported in lipoproteins. It is synthesized in many tissues from acetyl-CoA and is the precursor of all other steroids in the body. Cholesterol occurs in foods of animal origin such as egg yolk, meat, liver and brain. Cholesterol is a major constituent of

gallstones. However, its chief role in pathologic process is as a factor in the genesis of atherosclerosis of vital arteries, causing cerebrovascular, coronary, and peripheral vascular disease (Murray et al., 2006).

Cholesterol cannot travel alone through the blood stream, it has to combine with certain proteins. These proteins act like trucks, picking up the cholesterol and transporting it to different parts of the body. When this happens, the cholesterol and protein form a lipoprotein.

The two most important types of lipoproteins are high-density lipoproteins (HDL) and low-density lipoproteins (LDL). People call LDL cholesterol "bad cholesterol" and HDL cholesterol "good cholesterol" because of their very different effects on the body. Most cholesterol in the body are found in LDL, and this is the kind that is most likely to clog the blood vessels, keeping blood from flowing through the body the way it should (Murray *et al*., 2006).

On the other hand, HDL cholesterol removes cholesterol from the blood vessels and carries it back to the liver, where it can be processed and sent out of the body.

1.5.1. Biosynthesis

All animal cells manufacture cholesterol with relative production rates varying by cell type and organ function. About 20ï 25% of total daily cholesterol production occurs in the liver; other sites of higher synthesis rates include the intestines, adrenal glands, and reproductive organs. Synthesis within the body starts with one molecule of acetyl CoA and one molecule of acetoacetyl-CoA, which are hydrated to form 3-hydroxy-3-methylglutaryl CoA (HMG-CoA). This molecule is then reduced to mevalonate by the enzyme HMG-CoA reductase. This is the regulated, rate-limiting and irreversible step in cholesterol synthesis and is the site of action for the statin drugs (HMG-CoA reductase competitive inhibitors).

Mevalonate is then converted to 3-isopentenyl pyrophosphate in three reactions that require ATP. Mevalonate is decarboxylated to isopentenyl pyrophosphate, which is a key metabolite for various biological reactions. Three molecules of isopentenyl pyrophosphate condense to form farnesyl pyrophosphate through the action of geranyl transferase. Two molecules of f arnesyl pyrophosphate then condense to form squalene by the action of squalene synthase in the endoplasmic reticulum. Oxidosqualene cyclase then cyclizes squalene to form lanosterol. Finally, lanosterol is converted to cholesterol through a 19-step process (Rhodes et al.,1995).

1.5.2. Functions of cholesterol

Cholesterol has several functions. These include;

It builds and maintains cell membranes (outer layer), it prevents crystallization of hydrocarbons in the membrane.

It is essential for determining which molecules can pass into the cell and which cannot (cell membrane permeability).

It is involved in the production of sex hormones (androgens and estrogens).

It is essential for the production of hormones released by the adrenal glands (cortisol, corticosterone, aldosterone, etc).

It aids in the production of bile.

It converts sunshine to vitamin D.

It is important for the metabolism of fat soluble vitamins, including vitamins A, D, E, and K.

It insulates nerve fibers [htt://www Wikipedia, 2010].

Cholesterol cannot travel alone through the blood stream; it has to combine with certain proteins. These proteins act like trucks, picking up the cholesterol and transporting it to different parts of the body. When this happens, the cholesterol and protein form a lipoprotein. The two most important types of lipoproteins are high-density lipoproteins (HDL) and low-density lipoproteins (LDL). Most cholesterol in the body are found in LDL, and this is the kind that is most likely to clog the blood vessels, keeping blood from flowing through the body the way it should (Murray *et al*., 2006).

On the other hand, HDL cholesterol removes cholesterol from the blood vessels and carries it back to the liver, where it can be processed and sent out of the body.

1.6 High-Density Lipoprotein

High-density lipoprotein (HDL) is one of the five major groups of lipoproteins which, in order of sizes, largest to smallest, are chylomicrons, VLDL, IDL, LDL and HDL, which enable lipids like cholesterol and triacylglycerols to be transported within the water-based bloodstream. In healthy individuals, about thirty percent of blood cholesterol is carried by HDL (Kwiterovich, 2000).

Blood tests typically report HDL-C level, i.e., the amount of cholesterol contained in HDL particles. It is often contrasted with low density lipoprotein or LDL-cholesterol (LDL-C). HDL particles are able to remove cholesterol from within artery atheroma and transport it back to the liver for excretion or re-utilization, which is the main reason why the cholesterol carried within HDL particles (HDL-C) is sometimes called "good cholesterol". Those with

higher levels of HDL-C seem to have fewer problems with cardiovascular diseases, while those with low HDL-C cholesterol levels have increased rates for heart disease (Barter *et al*., 2007).

1.6.1 Structure and Functions of High Density Lipoprotein

HDL is the smallest of the lipoprotein particles. They are the most dense because they contain the highest proportion of protein and cholesterol. The most abundant apolipoproteins are apo A-I and apo A-II (Lin *et al*., 1998). The liver synthesizes these lipoproteins as complexes of apolipoproteins and phospholipid, which resemble cholesterol-free flattened spherical lipoprotein particles. They are capable of picking up cholesterol, carried internally, from cells by interaction with the ATP-binding cassette transporter A1 (ABCA1). 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 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 triacylglycerols 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. The triacylglycerols 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 [http://www.wikipedia., 2010].

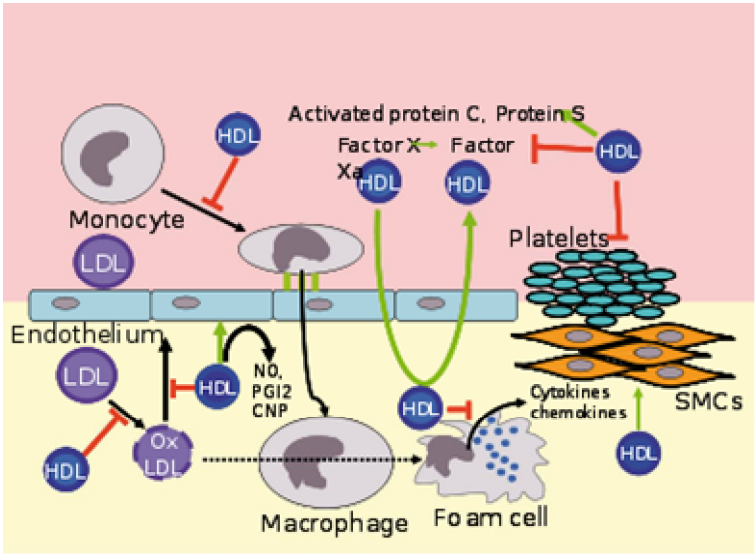


Fig 5: Structure of high density lipoproteins showing the reverse cholesterol transport [Der Gaag et al., 2001].

Several steps in the metabolism of HDL can contribute to the transport of cholesterol from lipid-laden macrophages of atherosclerotic arteries, to the liver for secretion into the bile. This pathway has been termed reverse cholesterol transport and is considered as the classical protective function of HDL toward atherosclerosis (Der Gaag *et al.*, 2001).

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. In the stress response, serum amyloid A, which is one of the acute-phase proteins and an apolipoprotein, is under the stimulation of cytokines (IL-1, IL-6). Cortisol produced in the adrenal cortex and carried to the damaged tissue incorporated into HDL particles. At the inflammation site, it attracts and activates leukocytes. In chronic inflammations, its deposition in the tissues manifests itself as amyloidosis.

It has been postulated that the concentration of large HDL-C particles reflects protective action, as opposed to the concentration of total HDL-C particles (Bairaktari *et al.*, 1999). This ratio of large HDL-C to total HDL-C particles varies widely and is measured only by more sophisticated lipoprotein assays using electrophoresis.

Men tend to have noticeably lower HDL-C levels, and lower cholesterol content, than women. Men also have an increased incidence of atherosclerotic heart disease. Alcohol consumption tends to raise HDL-C levels (Hendrik 1998), and moderate alcohol consumption is associated with lower rate of cardiovascular disease. Epidemiological studies have shown that high concentrations of HDL-C have protective value against cardiovascular diseases such

as ischemic stroke and myocardial infarction. Low concentrations of HDL-C increase the risk for atherosclerotic diseases.

Even people with very low LDL-C levels are exposed to increased risk if their HDL-C levels are not high enough (Hirano *et al*., 2008).

HDL-C is beneficial for a number of reasons. The most important is its ability to drive a process called "reverse cholesterol transportò (Der Gaag *et al*., 2001). HDL-C is a mop in that it helps to extract excess cholesterol deposited in blood vessel walls and deliver it back to the liver for elimination through the gastrointestinal tract ((Hendrik 1998). The higher the HDL-C, the greater the capacity to remove cholesterol and prevent dangerous blockages from developing in the blood vessels. HDL-C helps to keep your blood vessels widened (dilated), thereby promoting better blood flow. HDL-C also reduces blood vessel injury through its antioxidant and anti-inflammatory functions, among other effects.

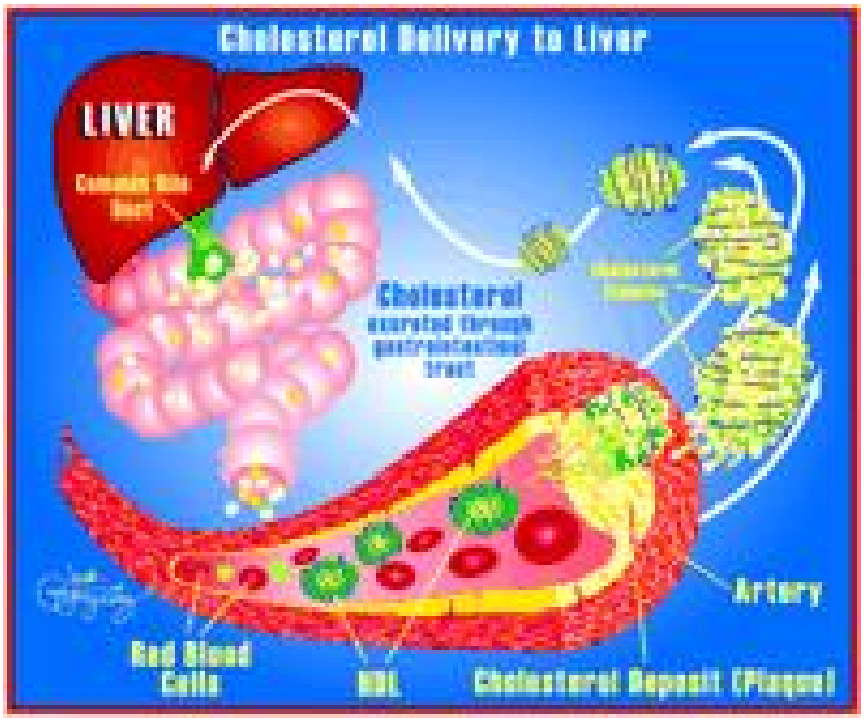


Fig. 6: Pathway for the reverse of cholesterol transport

Fig.6 illustrates the schematic depiction of reverse cholesterol transport. As HDL-C in blood penetrates atherosclerotic plaque in the vessel wall, it mobilizes and binds cholesterol and transports it back to the liver for elimination through the gastrointestinal tract [http://www.wikipedia, 2010].

1.6.2. Medications That Raise High Density Lipoprotein

For many patients, lifestyle modification may not be enough to achieve adequate elevations in HDL-C. Heredity plays an important role in regulating the level of HDL-C. Mutations in one or more genes can give some people a very high level of HDL-C and predispose others to very low levels of HDL-C. Many patients will require the combination of medication with lifestyle modification.

A number of medications can have an impact on blood levels of HDL-C (Spate-Douglas and Keyser, 1999). The statins have been shown to reduce the risk of heart attack and death in patients with high LDL-C and low HDL-C. Fibrates (gemfibrozil, fenofibrate) are effective therapy for patients with high triacylglycerols and low HDL-C. Niacin is the most potent drug currently available for raising HDL-C and has been shown to reduce the risk of heart attack and stroke in patients with heart disease. Several forms of niacin are available, but dietary-supplement niacin must not be substituted for the niacin that the doctor prescribes because the supplement can cause significant liver injury. When taking a statin and niacin in combination, patients with low HDL-C should not take vitamin E, vitamin C, or beta-carotene supplements because these agents appear to impair the ability of statins and niacin to raise HDL-C.

1.6.3. Diet and Lifestyle

Certain changes in lifestyle may have a positive impact on raising HDL-C levels:

Aerobic exercise

Weight loss

Nicotinic Acid supplementation

Smoking cessation

Removing trans fatty acids from the diet

Mild to moderate alcohol intake (Hendrik 1998).

Adding soluble fiber to diet

Using supplements such as omega 3 fish oil or flax oil.

Increasing intake of *cis*-unsaturated fats and cholesterol [http://www.wikipedia., 2010].

Most saturated fats increase HDL cholesterol to varying degrees but also raise total and LDL cholesterol. A high-fat, adequate-protein, low-carbohydrate ketogenic diet may have similar response to taking niacin as described above (lowered LDL and increased HDL) through beta-hydroxybutyrate coupling the Niacin receptor 1 (Meyers *et al*., 2003).

1.7. Low-Density Lipoprotein cholesterol

Low-density lipoprotein cholesterol(LDL-C) is one of the five major groups of lipoproteins, which in order of size, largest to smallest, are chylomicrons, VLDL, IDL, LDL and HDL, that enable lipids like cholesterol and triacylglycerol to be transported within the water-based bloodstream. Blood tests typically report LDL-C, the amount of cholesterol contained in LDL. In clinical context, mathematically calculated estimates of LDL-C are commonly used to estimate how much low density lipoproteins are driving progressions of atherosclerosis.
Direct LDL measurements are also available and better reveal individual issues but are less often promoted or done due to slightly higher costs and being available from only a couple of laboratories in the United States.

1.7.1. Biochemistry of Low density lipoprotein cholesterol

Each native LDL particle contains a single apolipoprotein B-100 molecule (Apo B-100, a protein with 4536 amino acid residues), which circulates the fatty acids, keeping them soluble in the aqueous environment. In addition, LDL-C has a highly-hydrophobic core consisting of a 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 a single copy of B-100 large protein (514 kD). LDL-C particles are approximately 22nm (0.00000087 in.) in diameter and have a mass of about 3 million daltons, but since LDL-C particles contain a changing number of fatty acids, they actually have a distribution of mass and size (Segrest *et al.*, 2001). Determining structure of LDL-C has been a tough task because of cits heterogeneous structure. First structure of LDL-C at human body temperature in native condition has been recently found using cryo-electron microscopy and it has resolution of 16 Angstrom (Kumar *et al.*, 2011).

1.7.2. LDL-C Subtype Patterns

LDL-C particles vary in size and density. Studies have shown that a pattern that has more small dense LDL-C particles, called Pattern B, equates to a higher risk factor for coronary heart disease (CHD) than does a pattern with more of the larger and less dense LDL-C particles (Pattern A). This is because the smaller particles are more easily able to penetrate the endothelium. Pattern I, for intermediate, indicates that most LDL particles are very close in size to the normal gaps in the endothelium (26 nm).
The correspondence between Pattern B and CHD has been suggested by some in the medical community to be stronger than the correspondence between the LDL number measured in the standard lipid profile test. Tests to measure these LDL subtype patterns have been more

expensive and not widely available, so the common lipid profile test has been used more commonly (Superko *et al*., 2002; Warnick *et al*., 1990).

1.7.3. Transport into the Cell

When a cell requires cholesterol, it synthesizes the necessary LDL-C receptors and inserts them into the plasma membrane. The LDL-C receptors diffuse freely until they associate with clathrin-coated pits. LDL-C particles in the blood stream bind to these extracellular LDL-C 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-C and their receptors to early endosomes, onto late endosomes to lysosomes. Here the cholesterol esters in the LDL-C are hydrolysed. The LDL-C receptors are recycled back to the plasma membrane. Because LDL-C particles can also transport cholesterol into the formation of plaques, increased levels are associated with atherosclerosis. Over time vulnerable plaques rupture, activate blood clotting and produce arterial stenosis, which if severe enough results in heart attack, stroke, and peripheral vascular disease symptoms and major debilitating events.

Increasing evidence has revealed that the concentration and size of the LDL particles more powerfully relates to the degree of atherosclerosis progression than the concentration of cholesterol contained within all the LDL particles. The healthiest pattern A, though relatively rare, is to have small numbers of large LDL particles and no small particles. Having small LDL particles, though common, is an unhealthy pattern B; high concentrations of small LDL-C particles (even though potentially carrying the same total cholesterol content as a low concentration of large particles) correlates with much faster growth of atheroma, progression of atherosclerosis and earlier and more severe cardiovascular disease events and death.

A hereditary form of high LDL-C is familial hypercholesterolemia. Increased LDL is termed hyperlipoproteinemia type II. LDL-C particles pose a risk for cardiovascular disease when they invade the endothelium and become oxidized, since the oxidized forms are more easily retained by the proteoglycans. A complex set of biochemical reactions regulates the oxidation of LDL-C particles, chiefly stimulated by presence of necrotic cell debris and free radicals in the endothelium.

1.7.4. Agents that effect the lowering of LDL-C

The mevalonate pathway serves as the basis for the biosynthesis of many molecules, including cholesterol. The enzyme, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase) is an essential component in the pathway.

(a) Pharmaceutical

Statins reduce high levels of LDL particles by inhibiting the enzyme HMG-CoA reductase in cells and the rate-limiting step of cholesterol synthesis. To compensate for the decreased cholesterol availability, synthesis of hepatic LDL receptors is increased, resulting in an increased clearance of LDL particles from the blood. Ezetimibe reduces intestinal absorption of cholesterol and thus can powerfully reduce LDL particle concentrations when combined with statins.

Niacin (B_3), lowers LDL-C by selectively inhibiting hepatic diacyglycerol acyltransferase 2, reducing triacylglycerol synthesis and VLDL-C secretion through a receptor HM74 and HM74A or GPR109A (Peterson et al, 2008).

Clofibrate is effective at lowering cholesterol levels, but has been associated with significantly increased cancer and stroke mortality, despite lowered cholesterol levels. Other, more recently developed and tested fibrates, e.g. fenofibric acid, have had a better track record and are primarily promoted for lowering VLDL particles (triacylglycerol), not LDL-C particles.

Some Tocotrienols, especially delta- and gamma-tocotrienols, are being promoted as statin alternative non-prescription agents to treat high cholesterol, having been shown in vitro to have an effect. In particular, gamma-tocotrienol appears to be another HMG-CoA reductase inhibitor and can reduce cholesterol production. As with statins, this decrease in intra-hepatic (liver) LDL levels may induce hepatic LDL receptor up-regulation, also decreasing plasma LDL levels (Peterson et al., 2008). As always, a key issue is how the benefits and complications of such agents will compare with the statins and molecular tools which have been analyze since the mid-1970s, in large numbers of human research and clinical trials.

(b) Dietary

The most effective approach has been minimizing visceral body fat stores. Visceral fat, which is more metabolically active than subcutaneous fat, has been found to produce many enzymatic signals, e.g. resistin, which increase insulin resistance and circulating VLDL particle concentrations, thus both increasing LDL particle concentrations and accelerating the development of Diabetes Mellitus.

Insulin induces HMG-CoA reductase activity, whereas glucagon diminishes it. While glucagon production is stimulated by dietary protein ingestion, insulin production is stimulated by dietary carbohydrate. The rise of insulin is determined by the digestion of carbohydrates into glucose and subsequent increase in serum glucose levels. In non-diabetics, glucagon levels are very low when insulin levels are high; however, those who have become diabetic no longer suppress glucagon output after eating.

A ketogenic diet may have similar response to taking niacin (lowered LDL and increased HDL) through beta-hydroxybutyrate, a ketone body, coupling the niacin receptor (HM74A).

Lowering the blood lipid concentration of triacylglycerol helps lower the concentration of small LDL particles, because fat rich VLDL particles convert in the bloodstream into small LDL particles. Fructose a component of sucrose upregulates hepatic VLDL synthesis.

1.8. Triacylglycerols

Triacylglycerols (TAG) are esters derived from glycerol and three fatty acids (Daley *et al.*,2004). It is the main constituent of vegetable oil and animal fats (Balch and Phyllis, 2006).

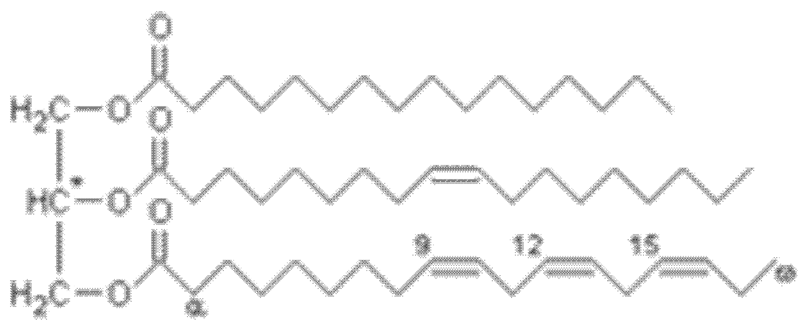


Fig. 7: The structure of triacylglycerol

In triacylglycerol, the hydroxyl groups of the glycerol join the carboxyl groups of the fatty acid to form ester bonds

$HOCH_2CH(OH)CH_2OH$ + RCO_2H + $R'CO_2H$ + $R''CO_2H$ Ÿ $RCO_2CH_2CH(O_2CR')CR''$ + $3H_2O$ ---(equation 2)

Fig. 8: Equation of esterification.

The three fatty acids (RCO_2H, $R'CO_2H$, $R''CO_2H$ in the above equation) are usually different, but many kinds of triacylglycerols are known. The chain lengths of the fatty acids in natural occurring triacylglycerols vary, but most contain 16, 18, or 20 carbon atoms. Natural fatty acids found in plants and animals are typically composed of only even numbers of carbon

atoms, reflecting the pathway for their biosynthesis from the two-carbon building-block, acetyl-CoA. Bacteria, however, possess the ability to synthesize odd- and branched-chain fatty acids. As a result, ruminant animal fat contains odd-numbered fatty acids, such as 15, due to the action of bacteria in the rumen. Many fatty acids are unsaturated, some are polyunsaturated, e.g., those derived from linoleic acid [htt://www Wikipedia, 2010].

1.8.1. Metabolism

The enzyme pancreatic lipase acts at the ester bond, hydrolyzing the bond and "releasing" the fatty acid. In triacylglycerol form, lipids cannot be absorbed by the duodenum. Fatty acids, monoacylglycerols, and some diacylglycerols are absorbed by the duodenum, once the triacylglycerols have been broken down.

Triacylglycerols are major components of very-low-density lipoprotein (VLDL) and chylomicrons. They play an important role in metabolism as energy sources and transporters of dietary fat. They contain as much twice of energy (9Kcal/g or 38KJ/g) as carbohydrates and proteins. In the intestine, triacylglycerols are split into monoacylglycerol and free fatty acids in a process called lipolysis. Lipases catalyze lipolysis. The triacylglycerols are rebuilt in the enterocytes from their fragments and packaged together with cholesterol and proteins to form chylomicrons. These are excreted from the cells and collected by the lymph system and transported to the large vessels near the heart before being mixed into the blood. Various tissues can capture the chylomicrons, releasing the triacylglycerols to be used as a source of energy. Fat and liver cells can synthesize and store triacylglycerols. When the body requires fatty acids as an energy source, the hormone glucagon signals the breakdown of the triacylglycerols by hormone-sensitive lipase to release free fatty acids. As the brain cannot utilize fatty acids as an energy source the glycerol component of triacylglycerols can be converted into glucose, via glyconeogenesis by conversion into dihydroxyacetone phosphate and then into glyceraldehyde 3-phosphate, and subsequently for glucose.

Triacylglycerols cannot pass through cell membranes freely. Special enzymes on the walls of blood vessels called lipoprotein lipases must break down triacylglycerols into free fatty acids and glycerol. Fatty acids can then be taken up by cells via the fatty acid transporter (FAT).

1.8.2. Role in disease

In the human body, high levels of triacylglycerols 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 triacylglycerols compared to that of LDL:HDL ratios is as

yet unknown. The risk can be partly accounted for by a strong inverse relationship between triacylglycerol levels and HDL-cholesterol level.

Diets high in carbohydrates, with carbohydrates accounting for more than 60% of the total caloric intake, can increase triacylglycerol levels. Insulin resistance is a primary suspect cause of this phenomenon of carbohydrate-induced hypertriglyceridemia (Hemat, 2003).

There is evidence that carbohydrate consumption causing a high glycemic index can cause insulin overproduction and increase triacylglycerols level in women.

Adverse changes associated with carbohydrate intake, including triacylglycerol levels, are stronger risk factors for heart disease in women than in men.

Triacylglycerol levels are also reduced by exercise, omega-3 fatty acids from fish, flax seed oil and other sources.

Carnitine has the ability to lower blood triacylglycerol levels. In some cases, fibrates have been used to bring down triacylglycerol levels substantially.

Heavy use of alcohol can elevate triacylglycerol levels (Despress, 2009).

1.9. Serum Total Protein

The total protein test measures the total amount of two classes of proteins found in the fluid portion of the blood: albumin and globulin.

Proteins are important parts of all cells and tissues. Albumin helps prevent fluid from leaking out of blood vessels. Globulins are an important part of your immune system.

The globulin in turn is made up of Ŭ1, Ŭ2, ϐ, and ɔ globulins. These fractions can be separated using protein electrophoresis, but the total protein test is a faster and cheaper test that estimates the total of all fractions together. The traditional method for measuring total protein uses the biuret reagent, but other chemical methods such as Kjeldahl method, dye-binding and refractometry are now available. The measurement is usually performed on automated analyzers along with other laboratory tests [htt://www Wikipedia, 2010].

1.10. Liver enzymes

1.10.1. Alanine (ALT) and Aspartate (AST)

AST and ALT are jointly known as transaminses. They are associated with inflammation and/or injury to liver cells, a condition known as hepatocellular liver injury. Damage to the liver typically results in a leak of AST and ALT into the bloodstream. Because AST is found in many other organs besides the liver, including the kidneys, the muscles and the heart, having a high level of AST does not always (but often does) indicate that there is a liver problem. For example, even vigorous exercise may elevate AST levels in the body. On the

other hand, because ALT is found primarily in the liver, high levels of ALT almost always indicate that there is a problem with the liver. Conversely, a normal ALT level does not necessarily mean that the liver is definitely normal. Despite what one might expect, high levels of transaminases in the blood do not always reveal just how badly the liver is inflamed or damaged. This is an extremely important point to keep in mind. The normal ranges for AST and ALT are around 0 to 40IU/L and 0 to 45 IU/L respectively. But someone who has an ALT level of 50 IU/L is not necessarily in a better condition than someone with an ALT level of 250 IU/L. This is because; these blood tests measure inflammation and damage to the liver at an isolated point in time. For instance, if the liver is inflamed on the day that blood was drawn ï letôs say if a patient consumes an alcoholic drink a few hours to blood being drawn- the levels of the transaminases may be much higher than if the alcohol had not been consumed. Following the same reasoning if the liver was damaged years before-by excessive alcohol use, the results of a blood test done today may be normal, but a damaged liver may still be present.

The ratio of the ALT and AST may also provide useful information regarding the extent and cause of liver disease. Most liver diseases are characterized by greater ALT elevations than AST elevations. Two exceptions to this rule exist. Both cirrhosis and /or alcohol abuse are associated with higher AST levels than ALT levels, often in a ratio of approximately 2:1[http://www.wikipedia., 2010].

Elevations of the transaminases occur due to so many causes that they give the doctor only a vague clue of the diagnosis. Additional testing is required in order to determine more precisely what is wrong with the liver. Some possible causes of elevated transaminase levels include the following:

Viral hepatitis, a fatty liver, alcoholic liver disease, drug/medication-induced liver disease, autoimmune hepatitis, herbal toxicity, genetic liver diseases, liver tumors, heart failure and strenuous exercise. [http://www.wikipedia., 2010].

1.10.2. Gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) (Cholestatic Liver Enzymes)

High levels of GGT and ALP hint at a possible blockage of the bile ducts, or of possible injury to, or inflammation of the bile ducts. This type of problem is characterized by an impairment or failure of bile flow, which is known as cholestasis. This type of liver injury is known as cholestatic liver injury a the disease is known as cholestatic liver disease. Intrahepatic cholestasis refers to bile duct blockage or injury within the liver. Intrahepatic

cholestasis may occur in people with primary biliary cirrhosis or liver cancer for example. Extrahepatic cholestasis refers to bile duct blockage or injury occurring outside the liver. Extrahepatic cholestasis may occur in people with gallstones.

When a blockage or inflammation of the bile ducts occurs, the GGT and ALP can overflow like a backed up sewer and seep out of the liver into the bloodstream. These enzymes typically become markedly elevated-approximately ten times the upper limit of normal.

GGT is found predominantly in the liver. ALP is mainly found in the bones and the liver but can also be found in many other organs, such as the intestines, kidneys, and placenta.Therefore, elevated levels of ALP will indicate that something is wrong with the liver only if the amount of GGT is raised as well. It should be noted that for unclear reasons, people who smoke cigarettes appear to have higher ALP and GGT than nonsmokers. Also, levels of ALP and GGT are most accurate after a twelve- hour fast. All these demonstrate the complexities that arise when evaluating abnormal liver function test.

Primary biliary cirrhosis, Primary sclerosing cholangitis, Nonalcoholic fatty liver disease, Alcoholic liver disease and Liver tumors increase the level of ALP [http://www.wikipedia., 2010].

1.11. JUSTIFICATION OF THE STUDY

ndThe justification of this study is to elucidate the effects of ethanol administration on some biochemical parameters. It is known that excessive ethanol in the body increases the level of these parameters and thus have adverse effects on the liver.

1.12. OBJECTIVES OF THE STUDY

The major objective of the present study is to investigate the effects of the alcohol (ethanol) administration on some biochemical parameters in rats.

1.12.1. Specific Objectives

The work is designed to achieve the following specific objectives:

i) To determine the effect of ethanol on the serum lipid profile (total cholesterol, high density lipoprotein and triacylglycerols) in rats.
ii) To determine the effect of ethanol on the activities of liver enzymes in rats.
iii) To determine the effect of ethanol on serum total protein concentrations in rats.
iv) To determine histopathology of the liver in rats.

CHAPTER TWO

MATERIALS AND METHODS

MATERIALS

Animals

A total of 25 animals (wistar rats 190-200g) were used. The animals were grouped so that the mean difference in the various groups would not vary significantly. Each group was administered with a specified concentration of 0%, 1%, 3%, 5% and 10% ethanol respectively. The treatment was carried out for three months and analysis of the parameters done on a four-week basis. The ethanol was administered intraperitoneally.

2.1.2. Chemicals

All chemicals used for this study were of analytical grade and products (of kits) of Randox Laboratories Limited, 55 Diamond Road, Crumlin Country Antrim, BT29 4QY, United Kingdom and Biosystem S.A Costa Brava 30 Barcelona (Spain). Quality system certified according to EN 150 13485 and EN 150 9001 standards.

2.1.3. Equipment/instrument

Capillary tubes; for collection of blood sample from the eyes of the rat into EDTA tubes.

Centrifuge (MSC bench); for spinning the blood samples to form serum which is used as sample test.

Micro pipettes; (0-500 micro litres) to pipette the test samples into the test tubes.

Sample tubes; for blood samples collection.

Syringe and needles (10ml); for administration of ethanol into the rats intraperitoneally.

Spectrophotometer (W.P.A, C.7.5, England); to measure the intensity of light with a specified wavelength.

Test tubes; for running the test in the laboratory.

2.1.4. Preparation of reagents

How the percentage of alcohol was determined. The various percentages were determined by mixing different (ml) of absolute alcohol in distilled water.

Ethanol (1%): Ethanol (1ml) was mixed with distilled water and made up to a volume of 100ml.

Etahanol (3%): Ethanol (3ml) was mixed with distilled water and made up to a volume of 100ml.

Ethanol (5%): Ethanol (5ml) was mixed with distilled water and made up to a volume of 100ml.

Ethanol (10%): Ethanol (10ml) was mixed with distilled water and made up to a volume of 100ml.

Reagents for lipid profile

Reagent for total cholesterol; BioSystems Cholesterol IVD REF/11505

Biosystem S.A Costa Brava 30, Barcelona (Spain)

Quality system certified according to EN 150 13485 and EN 150 9001 standard

Reagent kit for high density lipoprotein cholesterol;

Biosystem S.A Costa Brava 30, Barcelona (Spain)

Quality system certified according to EN 150 13485 and EN 150 9001 standard.

Reagent kit for Triacylglycerol;

Randox kit triacylglycerol standard let NUMBER: 832TR

Randox Laboratories Ltd, 55 Diamond Road Crumin, CO. Antrim, United Kingdom BT 29 4QY.

Total protein:

Reagent kit for Total protein was Randox Total protein kit made by Randox Laboratories Ltd, 55 Diamond Road Crumin, CO. Antrim, United Kingdom BT 29 4QY.

Reagents for liver enzymes.

Alanine aminotransferase;

Reagent kit for alanine aminotranferase was Randox alanine ammotransferase kit made by Randox Laboratories Ltd, 55 Diamond Road Crumin, CO. Antrim, United Kingdom BT 29 4QY.

Aspartate aminotransferase;

Reagent kit for aspartate aminotransferase was Randox enzyme aspartate aminotransferase kit.

Alkaline phosphatase;

Reagent kit for alkaline phosphatase was Randox enzyme alkaline phosphatase kit

2.2 Methods

2.2.1 Experiment Design

Group 1: Group 1 rats (control) were given water ad libitum.

Group 2: Group 2 rats were administered 1% ethanol.

Group 3: Group 3 rats were administered 3% ethanol.

Group 4: Group 4 rats were administered 5% ethanol.

Group 5: Group 5 rats were administered 10% ethanol.

All the rats were administered Intraperitoneally (I.P).

2.2.2 Collection of samples

The male wistar rats were bought from Faculty of Vetenary Medicine, University of Nigeria Nsukka (UNN). They rats were fed and housed in a central animal house (Department of Biochemistry). The alcohol treatment was given. The treatment was carried out for three months and the analysis of the parameters was done on a four week basis. The blood samples of various groups were collected with the use of capillary tubes through their eyes into the EDTA tube (sample tubes).

2.2.3 Preparation of samples

The blood samples collected from the treated rat and the control were spinned in a centrifuge to separate the blood components at 4000r.p.m for 10 minutes. The serum (Supernatant) was separated from the plasma with micropipette into another test tube.

2.2.4 Assays

2.2.4.1 Determination of total cholesterol level

The concentration of the serum total cholesterol was determined according to the method of Assman et al. (1984).

Principle

Cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-amino antipyrine in the presence of phenol and peroxidase.

$$\text{Cholesterol ester} + H_2O \xrightarrow[\text{esterase}]{\text{Cholesterol}} \text{cholesterol} + \text{Fatty acid} \quad \text{sé é é é é é .. (1)}$$

$$\text{Cholesterol ester} + H_2O \xrightarrow[\text{Oxidase}]{\text{Cholesterol}} \text{cholestene-3-one} + H_2O_2 \quad \text{.....(2)}$$

$$2H_2O_2 + \text{Phenol} + \text{4ï Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4\,H_2O_2 \quad \text{....(3)}$$

Reagents

Contents	**Initial Concentration of Solution**
4 ï Aminoantipyrine	0.30mmol/l
Cholesterol esterase	Ó0.2u/ml
Cholesterol oxidase	Ó0.1u/ml
Phenol	6.0mml/l
Peroxide	Ó0.5U/ml
Pipes buffer	80mmol/l
Standard	5.17mmol/l

Protocol

	Reagent Blank (B)	Standard (ST)	Sample S_1	S_2	S_3	S_4	S_5
Distilled water	10ul	-	-	-	-	-	-
Sample	-	10ul	-	-	-	-	-
Standard (ST)	-	-	10ul	10ul	10ul	10ul	10ul
Reagent	10ul	10ul	10ul	10ul	10ul	10ul	10ul

Procedure

Three sets of test tubes labeled reagent blank (B), standard (ST), and five sample tubes (S) were set up. Distilled water (10Ul) was delivered into the test tube (B) only and 10Ul of the standard solution was delivered into the test tube (ST). Also, 10Ul of serum sample was delivered into the test tubes (S). Then, 1.0ml of the reagent was added to all the three sets of test tubes. The contents of the test tubes were mixed and incubated in a water bath at 37^{o}C for 5 minutes. The absorbance of the sample (A_{sample}) was read against the reagent blank (B) within 60 minutes at 500nm. Also, the absorbance of the standard ($A_{standard}$) was read at 500nm. The concentration of cholesterol in the serum samples was calculated using the formula:

$$\text{Conc. of cholesterol in sample} = \frac{A_{Sample}}{A_{S\,\text{tan}\,dard}} \times \text{Conc. of standard (150mg/dl)}$$

2.2.4.1.2 Determination of high density lipoprotein (HDL)-cholesterol level

Concentration of HDL cholesterol was determined according to the method of Assman et al. (1984).

Principle

Very low density lipoprotein (VLDL) and low density lipoprotein (LDL) in the sample precipitate with phosphofungstate and magnesium ions. The supernatant contains HDL-cholesterol. The reactions described below.

Cholesterol ester + H_2O $\xrightarrow[\text{esterase}]{\text{Cholesterol}}$ Cholesterol + Fatty acidsé é é é é .. (1)

Cholesterol ester + H_2O $\xrightarrow[\text{Oxidase}]{\text{Cholesterol}}$ Cholestene-3-one + H_2O_2 .....(2)

$2H_2O_2$ + Phenol + 4ï Aminoantipyrine $\xrightarrow{\text{Peroxidase}}$ Quinoneimine + 4 H_2O_2 .....(3)

Reagents

Contents	**Initial Concentration of Solution**
a) Magnesium chloride	20mmol/l
Phosphotungstate	0.4mmol/l
b) 4 ï Aminoantipyrine	0.50mmol/l
Cholesterol esterase	Ó0.2u/ml
Cholesterol oxidase	Ó0.1u/ml
Dichlorophenolsulfonate	4mmol/l
Peroxidase	Ó1u/ml
Phosphate	35mmol/l
c) Standard	15mg/dl

Protocol

	Reagent Blank (B)	Standard (ST)	Sample S_1	S_2	S_3	S_4	S_5
Sample(serum)			0.2ml	0.2ml	0.2ml	0.2ml	0.2ml
Reagent(A1)			0.5ml	0.5ml	0.5ml	0.5ml	0.5ml l
Sample(supernatant)			50ul	50ul	50ul	50ul	50ul
Reagent(B)	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml
Distilled water	50ul						
Standard		50ul					

Procedure

Serum samples (0.2ml) were added to the five test tubes labeled samples (S). Then, 0.5ml of the reagent (A1) was added to each of the test tubes and the contents mixed thoroughly and left to stand for 10 minutes at room temperature.

The content of all the test tubes were centrifuged at 5000 r.p.m for 10 minutes and the supernatant was carefully collected. The reagent (B) was brought to room temperature.

Sample supernatant (50Ul) was added to the test tubes labeled samples (S). Then, 1.0ml of the reagent (B) was added to each of the test tubes and content mixed thoroughly. To another test tube labeled blank (B) was added 1.0ml of reagent B and 50Ul of distilled water only. Also to another test tube labeled standard (ST) reagent B 1.0ml and the HDL-cholesterol standard (ST) 50Ul were added only.

The constituents of all the test tubes were mixed and incubated for 10 minutes at room temperature (37^oC). Immediately after incubation, the absorbances of the samples (S) were read against the sample blank at a wavelength of 550nm. The HDC-cholesterol concentration in the sample was obtained from the formula below

$$\text{HDL-C} = \frac{A_{Sample}}{A_{S\,\text{tan}\,dard}} \times \text{Conc of standard (52.5mg/dl)}$$

2.2.4.1.3. Determination of low density lipoprotein-cholesterol level

The concentration of low density lipoprotein (LDL) was determined according to the method of Assmann *et al*. (1984).

Principle

Low density lipoproteinï cholesterol (LDLï cholesterol) can be determined as the difference between total cholesterol and cholesterol content of the supernatant after precipitation of the LDL fraction by polyvinyl sulphate in the presence of polyethyleneglycol monomethyl ether.

LDL-cholesterol = Total cholesterol ï HDL- cholesterol in the supernatant

Reagents

Content	**Initial Concentration of Solutions**
Precipitation Reagent:-	
Polyvinyl sulphate	0.7 g/L
EDTA Na_2SO_4	5.0 Mm
Polyethyleneglycol monomethyl ether	170 g/L
Stabilizers	

Protocol

	Sample S_1	S_2	S_3	S_4	S_5
Reagent	0.1ml	0.1ml	0.1ml	0.1ml	0.1ml
Sample	0.2mll	0.2mll	0.2mll	0.2ml	0.2ml

Procedure

Precipitation reaction

The precipitation solution (0.1ml) was carefully measured into 5 sample test tubes labeled (S) accordingly. The serum sample (0.2ml) was added to the labeled test tubes. The contents were thoroughly mixed and left to stand for 15 minutes at room temperature (25^{o}C). Then, the mixture was centrifuged at 2,000 × g for 15 minutes and the HDL-cholesterol concentration in the supernatant was measured at 500nm.

Calculations

The LDLï cholesterol concentration in the sample was calculated using the following general formula:

LDLï cholesterol (mg/dl) = Total cholesterol (mg/dl) ï supernatant cholesterol (HDL-C) (mg/dl).

2.2.4.1.4. Determination of triacylglycerol (TAG) level

The concentration of triacylglycerol was determined according to the method of Assmann *et al*. (1984) .

Principle

The triaclyglycerols are determined after enzymatic hydrolysis with lipases. The indicator was quinoneimine formed from hydrogen peroxide, 4 ï amniophenzaone and 4 ï chlorophenol under the catalytic influence of a peroxidase.

Triacylglycerol + H_2O + Lipases Ÿ Glycerol + Fatty acids- - - - - - - - _ - - - - - - - - - -(1)

Glycerol + ATP+ Glycerol kinaseŸ Glycerol-3-phosphate + ADP- - - - -- - - - - - - - - - -(2)

Glycerol-3-phosphate + O_2 + GPOŸ Dihydroxyacetone + Phosphate +H_2O_2 - - - - - - -- (3)

H_2O_2 + 4ï Amniophenzaone + 4ï Chlorophenol peroxidaseŸ Quinoneimine + HCl + 4H_2O-- (4)

GPO (Glycerol -3- phosphate oxidase).

Reagents

Contents	**Initial Concentration of Solution**
Buffer	
Pipes buffer	40.0mol/l, pH 7.6
4 ï chlorophenol	5.5mmol/l
Magnesium ions	17.5mmol/l
Enzyme Reagent	
4 ï Amniophenzaone	0.5mmol/l
ATP	1.0mmol/l
Lipases	Ó150U/ml
Glycerol kinase	Ó0.4U/ml
Gylcerol -3- phosphate oxidase	Ó1.5U/ml
Peroxidase	Ó0.5U/ml
Standard	2.29mmol/l (200mg/dl)

Protocol

	Reagent Blank (B)	Standard (ST)	Sample S_1	S_2	S_3	S_4	S_5
Sample(serum)	-	-	10ul	10ul	10ul	10ul	10ul
Standard (ST)	-	10ul	-	-	-	-	-
Reagent (Enz reagent + buffer sol)	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml

Procedure

Three sets of test tubes labeled reagent blank (B), standard (ST), and five sample tubes (S) were set up. Enzyme reagent (15ml) was reconstituted with 15ml of the buffer solution and the new solution stored in the refrigerator($6^{O}c$). Serum sample (10µl) was delivered into the test tubes (S) while 10µl of the standard solution was delivered into the test tube (ST). Then, 1.0ml of the reconstituted enzyme reagent was added to all the three sets of test tubes. The contents of the test tubes were mixed and incubated in a water bath at $37^{o}C$ for 5 minutes. The absorbance of the sample (A_{sample}) and standard ($A_{standard}$) were measured against the reagent blank (B) within 60 minutes at 500nm. The concentration of triacylglycerol in the serum samples was calculated using the formula:

$$\text{Conc of triacylglycerol in sample} = \frac{A_{Sample}}{A_{S\tan dard}} \times \text{Conc of standard (150mg/dl)}$$

2.2.4.2. Determination of Serum total protein level

The concentration of serum total protein was determined according to the method of Lowry et al.,

Principle

Cupric ions, in alkaline medium interact with protein peptide bonds resulting in the formation of a coloured complex. All substances with three or more peptide bonds give the reaction, and the intensity of the purple colour produced is proportional to the number of such bonds present and hence to the amount of protein.

Reagents

Contents **Initial Concentration of solution**

a)Biuret reagent

Sodium hydroxide 100mmol/l

Na K-tartrate 16mmol/l

Potassium iodide 15mmol/l

Cupric sulphate 6mmol/l

b) Blank reagent

Sodium hydroxide 100mmol/l

Na K- tartrate 16mmol/l

c) Standard

Protein 60g/l (6.0g/dl

Protocol

	Reagent Blank (B)	Standard (ST)	Sample S_1	S_2	S_3	S_4	S_5
Distilled water	0.02ml	-	-	-	-	-	-
Sample	-	-	0.02ml	0.02ml	0.02ml	0.02ml	0.02ml
Standard (ST)	-	0.02ml	-	-	-	-	-
Reagent (Biuret)	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml	1.0ml

Procedure (Biuret Method)

The Biuret reagent was reconstituted by diluting 100ml of the reagent with 400ml of distilled water. The blank reagent was also reconstituted by diluting 100ml of the reagent with 400ml of double distilled water. Three sets of test tubes labeled reagent blank (B), standard (ST) and sample tubes (S) were set up. Distilled water (0.02ml) was delivered into the reagent blank

test tubes and 0.02ml of standard solution added into the standard test tube. Then, 0.02ml of serum sample was added into the set of test tubes labeled (S). This was followed by the addition of 1.0ml of the reconstituted Biuret reagent into all the test tubes. The contents of the test tubes were mixed and incubated for 30minutes at 25^{o}C (room temperature). The absorbance of the sample (A sample) and of the standard (A standard) was measured against the reconstituted reagent blank at 540nm. The total protein concentration was calculated as follows;

$$\text{Total Protein Conc.} = \frac{A_{Sample}}{A_{Standard}} \times \text{Conc. of standard (5.85g/dl)}$$

2.2.4.3. Determination of liver enzymes

2.2.4.3.1. Determination of alanine aminotransferase activity

The Alanine aminotransferase activity was determined with a Randox Commercial Enzyme kit according to the method of Reitman and Frankel (1957)

Principle

Alanine aminotransferase assay is based on the principle that pyruvate is formed from:

αï Oxoglutarate + L-alanine ⟶ L-glutamate + pyruvate

Alanine aminotransferase is measured by monitoring the concentration of pyruvate hydrazine formed with 2,4ï dinitrophenyl hydrazine.

Reagents

Contents	**Initial concentrations of solutions**
Buffer	
Phosphate buffer	100mmol/l pH 7.4
Lï alanine	200mmol/l
αï oxoglutarate	2.0mmol/l
2, 4ï dinitrophenyl hydrazine	2.0mmol/l
Sodium hydroxide solution	0.4mol/l

Protocol

	Reagent Blank (B)	Sample S_1	S_2	S_3	S_4	S_5
Buffer	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml
Sample(serum)	-	0.1ml	0.1ml	0.1ml	0.1ml	0.1ml
Reagent (2,4-dinitrophenyl-hyrazine)	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml

Serum (sample)	0.1ml	-	-	-	-	-
NaOH(0.4mol/l)	5.0ml	5.0ml	5.0ml	5.0ml	5.0ml	5.0ml

Procedure

Measurement against Sample Blank

The ALT substrate and phosphate buffer (0.5ml each) was pipetted into two sets of test tubes labeled B (sample blank) and five S (sample test) tubes respectively. The serum (0.1ml) sample was added to the sample test (S) only and mixed properly, then incubated for exactly 30min in a water bath at a temperature of 37^{o}C.

2,4 ï dinitrophenyl hydrazine (0.5ml) was added to both test tubes labeled S (sample test) and B (sample blank) immediately after the incubation. Also, 0.1ml of serum sample was added to the sample blank (B) only. The entire medium was mixed thoroughly and allowed to stand for exactly 20min at 25^{o}C. After that, 5.0ml of sodium hydroxide (NaOH) solution (0.4mol/l) was added to both test tubes and also mixed thoroughly. Absorbance of the sample was read at a wavelength of 550nm after 5min.

Measurement against Reagent Blank

The ALT substrate and phosphate buffer (0.5ml each) was pipetted into both the reagent blank (B) and five sample (S) test tubes respectively. The serum sample 0.1ml was added to the sample (S) test tubes only and mixed thoroughly. Then 0.1ml of distilled water was added to the reagent blank (B). The entire medium was mixed and incubated for exactly 30min in a water bath at 37^{o}C. Immediately after incubation, 2,4 ï dinitrophenyl ï hydrazine (0.5ml) was added to both reagent blank and sample (S) test tubes. The contents of the tubes were mixed thoroughly and allowed to stand for exactly 20min at 25^{o}C. Finally, 5.0ml of sodium hydroxide solution (0.4 mol/l) was added to both the blank and reagent test tubes respectively. Each was mixed thoroughly and absorbance of sample was read at a wavelength of 550nm against the reagent blank after 5min..

2.2.4.3.2. Determination of Aspartate aminotransferase Activity

The Aspartate aminotransferase activity was determined with a Randox Commercial Enzyme kit according to the method of Reitman and Frankel (1957).

Principle

This method is based on the principle that oxaloacetate is formed from the reaction below:

αï Oxoglutarate + L ï aspartate $\longrightarrow$ L-glutamate + oxaloacetate

Glutamic-oxaloacetic acid transaminase (aspartate aminotransferase) activity was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenyl hydrazine.

Reagents

Contents	**Initial concentration of reagents**
Buffer	
Phosphate buffer	100mmol/l, pH 7.4
Lï Aspartate	100mmol/l
αï Oxoglutarate	2mmol/l
2, 4ï dinitrophenyl hydrazine	2mmol/l
Sodium hydroxide solution	0.4mol/l

Protocol

	Reagent Blank (B)	Sample S_1	S_2	S_3	S_4	S_5
Buffer	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml
Sample	-	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml
Distilled H_2O	0.1ml	-	-	-	-	-
Reagent (2,4-dinitrophenyl-hyrazine)	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml
NaOH(0.4mol/l)	5.0ml	5.0ml	5.0ml	5.0ml	5.0ml	5.0ml

Procedure

Measurement against Reagent Blank

The AST substrate and phosphate buffer (0.5ml each) was pipetted into both the reagent blank (B) and sample (S) test tubes respectively. The serum sample (0.5ml) was added to the sample (S) test tubes only and mixed thoroughly. Then 0.1ml of distilled water was added to the reagent blank (B). The entire reaction medium was well mixed and incubated for 30min in a water bath at 37^{o}C.

Immediately after incubation, 2,4-dinitrophenyl-hydrazine (0.5ml) was added to the reagent blank (B) and the sample (S) test tubes, mixed thoroughly and allowed to stand for exactly 20min at 25^{o}C. Finally, 5.0ml of sodium hydroxide (0.4mol/l) solution was added to both the blank and the reagent test tubes respectively and mixed thoroughly.

The absorbance of sample was read at a wavelength of 550nm against the reagent blank after 5 min.

2. Measurement against Sample Blank

The AST substrate and phosphate buffer (0.5ml each) was pipetted into the sample blank (B) and sample (S) test tubes respectively. The serum sample (0.1ml) was added to the sample test (S) only, mixed immediately and then incubated in a water bath for exactly 30min at 37^{o}C.

2,4 ï Dinitrophenylhyrazine was added to both sample blank (B) and sample (S) test tubes immediately after incubation. Also, 0.1ml of the sample was added to the sample blank (B) only. Each medium was mixed and allowed to stand for exactly 20min at 25^{o}C. Finally, 5.0ml of sodium hydroxide (NaOH) solution (0.4mol/l) was added to both the sample blank (B) and sample (S) test tubes and mixed thoroughly. Absorbance of the sample was read at a wavelength of 550nm against the sample blank after 5 min.

2.2.4.3.3. Determination of Alkaline phosphatase activity

This was done using the Randox Commercial Enzyme Kit which is based on the phenolphthalein monophosphate method of Klein *et al.* (1960)..

Principle

Serum alkaline phosphatase hydrolyzes a colourless substrate of phenolphthalein monophosphate giving rise to phosphoric acid and phenolphthalein which at alkaline pH values, turns to pink colour that can be photometrically determined.

The concentrations in the reagent solution are;

2-Amino-2-methly-1-propanol	pH 11
Phenolphthalein monophosphate	63mM
Na_2HPO_4	80mM

Stabilizers and preservatives

Protocol

	Reagent Blank (B)	Sample S_1	S_2	S_3	S_4	S_5
Distilled H_2O	1ml	1ml	1ml	1ml	1ml	1ml
Chromogenic substance	1 drop	1 drop	1 drop	1 drop	1 drop	1 drop
Standard(80mM)	0.1ml	-	-	-	-	-
Sample(serum)	-	0.1ml	0.1ml	0.1ml	0.1ml	0.1ml
Reagent(phenolphthalein monophosphate)	5.0ml	5.0ml	5.0ml	5.0ml	5.0ml	5.0ml

Procedure

Distilled water (1ml) was pipetted into 2 sets of test tubes labeled ST (standard) and five S (sample) tubes respectively. Then one drop of each of the chromogenic substrates was added to the distilled water in the two sets of test tubes. Their contents were mixed and incubated at 37^{o}C for 5min.

A standard solution of 80mM (alkaline phosphate 0.1ml) was added to the standard test tube (ST) only, followed by the addition of 0.1ml of the serum sample to the sample test tube (S). The contents of the test tubes were mixed and incubated at 37^{o}C for 20 min in a water bath. A colour developer (phenolphthalein monophosphate) (5.0ml each) was added to both sets of test tubes and mixed thoroughly.

Absorbance of the sample against the blank (water) was read at a wave length of 550nm.

The activity of alkaline phosphatase in the serum was obtained from the formula below.

$$\frac{\text{SA O.D}}{\text{ST O.D}} \times \text{Conc of standard (30U/l)} = \text{U/l of Alkaline phosphatase}$$

SA O.D = Sample Optical Density

ST O.D = Standard Optical Density

2.3. Statistical Analysis

The results were expressed as mean $\pm$ SD and tests of statistical significance were carried out using two-way analysis of variance (ANOVA) and p values 0.05 were considered significance. The means were separated using Duncan Multiple Test. t-Test and correlation were also performed. The statistical package used was statistical package for social science SPSS: Version 18.

CHAPTER THREE

RESULTS

3.1 Effect of varying ethanol concentration on the total cholesterol levels of albino wistar rats

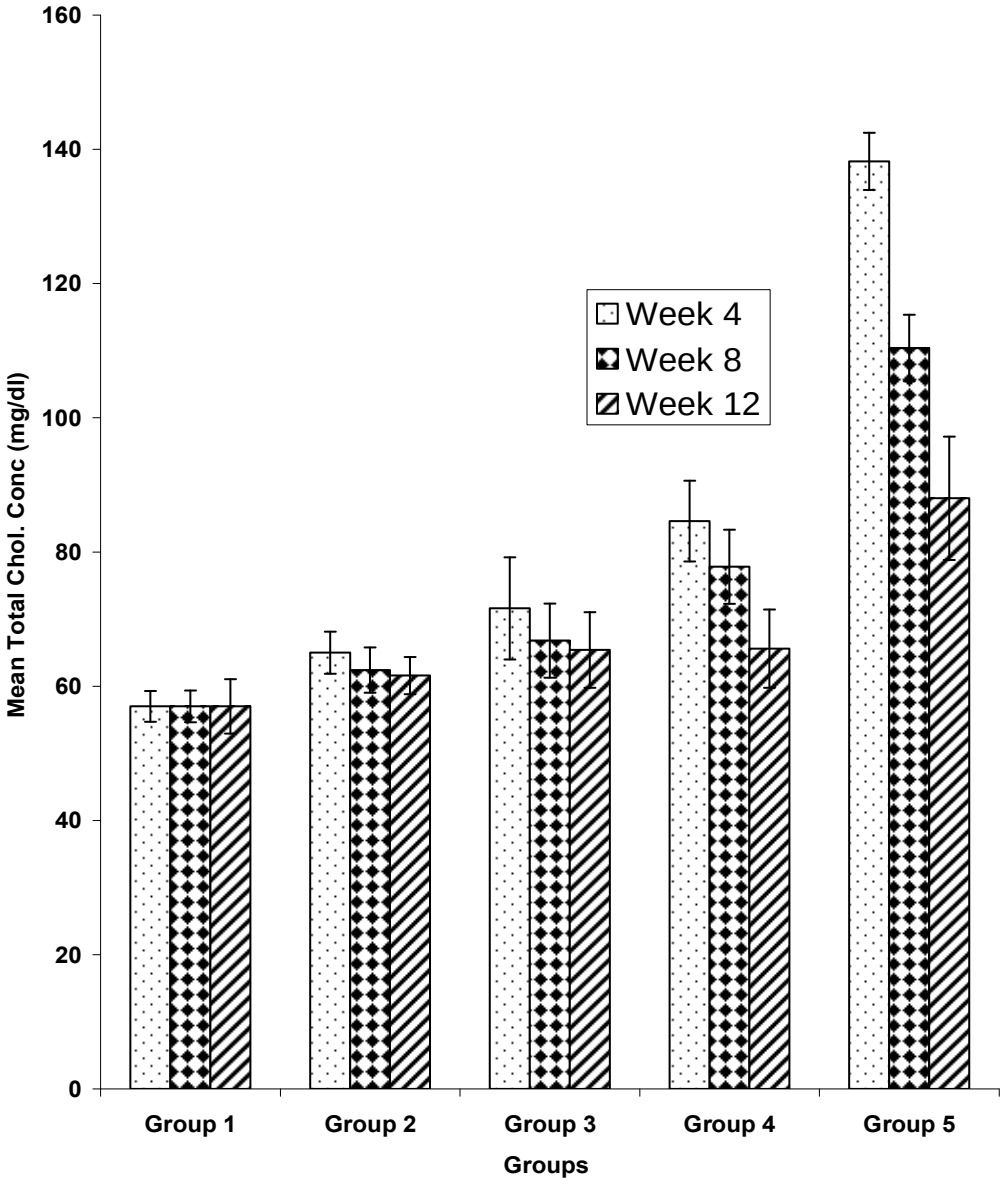


Fig.9: Effect of varying ethanol concentration on the total cholesterol levels of albino wistar rats

Group 1: Rats were given water *ad libitum.*
Group 2: Rats were administered 1% ethanol
Group 3: Rats were administered 3% ethanol
Group 4: Rats were administered 5% ethanol
Group 5: Rats were administered 10% ethanol

Fig.9 shows the effect of varying ethanol concentration on the total cholesterol levels of the rats. Result obtained showed a significant increase in the total cholesterol levels ($p<0.05$) in all the groups when compared to the control.

The correlation analysis pointed a significant difference at the 0.05 level. Comparison within the group revealed a significant increase ($p<0.05$) of cholesterol levels among the groups that were treated with ethanol when compared with the control. The increase was at its maximum at week four when compared to the control.

However, the increase in cholesterol level decreased at week eight and twelve of other groups when compared to the levels at week four.

3.2 Effect of varying ethanol concentrations on the high density lipoprotein cholesterol levels of albino wistar rats

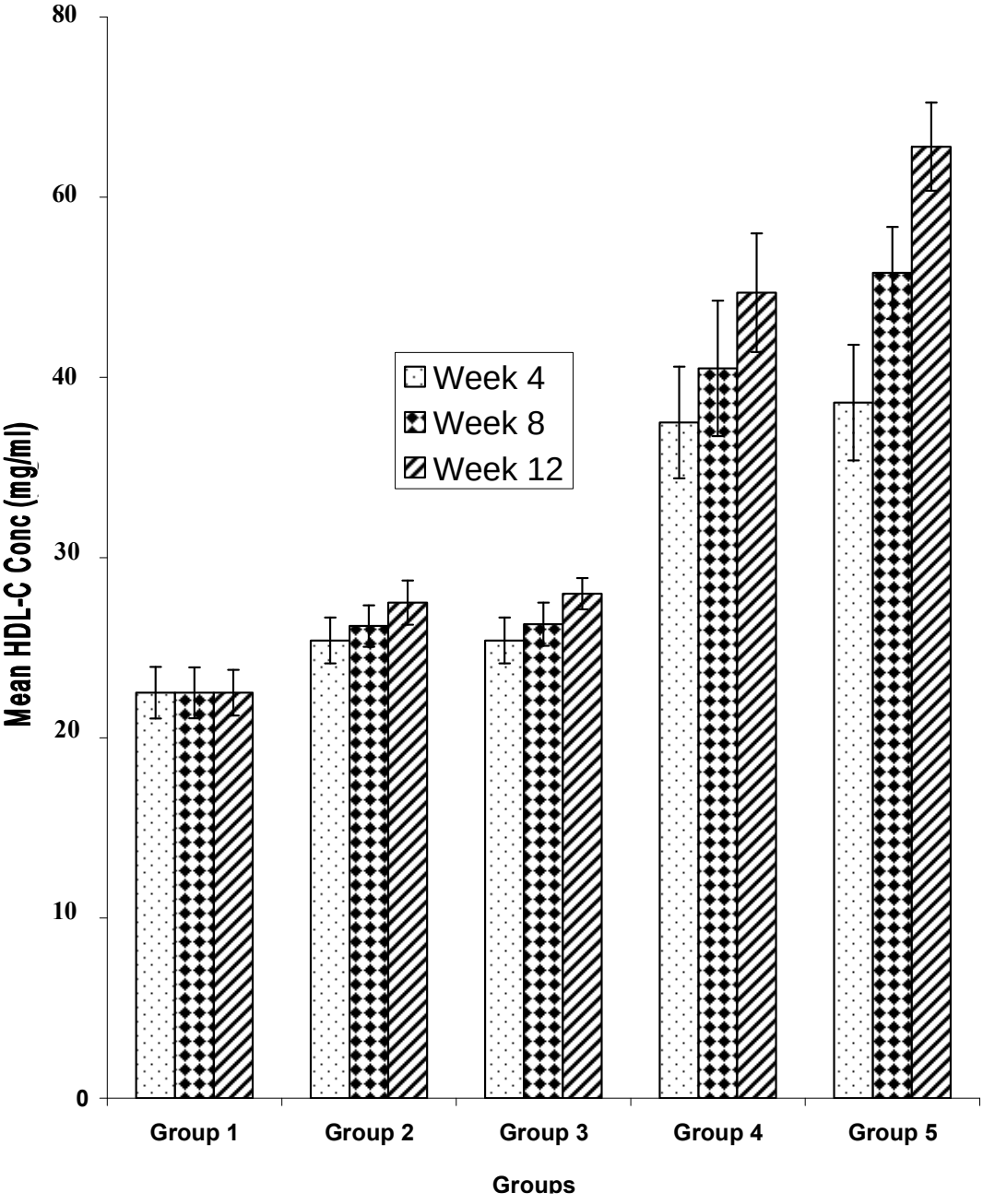


Fig.10: Effect of varying ethanol concentration on the high density lipoprotein cholesterol levels of albino wistar rats

Group 1: Rats were given water *ad libitum.*
Group 2: Rats were administered 1% ethanol
Group 3: Rats were administered 3% ethanol
Group 4: Rats were administered 5% ethanol
Group 5: Rats were administered 10% ethanol

Fig.10 made visible the effect of varying ethanol concentration on the High density lipoprotein cholesterol levels of rats. Results obtained indicated a dose dependent significant increase in HDL-cholesterol levels ($P<0.05$) in all the groups when compared to the control.

The correlation analysis revealed a significant difference at the 0.05 level of HDL-cholesterol.

Comparison within the groups indicated a rise of high density lipoprotein cholesterol level in all the groups. The increase was marked in group five at week twelve. The level of HDL increased with increasing percentage of moderate alcohol administered. There was an age dependent rise in the level of HDL in all groups with marked elevation noted in groups four and five when compared with control ($p<0.05$). No marked increase in HDL was noted for groups two and three hence ($p > 0.05$) due to low ethanol concentration.

3.3 Effect of varying ethanol concentration on the low density lipoprotein cholesterol of albino wistar rats

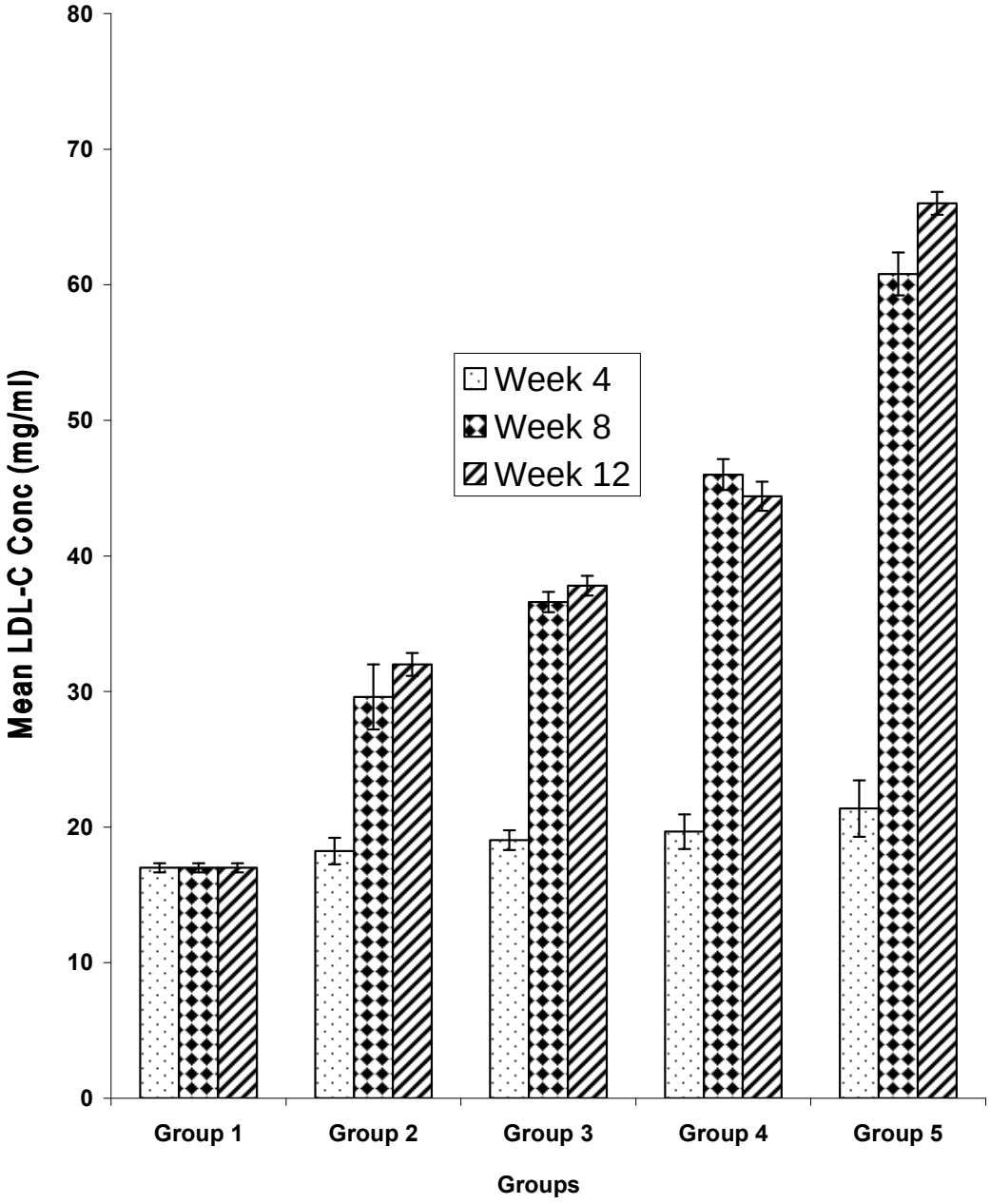


Fig.11: Effect of varying ethanol concentration on the low density lipoprotein cholesterol levels of albino wistar rats

Group 1: Rats were given water *ad libitum.*
Group 2: Rats were administered 1% ethanol
Group 3: Rats were administered 3% ethanol
Group 4: Rats were administered 5% ethanol
Group 5: Rats were administered 10% ethanol

Fig.11 portrays the effect of varying ethanol concentration on the low density Lipoprotein cholesterol level of the rats. Results obtained showed a dose dependent significant increase in the low density lipoprotein cholesterol level ($p<0.05$) in week eight and twelve in all the groups when compared to the control.

The correlation analysis also revealed a significant difference at the 0.05 level. Comparison within the groups showed there was a significant increase ($p<0.05$) in all the groups. However, there was no significant increase ($p>0.05$) in low density lipoprotein cholesterol levels at week four within all the groups when compared to the control. This has been attributed to short duration of ethanol.

3.4 Effect of varying ethanol concentration on the triacylglycerol levels of albino wistar rats

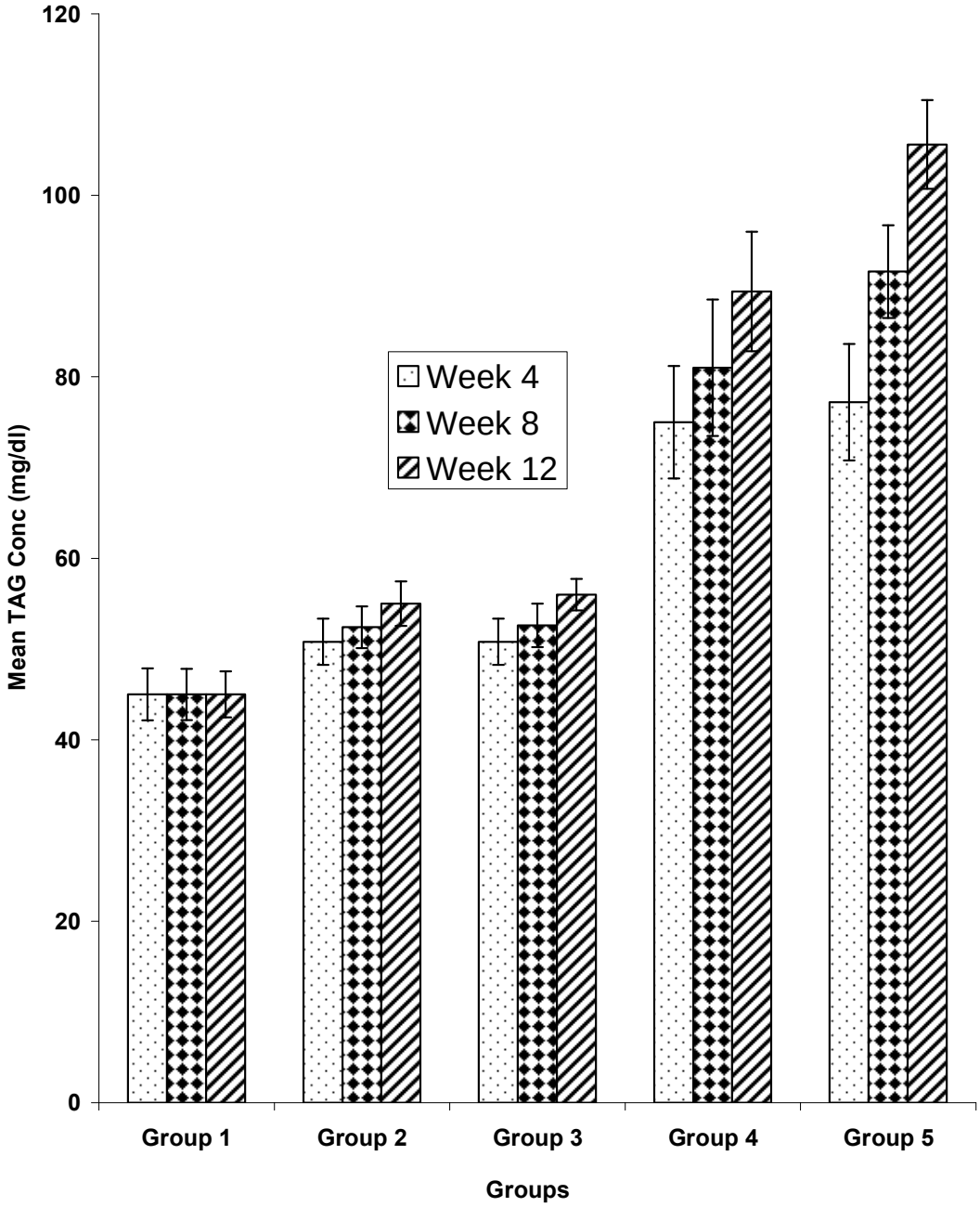


Fig.12: Effect of varying ethanol concentration on the triacylglycerol levels of albino wistar rats

Group 1: Rats were given water *ad libitum*.
Group 2: Rats were administered 1% ethanol
Group 3: Rats were administered 3% ethanol
Group 4: Rats were administered 5% ethanol
Group 5: Rats were administered 10% ethanol

Fig.12 shows the effect of varying ethanol concentration on the triacylglycerol levels of the rats. Results obtained showed a dose dependent significant increase in triacylglycerol ($p<0.05$) in all the groups when compared to the control.

The correlation analysis showed a significant difference at the 0.05 level. Comparison within the groups showed that there was increase in triacylglycerol levels across the group and it was dose dependent. The higher the ethanol intake, the more triacylglycerol levels accumulate.

Although there was increase across the group, groups two and three showed non significant increase ($p>0.05$) when compared to the control. This has been attributed to low ethanol concentration.

3.5 Effect of varying ethanol concentration on the total protein levels of albino wistar rats

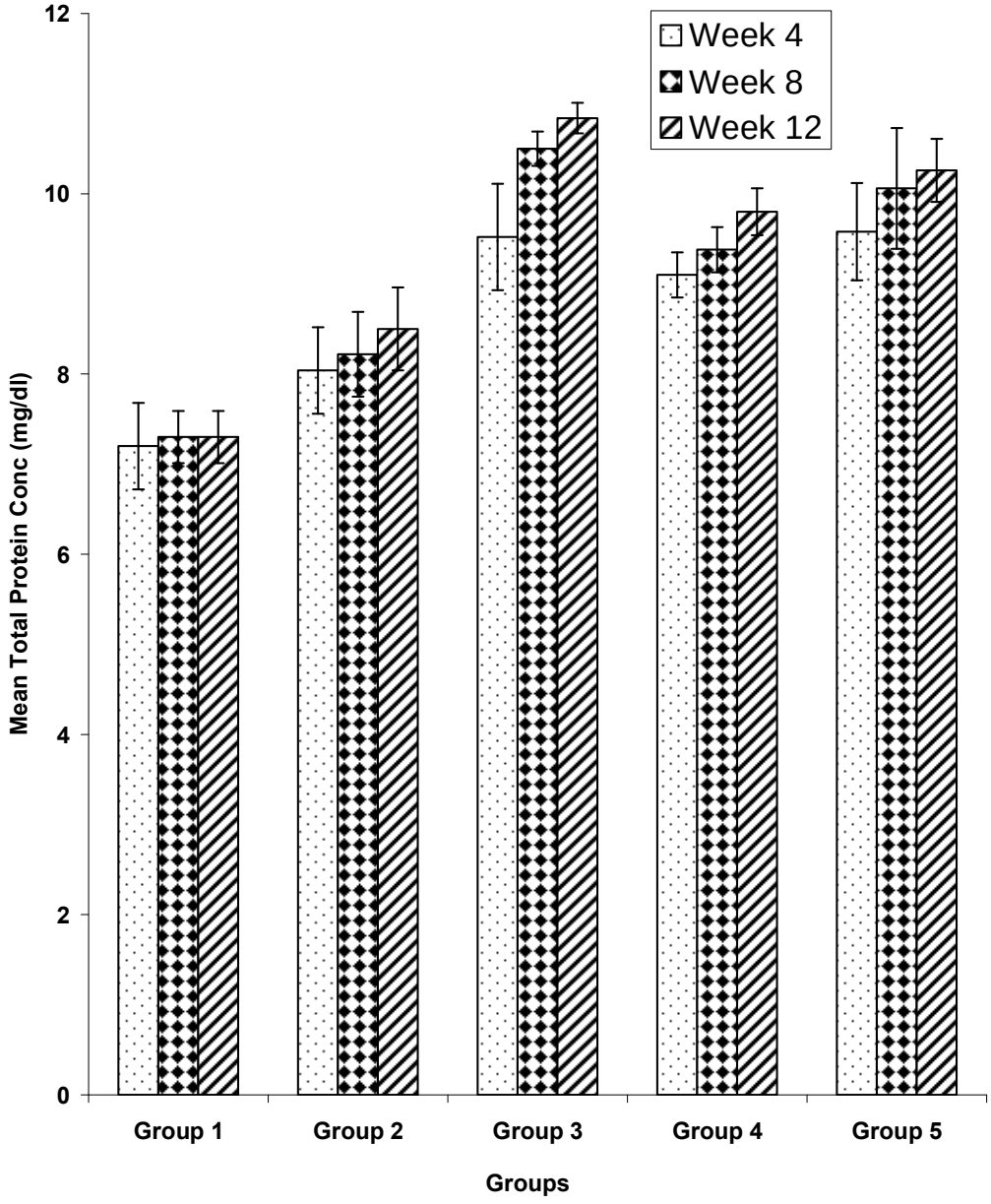


Fig.13: Effect of varying ethanol concentration on the serum total protein levels of albino wistar rats

Group 1: Rats were given water *ad libitum*.
Group 2: Rats were administered 1% ethanol
Group 3: Rats were administered 3% ethanol
Group 4: Rats were administered 5% ethanol
Group 5: Rats were administered 10% ethanol

Fig.13 shows the effect of varying ethanol concentration on the total protein levels of the rats. Results obtained showed a dose dependent significant increase in the total protein levels ($p<0.05$) in all the groups when compared to the control.

The correlation analysis marked a significant difference at the 0.05 level.

Comparison within the groups revealed that there was significant increase ($p<0.05$) in total protein levels in all the groups. The total protein levels increased more at week twelve in all the groups when compared to the control and group three had the maximum increase inrelative to group five. This could be as a result of experimental erro. Furthermore the increase was also observed in week eight in all the groups when compared to the control.

3.6 Effect of varying ethanol concentration on the alanine aminotransferase activity of albino wistar rats

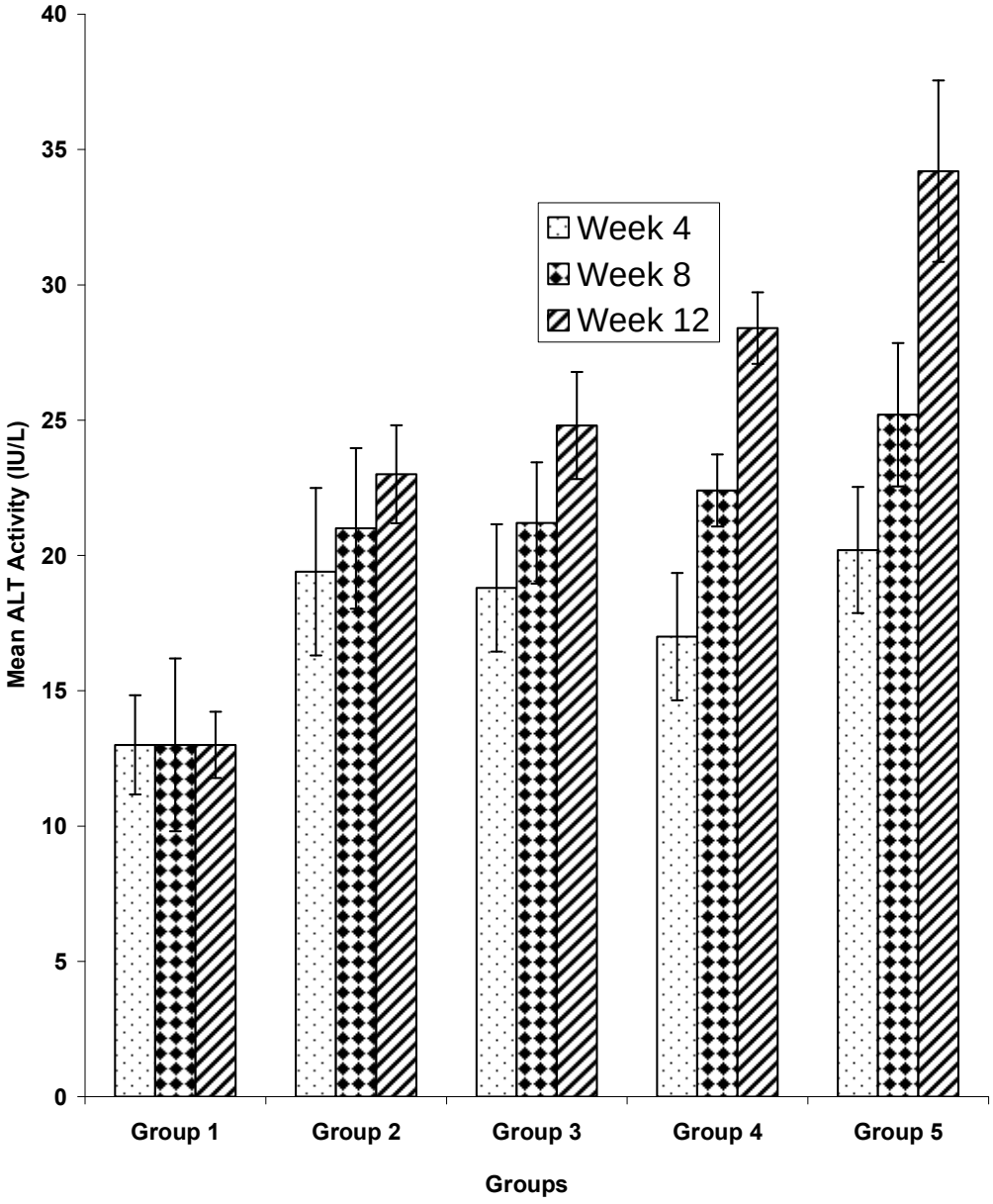


Fig.14: Effect of varying ethanol concentration on the alanine aminotransferase activity of albino wistar rats

Group 1: Rats were given water *ad libitum*.
Group 2: Rats were administered 1% ethanol
Group 3: Rats were administered 3% ethanol
Group 4: Rats were administered 5% ethanol
Group 5: Rats were administered 10% ethanol

Fig.14 shows the effect of varying ethanol concentration on the alanine aminotransferase activity of the rats. Results obtained revealed a significant increase in the alanine aminotransferase activity ($p<0.05$) in all the groups when compared to the control.

The correlation analysis also marked a significant difference at the 0.05 level.

Comparison within the groups indicated a rise of ALT activity in all the groups. The increase was marked in group five at week twelve. The level of ALT increased with increasing percentage of moderate alcohol administered. There was a marked dependent rise in the level of ALT in all groups with marked elevation noted in groups four and five when compared with control ($p<0.05$). No marked increase in ALT activity was noted much at week four ($p>0.05$) in all the group

However, the week four of all the groups increased a little when compared to the activity of the enzyme alanine aminotransferase to the control (group one).

This has been attributed to short duration of ethanol.

3.7 Effect of varying ethanol concentration on the aspartate aminotransferase activity of albino wistar rats

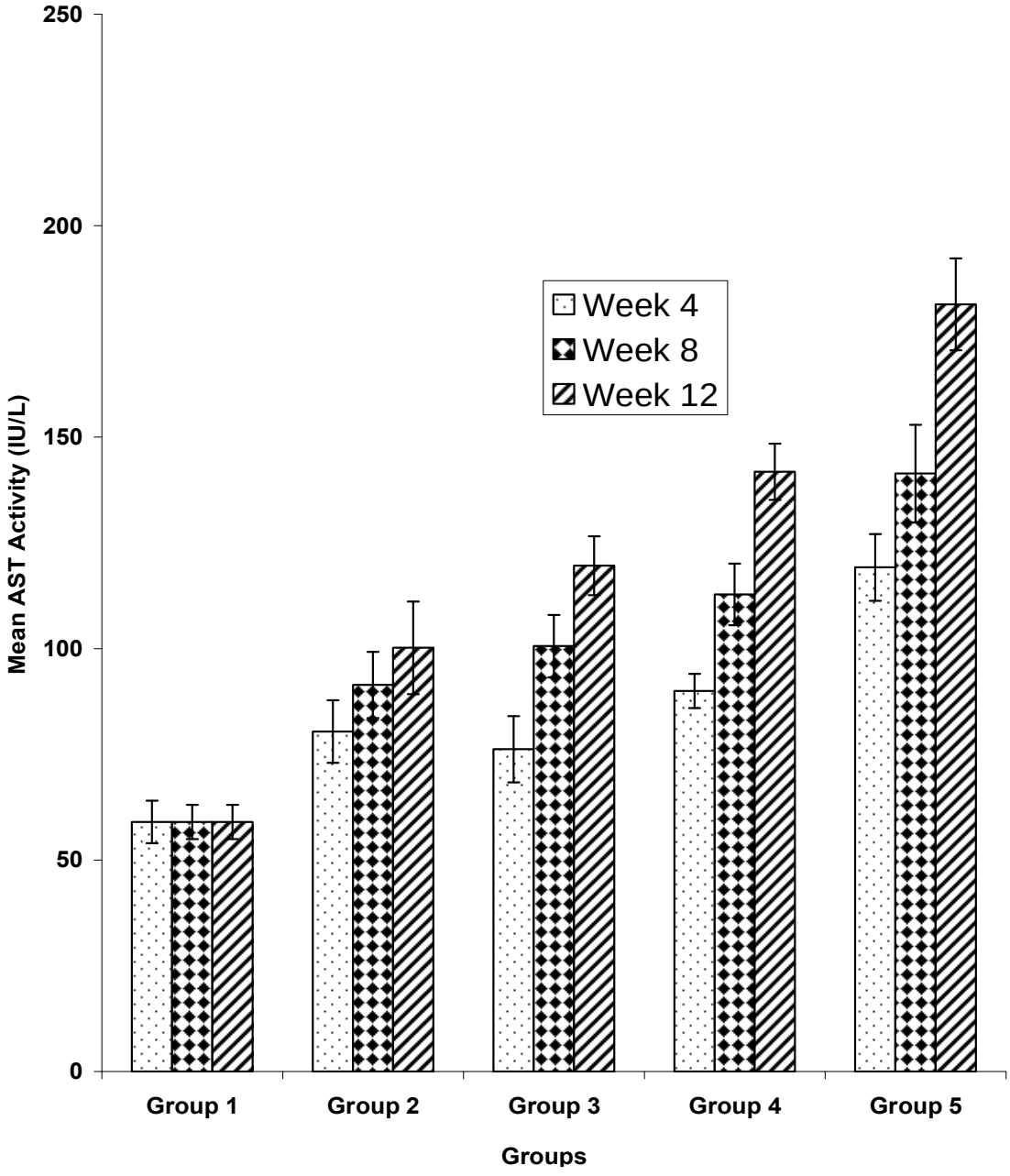


Fig.15: Effect of varying ethanol concentration on the aspartate aminotransferase activity of albino wistar rats

Group 1: Rats were given water *ad libitum*.
Group 2: Rats were administered 1% ethanol
Group 3: Rats were administered 3% ethanol
Group 4: Rats were administered 5% ethanol
Group 5: Rats were administered 10% ethanol

Fig. 15 shows the effect of varying ethanol concentration on the aspartate aminotransferase activity of the rats. Results obtained revealed a significant increase in the activity ($p<0.05$) in all the groups when compared to the control.

The correlation analysis revealed a significant difference at the 0.05 level.

Comparison within the groups showed that there was significant increase ($p<0.05$) increase in aspartate aminotransferase activity in the entire group. The increase waa dose dependent ie the higher the concentration of ethanol administered, the more the activity of aspartate aminotransferase in the liver. Along the group, week twelve of group five had the maximum increase and the activity of the enzyme decrease down the group when compared to the control

However, the week four of groups twq and three had non significant increase ($p>0.05$) when compared to the activities of the enzyme aspartate aminotransferase to other groups due short duration of ethanol.

3.8 Effect of varying ethanol concentration on the alkaline phosphatase activity of albino wistar rats

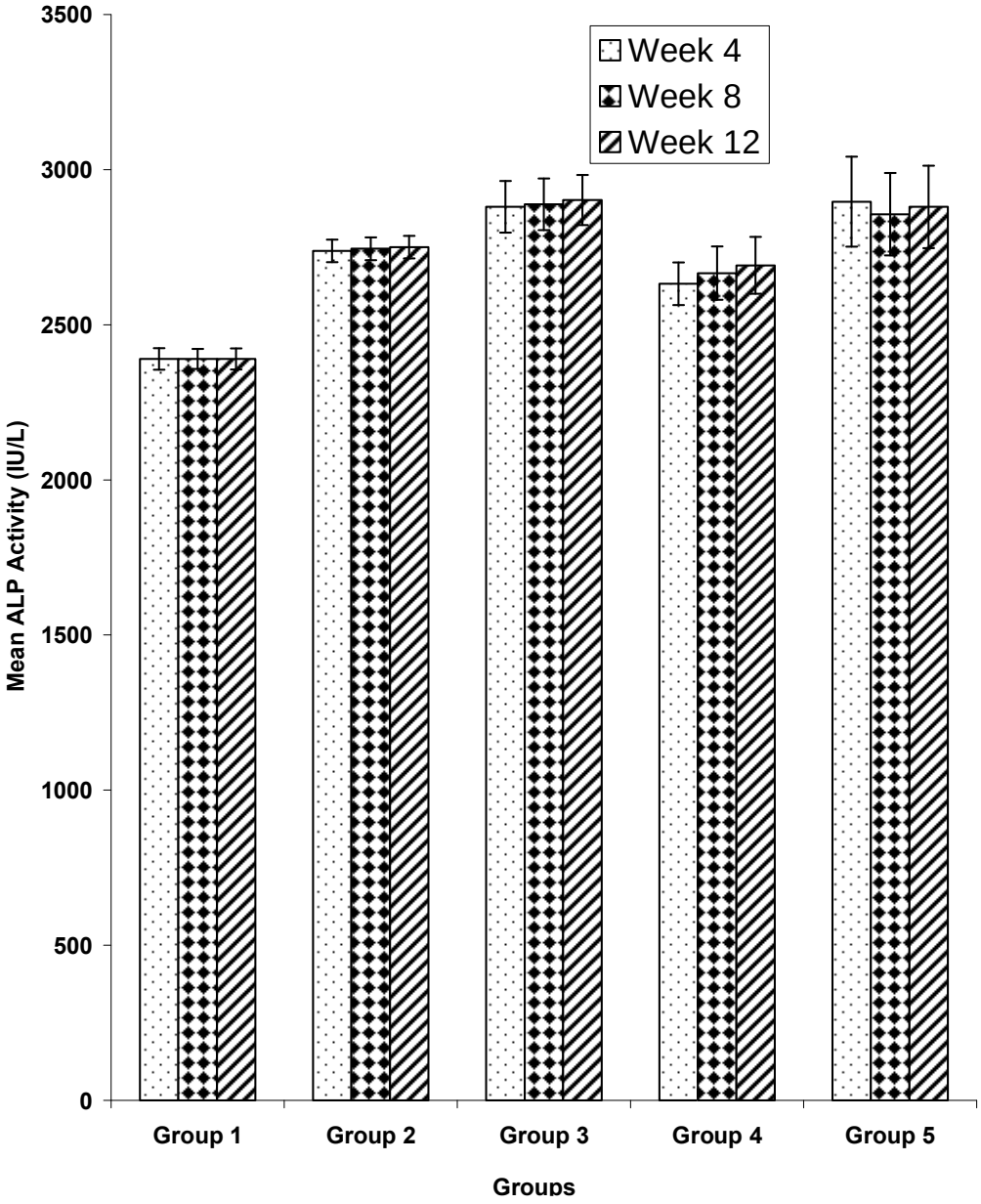


Fig.16: Effect of varying ethanol concentration on the alkaline Phosphatase activity of albino wistar rats

Group 1: Rats were given water *ad libitum.*
Group 2: Rats were administered 1% ethanol
Group 3: Rats were administered 3% ethanol
Group 4: Rats were administered 5% ethanol
Group 5: Rats were administered 10% ethanol

Fig.16 shows the effect of varying ethanol concentration on the alkaline phosphatase activity of the rats. Results obtained revealed an increase in the alkaline phosphatase activity ($p<0.05$) in all the groups when compared to the control.

The correlation analysis also showed a difference at the 0.05 level.

Comparison within the groups showed that there was significant increase ($p<0.05$) in alkaline phosphatase activity of the liver enzymes in the liver of the treated rats when compared to the control.

Although there was an increase, it was observed that there was non significant increase ($p>0.05$) in alkaline phosphatase activity in a group at the week level.

CHAPTER FOUR

4.1 Discussion

The effects of alcohol administration on cholesterol and lipoprotein metabolism have substantial public health implications in both human and animal models. Alcohol has many biological actions and adverse effects on lipid metabolism and cardiovascular diseases which are also well known, especially in individuals with familial predisposition to hyperlipidemia (Latour et al., 1999). The result on varying concentration of ethanol total cholesterol level indicated a dose dependent significant increase ($p<0.05$) in all the groups at week four when compared to control. This increased result in week four is consistent with the finding of Wu et al., 2001, who showed total cholesterol levels increased significant ($p<0.05$) with administration of ethanol. The increased cholesterol level during ethanol administration may be attributed to induction of alphaï hydroxyl methyl glutaryl CoA (HMG CoA) reductase activity, which is the rate limiting step in cholesterol biosysnthesis (Ashakumari and Vijyammal 1993). The decrease in cholesterol levels at weeks eight and twelve is consistent with the findings of Hendriks et al., 1997. This has been attributed to increased level of HDL-C which functions in reverse cholesterol transport (Der Gaag et al., 2001).

The effect of varying ethanol concentration on high density lipoprotein level of the rats. The result indicated a dose dependent significant increase ($p<0.05$) with treated rats when compared to control. This result showed that administration of ethanol caused relative increase in HDL-C level of rats. It is generally believed that moderate alcohol consumption reduces coronary heart disease risk because observational studies show it is associated with elevated HDL-C, decreased cardiovascular events (Latour et al., 1999).

The effect of varying ethanol concentration on low density lipoprotein level of the rats; revealed a dose significant increase ($p<0.05$) with high concentration and long duration of ethanol administration at weeks eight and twelve of all the groups. However, no significant increase ($p>0.05$) in LDL-C level of the rats was observed at week four and this has been attributed to low concentration of ethanol. The above result is consistent with the works and findings of Corella *et al*. (2001) who observed that LDL-C level is significantly high in drinkers compared to nondrinkers in men with the *E4* allele. in addition, alcohol intake was significantly associated with increased HDL-C and reduced LDL-C levels in a dose-dependent manner (Latour *et al.*, 1999). Hence, the findings of Latour *et al*. (1999) are to a large extent inconsistent with the findings of this result.

The result obtained from varying the ethanol concentration on triacylglycerol level of the rat;.showed a dose significant increase ($p<0.05$) with high concentration and long duration of ethanol administration in all the groups. However, there was no significant increase ($p>0.05$) in triacylglycerol level of the rats at weeks eight and twelve of group two. This has been attributed to low concentration of ethanol administered to the rats. This work agrees with the findings of Wiel (2012) who showed that ethanol increases the synthesis of large VLDL particles in the liver, which is the main source of triacylglycerol. Oxidation of ethanol by alcohol dehydrogenase leads to excess production of NADH (Murray et al., 2006).

The NADH generated competes with reducing equivalent from other substrates NAD^+ requiring reactions. The inhibition of fatty acid oxidation causes elevated triacylglycerol levels in the liver. These triacylglycerol accumulate forming fatty deposits and fatty liver (Baraona et al., 1983).

The effect of varying ethanol concentration on total protein level of the rats, revealed a dose significant increase ($p<0.05$) with ethanol administration in all the groups. The increase has been related with the result obtained by Burnham et al.,(2003) in which ethanol administration had significantly ($p<0.05$) elevated protein levels due to damage of the hepatocytes during metabolic disorders.

The result obtained from varying the ethanol concentration on liver enzymes of the rats, indicated a dose significant increase ($p<0.05$) with ethanol administration on liver enzymes such as alanine aminotransferase, aspartate aminotransferase and alkaline aminotransferase when compared to untreated rats (control). It is known that increase in the activities of ALT, AST and ALP was drawn from severe damage to the hepatocytes during chemical assaults or metabolic disorders which may cause these enzymes to leak into the plasma (Goldlerg and Watts 1965). Damage to the liver typically results in a leak of ALT and AST into the blood stream. Most liver diseases are characterized by greater ALT elevation than AST elevations. Cirrhosis and alcohol abuse are associated with AST activity than ALT activity.

4.2 Conclusion

It was observed from this research that excessive intake of ethanol caused some metabolic disorders and has adverse effects on total cholesterol, low density cholesterol, triacylglycerol, total protein and liver enzymes. Although, moderate consumption of ethanol, elevates high density lipoprotein cholesterol. Therefore, it is recommended that alcohol consumption by man should be properly taken care of.

4.3 Suggestions for further studies

(i) Other animal models can be used in order to compare with that of rats and human.

(ii) Other types of alcohol (such as methanol, propanol, etc) should be used to compare with the effect of ethanol.

REFERENCES

Agarwal, D.P. and Seitz, H.K. (2001). ñ**Alcohol in Health and Disease"**. Marcel Dekker, New York. Pp 1ï 28.

Albers, J. J., Warmick, G. R. and Cheng, M. C. (1978). ñDetermination of high density lipoprotein (HDL)ï cholesterolò. *Lipids*, 13: 926ï 932.

Allain, C. C., Poon, L. S., Chan, C. S. G., Richmond, W. and Fu, P. C. (1976). Enzymaticdetermination of serum total cholesterol.ò *Clinical Chemistry, 20 470-475*

Assmann, G., Jabs, H. U., Kohnert, U., Nolte, W. and Schriewer, H. (1984). ñDetermination of low density lipoprotein (LDL)ï cholesterolò. *Clinica Chimica Acta*, 140: 77ï 83.

Assmann, A., Bonifacic, M., Briviba, K., Sies, H. and Asmus, K. D. (2000).ò One-electron reduction stion of selenomethionine oxideò. *Free Radical Research*, 32: 371ï 376.

Ashkumari, L., and Vijyammal, P.(1993). Additive effect of alcohol and nicotine on lipid metabolism in rats. Indian J ExpBiol; 31:270-4.

Bairaktari, E., Elisaf, M., Katsaraki, A., Tsimihodimos, V., Tselepis, A.D., Siamopoulos, K.C. and Tsolas, O. (1999). ñHomogenous HDL-cholesterol assay versus ultracentrifugaration/dextran sulfate-Mg^{2+} precipitation and dextran sulphate-Mg^{2+} precipitation in healthy population and in Hemodialysis patientsò. *Clinical Biochemistry*, 32(5): 339.

Balch, P.A.and Phylis, K. (2006). ñPrescription for Nutritional Healing.ò 4th Ed. New York: Avery P. 54 Carnitine.

Baraon, E., Savolainen, M., KarsentyC, Leo, M.A. and Lieber, C.S.(1993). Pathogenesis of alcoh olichypertriglyceridaemia. Trans Assoc Am Physicians, **96**:306-315.

Barter, Philip, Gotto, Anthonio M.; LaRosa, John C. Maroni, Jaman; Szarek, Michael; Grundy, Scott M.; Kastelein, John J.P.; Bittner, Vera et al. (2007) ñHDL Cholesterol, Very Low Levels of LDL Cholesterol and Cardiovascular Eventsò. New England of Medicine. Pp 357.

Burnham, E.L., Brown, L.A., Halls, L. and Moses, M. (2003). ñEffects of chronic alcohol abuse on alveolar epithelial barrier function and glutathione homeostasisò. *Alcohol Clin. Exp. Res*., 27: 1167ï 1172.

Carrasco, M.P., Jimenez-Lopez, J.L. and Segovia, C. (2002). ñComparative study of the effects of short and long-term ethanol treatment and alcohol withdrawal on phospholipid biosynthesis in rat hepatocytesò, 491-497.

Corella, D., Tucker, K., Lahoz, C., Cupples, A., Wilson, P.W.F., Schaefer, E.J. and Ordovas, J.M. (2001). ñAlcohol drinking determines the effect of the ***APOE*** locus on LDL-cholesterol concentrations in menò: The Framingham Offspring Study. *Am. J. Clin. Nutr*., 73(4): 736 ï 745.

Dai, J. and Miller, B.A. (1997). ñAlcohol feeding impedes early atherosclerosis in Low-density lipoprotein receptor knockout mice: factors in addition to high-density lipoprotein-apolipoprotein AI are involvedò. *Alcohol Clinical Experiment Research*, 21: 11-18.

Daley, C.A., Abbot, A., Doyle, P., Nader, G. and Larson, S. (2004). ñA literature Review of the value-added nutrients found in grass-fed beef productsò. California State University, Chico (College of Agriculture).

Der Gaag, M., Van Tol, A., Vermunt, S., Scheek, L., Schaafsma, G., and Hendriks, H. (2001)."Alcohol consumption stimulates early steps in reverse cholesterol transport". *Journal of Lipid Research*, **42**(12): 2077ï 2083.

Despres, J.P. (2009). ñThe Atherogenic Triad of New Metabolic RiskFactors: Importance of Waist and Fasting Triglycerides as Screening Toolsò. Visceral Adipose Tissue and Cardiometabolic Risk: Does It Really Matter?

Garrett,H.,and Grisham, M. (2005).òThe structure of alcoholò. Biochemistry (3rd edition): 208.

*G*oldberg, D.M. and Watts, C. (1965). Serum enzyme changes as evidence of liver reaction to oral alcohol. Gastroenterol; 49: 256-61.

Gomes, de Sá R.M.F., da Costa G.L.C., de Alencar, F.C.D. and Strauss, E. (2005*).* *"*Alcoholic intake predisposes to more interface hepatitis in chronic hepatitis Cò*. Ann Hepatol*., **4**: 176–183.

Hemat, R.A. S (2003). ñPrinciples of Orthomolecularism. Urotextò. P.254.

Hendriks, H., Veenstra, J., van Tol, A., Groener, J. and Schaafsma, G. (1998). "Moderate doses of alcoholic beverages with dinner and postprandial high density lipoprotein composition". *Alcohol and alcoholism (Oxford, Oxfordshire)*, **33**(4): 403ï 410.

Hirano, T., Nohtomi, K., Koba, S., Muroi, A.and Ito, Y. (2008). "A simple and precise method for measuring HDL-cholesterol Subfractions by a single precipitation followed by homogenous HDL-cholesterol assay". *The Journal of Lipid Research*, **49**(5): 1130.

http://www.wikipedia. The effects of alcohol on the liver. Accessed from the internet on the 20th October, 2010.

Inaba,, D., and Cohen, B. (2004). *Physical and mental effects of psychoactive drugs* (5). p 185.

Kazemi-Shirazi, L., Veloso, M.P., Frommlet, F., Steindl-Munda, P., Wrba, F., Zehetmayer, S., Marsik, C., Ferenci P. (2008). *"*Differentiation of nonalcoholic from alcoholic steatohepatitisò: Are routine laboratory markers useful? *Wien Klin Wochenschr,* **120**: 25–30.

Kind, A. and King, S.A. (1954). ñAlkaline phosphates estimationò. *Journal of Clinical Pathology*. 7:322-323.

Klein, B., Read, P.A, and Babson,A.L.(1960). Rapid method for the quantity determination of serumalkaline phosphatase. Clinical Chemistry,6:269-275.

Kritchersky, S. (1970). The role of cholesterol as vehicle in experimental atherosclerosis. *A.M.J. Clin. Nutrition*, 23:1105-10.

Kumar, V., Butcher, S.J., Katrina, O., Engelhardt, P., Heikkonen, J., Kaski, K., Ala-Korpela, M. and Kovanen, P.T. (2011). "Three-Dimensional CryoEM reconstruction of native LDL particles to 16Å resolution at physiological body Temperatureò.

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

Latour, M.A., Patterson, B.W., Kitchens, R.T., Ostlund, R.E., Hopkins, D. and Schonfeld, G. (1999). ñEffects of Alcohol and Cholesterol Feeding on Lipoprotein Metabolism and Cholesterol Absorption in Rabbitsò. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **19:** 598-604.

Lédinghen V., Combes M., Trouette H., Winnock M., Amouretti M., de Mascarel A., Couzigou P. (2004). « Should a liver biopsy be done in patients with subclinical chronically elevated transaminasesò? *Eur. J. Gastroenterol. Hepatol.*, **16**: 879–883.

Lowry, O.H.,,Rosebrough, N.J., Farr A.L., and Randall R.J.(1951). Protein measurement with the Folin phenol reagent. J Biol Chem; 193: 265-275.

Lin, M., Hoke, C., Ettinger, B. (1998). "Evaluation of Homogenous High-Density Lipoprotein Cholesterol Assay on a BM/Hitachi 747-200 Analyzerò. *Clinical Chemistry* 44 (5): 1050.

Longstreth, F. and Zieve, D. (2009). "Alcoholic Liver Disease". Bethesda, MD: US National Library of Medicine & National Institutes of Health. Archived from the original on 22 January 2010. Retrieved 27 January 2010.

Masters, S. B. (2001). ñThe alcoholsò: Basic and Clinical Pharmacology. Katzung B. G. ed., 8th edition. Appleton and Lange. Pp.115.

Mello, T., Ceni, E., Surrenti, C. and Galli, A. (2007). ñAlcohol induced hepatic fibrosis: role acetaldehydeò. Mol Aspects Med; 29: 17-21.

Menon, K.V., Gores, G.J. and Shah, V.H. (2001). "Pathogenesis, diagnosis, and treatment of alcoholic liver disease" (PDF). *Mayo Clin. Proc.* **76** (10): 1021ï 1029.

Meyers, C. Daniel; Carr, Molly C.; Park, Sang; Brunzell, John D. (2003).òVarying Cost and Free Nicotinic Acid Content in Over ï the-Counter Niacin Preparations for Dyslipidemiaò. *Annals of Internal Medicine* 139 (12):996

O'Shea, R.S., Dasarathy, S.,, and McCullough, A.J. (January 2010). "Alcoholic liver disease"

. *Hepatology* **51** (1): 307ï 328.

Peterson, M.M., Mack, J.L. and Hall, P.R. (2008). ñApolipoprotein B is an innate barrier against invasive staphylococcus aureus infectionò. *Cell Host & Microbe* 4 (6): 555ï 566.

Reitman, S., and Frankel, S. (1957). ñDetermination of alanine aminotransferaseò. Activity. American Journal of Clinical Pathology. 28: 56-63.

Reuben, A. (2008). ñAlcohol and the liverò. Curr. Opin. Gastroenterol; 24: 328-338.

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

Segrest JP, Jones MK, De Loof H, Dashti N (2001). ñStructure of apolipoprotein B-100 in low density lipoproteinsò. *Journal of Lipid Research* 42 (9): 1346ï 1367.

Seppa, K., Sillanaukee, P., Pitkajarvi, T., Nikkila, M. and Koivula, T. (1992). ñModerate and heavy alcohol consumption have no favourable effect on lipids valuesò. Arch Intern Med; 152: 263-265.

Slater, R. J. (1986). ñExperiments in Molecular Biologyò. Clifton, New Jersey: Humana Press. p. 269.

Spate-douglas, T; and Keyser, R.E. (1999). "Exercise intensity: its effect on the high-density lipoprotein profile". Archives of physical medicine and rehabilitation 80 (6): 691-695.

Superko, H.R., Nejedly, M. and Garrett, B. (2002). "Small LDL and its clinical importance as a new CAD risk factor: a female case study". Progress in Cardiovascular Nursing 17 (4): 167ï 173.

Teschke, R., Neuefeind, M., Nishimura, M., Strohmeyer, G. (1983*). "*Hepatic gamma-glutamyltransferase activity in alcoholic fatty liver: Comparison with other liver enzymes in man and ratsò. *Gut,* **24**: 625–*6*30.

VŁdan, R., Gheorghe, L., Becheanu, G., Iacob, R., Iacob, S. and Gheorghe, C. (*2003*). *"*Predictive factors for the severity of liver fibrosis in patients with chronic hepatitis C and moderate alcohol consumptionò. *Rom. J. Gastroenterol.,* **12**: 183–187

Warnick, G.R., Knopp, R.H., Fitzpatrick, V. and Branson, L. (1990). ñEstimating low density Lipoprotein cholesterol by the Friedewald equation is adequate for classifying patients on the basis of nationally recommended cut pointsò. *Clinical Chemistry*, **36**(1): 15-19.

Wiel, A. (2012): ñThe Effect of Alcohol on Postprandial and Fasting Triglycerolsò. *International Journal of Vascular Medicine*, 2012: 1ï 4.

Wu, D.M., Pai, L., Sun, P.K., Hsu, L.L. and Sun, C.A. (2001). Joint effects of alcohol consumption and cigarette smoking on atherogenic lipid and lipoprotein profiles:

Results from a study of Chinese male population in Taiwanò. *Eur. J. Epidemiol*., 17(7):629-635.

APPENDICES

Table 1: Serum Normal Values of ALT
Activity of ALT in serum was obtained from the Table below.

Absorbance	U/L	Absorbance	U/L
0.025	4	0.275	48
0.050	8	0.300	52
0.075	12	0.325	57
0.100	17	0.350	62
0.125	21	0.370	67
0.150	25	0.400	72
0.175	29	0.425	77
0.200	34	0.450	83
0.225	39	0.475	88
0.250	43	0.500	94

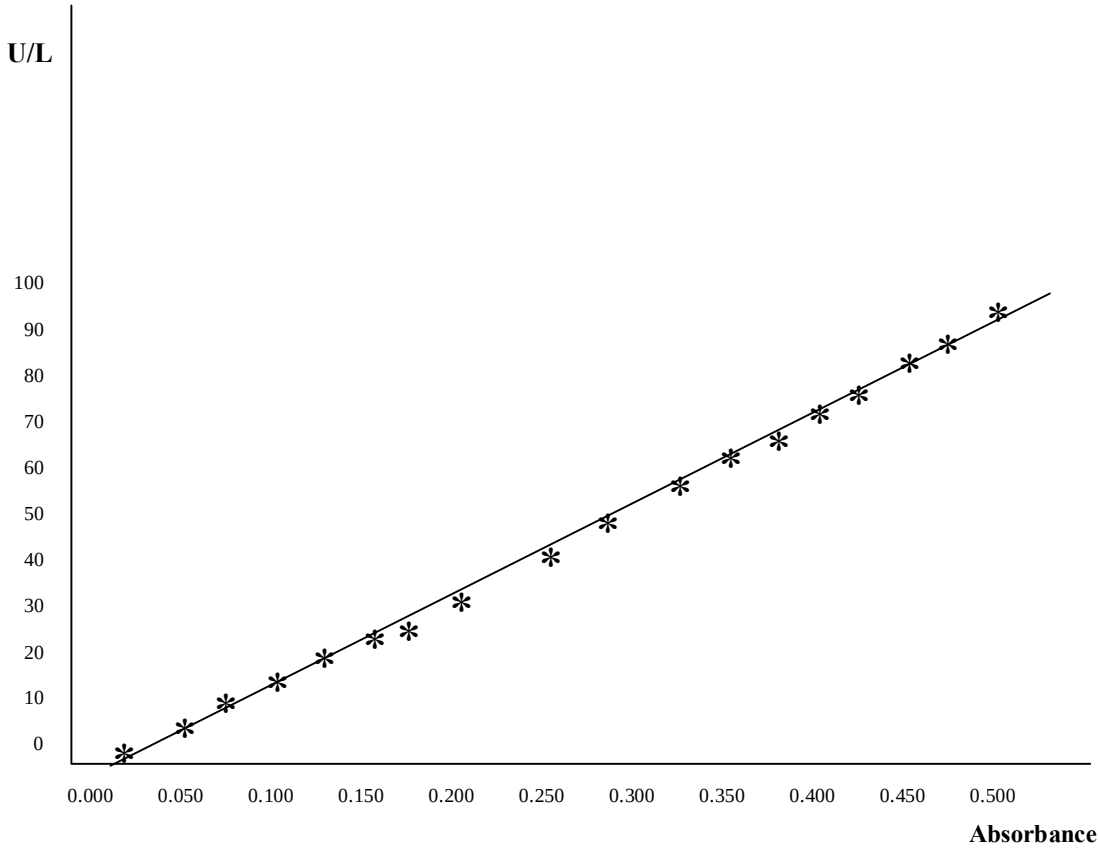
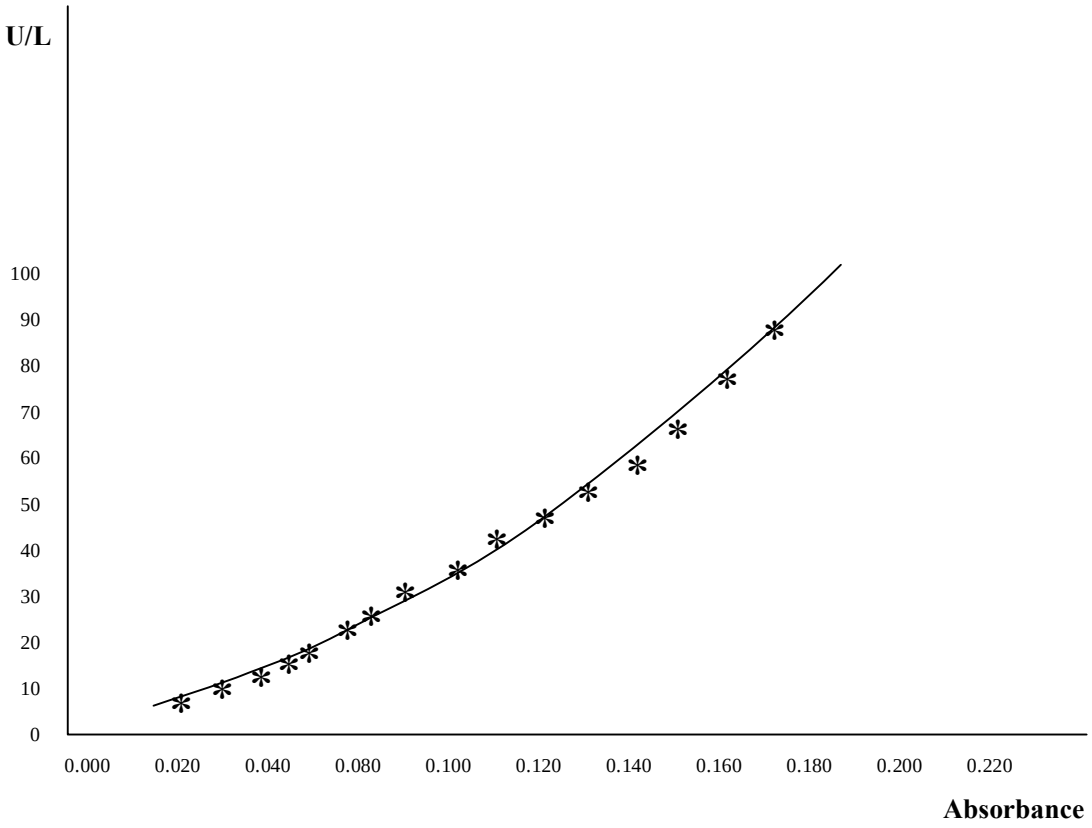


Table 2: Serum Normal Values of AST
The activity of AST in the serum was obtained from the Table below.

Absorbance	U/L	Absorbance	U/L
0.020	7	0.120	47
0.030	10	0.130	52
0.040	13	0.140	59
0.050	16	0.150	67
0.060	19	0.160	76
0.070	23	0.170	89
0.080	27		
0.090	31		
0.100	36		
0.110	41		



One way {Week 4 of Administration}

Descriptive

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
T_Chol	Group 1 (Control)	5	57.0000	.00000	.00000	57.0000	57.0000	57.00	57.00
	Group 2 (1% Contamination)	5	65.0000	7.00000	3.13050	56.3084	73.6916	57.00	74.00
	Group 3 (3% Contamination)	5	71.6000	17.03819	7.61971	50.4443	92.7557	50.00	95.00
	Group 4 (5% Contamination)	5	84.6000	13.46477	6.02163	67.8813	101.3187	67.00	99.00
	Group 5 (10% Contamination)	5	138.2000	9.52365	4.25911	126.3748	150.0252	125.00	150.00
	Total	25	83.2800	31.18563	6.23713	70.4072	96.1528	50.00	150.00
HDL	Group 1 (Control)	5	78.0000	.00000	.00000	78.0000	78.0000	78.00	78.00
	Group 2 (1% Contamination)	5	64.8000	4.43847	1.98494	59.2889	70.3111	60.00	69.00
	Group 3 (3% Contamination)	5	45.2000	3.56371	1.59374	40.7751	49.6249	40.00	49.00
	Group 4 (5% Contamination)	5	33.4000	3.04959	1.36382	29.6134	37.1866	30.00	37.00
	Group 5 (10% Contamination)	5	32.8000	1.64317	.73485	30.7597	34.8403	31.00	34.00
	Total	25	50.8400	18.42480	3.68496	43.2346	58.4454	30.00	78.00
LDL	Group 1 (Control)	5	17.0000	.00000	.00000	17.0000	17.0000	17.00	17.00
	Group 2 (1% Contamination)	5	26.8000	2.16795	.96954	24.1081	29.4919	23.00	28.00
	Group 3 (3% Contamination)	5	37.2000	1.64317	.73485	35.1597	39.2403	35.00	39.00
	Group 4 (5% Contamination)	5	43.2000	2.86356	1.28062	39.6444	46.7556	40.00	47.00
	Group 5 (10% Contamination)	5	62.8000	4.65833	2.08327	57.0159	68.5841	57.00	68.00
	Total	25	37.4000	16.05459	3.21092	30.7730	44.0270	17.00	68.00
TAG	Group 1 (Control)	5	45.0000	.00000	.00000	45.0000	45.0000	45.00	45.00
	Group 2 (1% Contamination)	5	50.8000	5.71839	2.55734	43.6997	57.9003	43.00	56.00
	Group 3 (3% Contamination)	5	50.8000	5.71839	2.55734	43.6997	57.9003	43.00	56.00
	Group 4 (5% Contamination)	5	75.0000	13.83835	6.18870	57.8174	92.1826	58.00	87.00
	Group 5 (10% Contamination)	5	77.2000	14.34225	6.41405	59.3918	95.0082	60.00	91.00
	Total	25	59.7600	16.36123	3.27225	53.0064	66.5136	43.00	91.00
Protein	Group 1 (Control)	5	7.3000	.00000	.00000	7.3000	7.3000	7.30	7.30
	Group 2 (1% Contamination)	5	8.0400	1.06442	.47603	6.7183	9.3617	7.10	9.80
	Group 3 (3% Contamination)	5	9.5200	1.32363	.59195	7.8765	11.1635	7.30	10.50
	Group 4 (5% Contamination)	5	9.1000	.56125	.25100	8.4031	9.7969	8.30	9.80
	Group 5 (10% Contamination)	5	9.5800	1.20499	.53889	8.0838	11.0762	8.30	10.90
	Total	25	8.7080	1.26850	.25370	8.1844	9.2316	7.10	10.90
ALT	Group 1 (Control)	5	13.0000	.00000	.00000	13.0000	13.0000	13.00	13.00
	Group 2 (1% Contamination)	5	19.4000	6.91375	3.09192	10.8154	27.9846	12.00	30.00
	Group 3 (3% Contamination)	5	18.8000	5.26308	2.35372	12.2650	25.3350	12.00	25.00
	Group 4 (5% Contamination)	5	17.0000	5.24404	2.34521	10.4887	23.5113	13.00	26.00
	Group 5 (10% Contamination)	5	20.2000	11.92476	5.33292	5.3934	35.0066	10.00	36.00
	Total	25	17.6800	6.90845	1.38169	14.8283	20.5317	10.00	36.00
AST	Group 1 (Control)	5	59.0000	.00000	.00000	59.0000	59.0000	59.00	59.00
	Group 2 (1% Contamination)	5	80.4000	16.54690	7.40000	59.8543	100.9457	59.00	98.00
	Group 3 (3% Contamination)	5	76.2000	17.54138	7.84474	54.4195	97.9805	59.00	98.00
	Group 4 (5% Contamination)	5	90.0000	9.02774	4.03733	78.7906	101.2094	76.00	98.00
	Group 5 (10% Contamination)	5	119.2000	17.64086	7.88923	97.2960	141.1040	89.00	131.00
	Total	25	84.9600	23.92955	4.78591	75.0824	94.8376	59.00	131.00
ALP	Group 1 (Control)	5	2390.0000	.00000	.00000	2390.0000	2390.0000	2390.00	2390.00
	Group 2 (1% Contamination)	5	2738.6000	81.71169	36.54258	2637.1415	2840.0585	2625.00	2817.00
	Group 3 (3% Contamination)	5	2880.2000	186.44222	83.37949	2648.7014	3111.6986	2585.00	3095.00

. The mean difference is significant at the 0.05 level.

One way {Week 8 of Administration}

Descriptive

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
T_Chol	Group 1 (Control)	5	57.0000	.00000	.00000	57.0000	57.0000	57.00	57.00
	Group 2 (1% Contamination)	5	62.4000	7.53658	3.37046	53.0421	71.7579	55.00	75.00
	Group 3 (3% Contamination)	5	66.8000	12.35718	5.52630	51.4565	82.1435	48.00	80.00
	Group 4 (5% Contamination)	5	77.8000	12.31666	5.50818	62.5069	93.0931	60.00	89.00
	Group 5 (10% Contamination)	5	110.4000	11.05893	4.94571	96.6685	124.1315	92.00	118.00
	Total	25	74.8800	21.39766	4.27953	66.0475	83.7125	48.00	118.00
HDL	Group 1 (Control)	5	78.0000	.00000	.00000	78.0000	78.0000	78.00	78.00
	Group 2 (1% Contamination)	5	58.4000	4.15933	1.86011	53.2355	63.5645	54.00	63.00
	Group 3 (3% Contamination)	5	42.0000	1.41421	.63246	40.2440	43.7560	40.00	43.00
	Group 4 (5% Contamination)	5	37.2000	4.43847	1.98494	31.6889	42.7111	32.00	43.00
	Group 5 (10% Contamination)	5	33.6000	2.88097	1.28841	30.0228	37.1772	30.00	37.00
	Total	25	49.8400	17.01147	3.40229	42.8180	56.8620	30.00	78.00
LDL	Group 1 (Control)	5	17.0000	.00000	.00000	17.0000	17.0000	17.00	17.00
	Group 2 (1% Contamination)	5	29.6000	5.36656	2.40000	22.9365	36.2635	23.00	38.00
	Group 3 (3% Contamination)	5	36.6000	1.67332	.74833	34.5223	38.6777	35.00	39.00
	Group 4 (5% Contamination)	5	46.0000	2.54951	1.14018	42.8344	49.1656	43.00	49.00
	Group 5 (10% Contamination)	5	60.8000	3.56371	1.59374	56.3751	65.2249	57.00	65.00
	Total	25	38.0000	15.39751	3.07950	31.6442	44.3558	17.00	65.00
TAG	Group 1 (Control)	5	45.0000	.00000	.00000	45.0000	45.0000	45.00	45.00
	Group 2 (1% Contamination)	5	52.4000	5.12835	2.29347	46.0323	58.7677	46.00	58.00
	Group 3 (3% Contamination)	5	52.6000	5.36656	2.40000	45.9365	59.2635	46.00	58.00
	Group 4 (5% Contamination)	5	81.0000	16.80774	7.51665	60.1304	101.8696	61.00	97.00
	Group 5 (10% Contamination)	5	91.6000	11.43678	5.11468	77.3994	105.8006	79.00	103.00
	Total	25	64.5200	20.66422	4.13284	55.9902	73.0498	45.00	103.00
Protein	Group 1 (Control)	5	7.3000	.00000	.00000	7.3000	7.3000	7.30	7.30
	Group 2 (1% Contamination)	5	8.2200	1.05214	.47053	6.9136	9.5264	7.20	9.90
	Group 3 (3% Contamination)	5	10.5000	.41833	.18708	9.9806	11.0194	9.80	10.90
	Group 4 (5% Contamination)	5	9.3800	.56303	.25179	8.6809	10.0791	8.70	10.10
	Group 5 (10% Contamination)	5	10.0600	1.49097	.66678	8.2087	11.9113	8.10	11.80
	Total	25	9.0920	1.44566	.28913	8.4953	9.6887	7.20	11.80
ALT	Group 1 (Control)	5	13.0000	.00000	.00000	13.0000	13.0000	13.00	13.00
	Group 2 (1% Contamination)	5	21.0000	6.63325	2.96648	12.7637	29.2363	14.00	31.00
	Group 3 (3% Contamination)	5	21.2000	5.01996	2.24499	14.9669	27.4331	15.00	27.00
	Group 4 (5% Contamination)	5	22.4000	7.43640	3.32566	13.1665	31.6335	15.00	34.00

	Group 5 (10% Contamination)	5	25.2000	12.63725	5.65155	9.5088	40.8912	15.00	40.00
	Total	25	20.5600	8.03679	1.60736	17.2426	23.8774	13.00	40.00
AST	Group 1 (Control)	5	59.0000	.00000	.00000	59.0000	59.0000	59.00	59.00
	Group 2 (1% Contamination)	5	91.4000	17.54423	7.84602	69.6160	113.1840	76.00	118.00
	Group 3 (3% Contamination)	5	100.6000	16.50152	7.37970	80.1107	121.0893	89.00	129.00
	Group 4 (5% Contamination)	5	112.8000	16.23884	7.26223	92.6368	132.9632	98.00	131.00
	Group 5 (10% Contamination)	5	141.4000	25.79341	11.53516	109.3733	173.4267	108.00	175.00
	Total	25	101.0400	31.73783	6.34757	87.9393	114.1407	59.00	175.00
ALP	Group 1 (Control)	5	2390.0000	.00000	.00000	2390.0000	2390.0000	2390.00	2390.00
	Group 2 (1% Contamination)	5	2745.0000	82.83417	37.04457	2642.1478	2847.8522	2630.00	2829.00
	Group 3 (3% Contamination)	5	2888.4000	186.17008	83.25779	2657.2393	3119.5607	2594.00	3104.00
	Group 4 (5% Contamination)	5	2666.6000	192.06587	85.89447	2428.1187	2905.0813	2523.00	3004.00
	Group 5 (10% Contamination)	5	2856.4000	296.66193	132.67125	2488.0456	3224.7544	2536.00	3299.00
	Total	25	2709.2800	246.60199	49.32040	2607.4877	2811.0723	2390.00	3299.00

*. The mean difference is significant at the 0.05 level.

One way {Week 12 of Administration}

Descriptive

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
T_Chol	Group 1 (Control)	5	57.0000	.00000	.00000	57.0000	57.0000	57.00	57.00
	Group 2 (1% Contamination)	5	61.6000	6.18870	2.76767	53.9157	69.2843	56.00	72.00
	Group 3 (3% Contamination)	5	65.4000	12.58173	5.62672	49.7777	81.0223	48.00	81.00
	Group 4 (5% Contamination)	5	65.6000	13.03073	5.82752	49.4202	81.7798	45.00	80.00
	Group 5 (10% Contamination)	5	88.0000	20.53047	9.18150	62.5081	113.4919	67.00	108.00
	Total	25	67.5200	15.83540	3.16708	60.9835	74.0565	45.00	108.00
HDL	Group 1 (Control)	5	78.0000	.00000	.00000	78.0000	78.0000	78.00	78.00
	Group 2 (1% Contamination)	5	57.6000	1.67332	.74833	55.5223	59.6777	55.00	59.00
	Group 3 (3% Contamination)	5	44.6000	2.70185	1.20830	41.2452	47.9548	40.00	47.00
	Group 4 (5% Contamination)	5	39.6000	5.77062	2.58070	32.4348	46.7652	30.00	45.00
	Group 5 (10% Contamination)	5	32.6000	2.07364	.92736	30.0252	35.1748	30.00	35.00
	Total	25	50.4800	16.57589	3.31518	43.6378	57.3222	30.00	78.00
LDL	Group 1 (Control)	5	17.0000	.00000	.00000	17.0000	17.0000	17.00	17.00
	Group 2 (1% Contamination)	5	32.0000	1.87083	.83666	29.6771	34.3229	30.00	35.00
	Group 3 (3% Contamination)	5	37.8000	1.64317	.73485	35.7597	39.8403	35.00	39.00
	Group 4 (5% Contamination)	5	44.4000	2.40832	1.07703	41.4097	47.3903	41.00	47.00
	Group 5 (10% Contamination)	5	66.0000	1.87083	.83666	63.6771	68.3229	63.00	68.00
	Total	25	39.4400	16.48252	3.29650	32.6364	46.2436	17.00	68.00
TAG	Group 1 (Control)	5	45.0000	.00000	.00000	45.0000	45.0000	45.00	45.00
	Group 2 (1% Contamination)	5	55.0000	5.47723	2.44949	48.1991	61.8009	47.00	61.00
	Group 3 (3% Contamination)	5	56.0000	3.87298	1.73205	51.1911	60.8089	52.00	61.00
	Group 4 (5% Contamination)	5	89.4000	14.74110	6.59242	71.0965	107.7035	70.00	101.00
	Group 5 (10% Contamination)	5	105.6000	10.94532	4.89490	92.0096	119.1904	91.00	116.00
	Total	25	70.2000	24.97999	4.99600	59.8888	80.5112	45.00	116.00
Protein	Group 1 (Control)	5	7.3000	.00000	.00000	7.3000	7.3000	7.30	7.30
	Group 2 (1% Contamination)	5	8.5000	1.02713	.45935	7.2246	9.7754	7.50	10.20
	Group 3 (3% Contamination)	5	10.8400	.37148	.16613	10.3787	11.3013	10.20	11.10
	Group 4 (5% Contamination)	5	9.8000	.59161	.26458	9.0654	10.5346	9.30	10.70
	Group 5 (10% Contamination)	5	10.2600	.77330	.34583	9.2998	11.2202	9.20	11.20
	Total	25	9.3400	1.43527	.28705	8.7475	9.9325	7.30	11.20
ALT	Group 1 (Control)	5	13.0000	.00000	.00000	13.0000	13.0000	13.00	13.00
	Group 2 (1% Contamination)	5	23.0000	6.28490	2.81069	15.1963	30.8037	17.00	33.00
	Group 3 (3% Contamination)	5	24.8000	4.43847	1.98494	19.2889	30.3111	18.00	30.00
	Group 4 (5% Contamination)	5	28.4000	7.43640	3.32566	19.1665	37.6335	20.00	40.00

	Group 5 (10% Contamination)	5	34.2000	14.20211	6.35138	16.5657	51.8343	20.00	50.00
	Total	25	24.6800	10.17235	2.03447	20.4811	28.8789	13.00	50.00
AST	Group 1 (Control)	5	59.0000	.00000	.00000	59.0000	59.0000	59.00	59.00
	Group 2 (1% Contamination)	5	100.2000	24.47856	10.94715	69.8059	130.5941	59.00	118.00
	Group 3 (3% Contamination)	5	119.6000	15.58204	6.96850	100.2523	138.9477	108.00	146.00
	Group 4 (5% Contamination)	5	141.8000	14.85598	6.64379	123.3539	160.2461	129.00	158.00
	Group 5 (10% Contamination)	5	181.4000	24.27550	10.85633	151.2580	211.5420	146.00	211.00
	Total	25	120.4000	44.90546	8.98109	101.8639	138.9361	59.00	211.00
ALP	Group 1 (Control)	5	2390.0000	.00000	.00000	2390.0000	2390.0000	2390.00	2390.00
	Group 2 (1% Contamination)	5	2750.4000	81.03579	36.24031	2649.7808	2851.0192	2638.00	2833.00
	Group 3 (3% Contamination)	5	2902.0000	180.73738	80.82821	2677.5849	3126.4151	2617.00	3112.00
	Group 4 (5% Contamination)	5	2691.6000	205.68860	91.98674	2436.2039	2946.9961	2550.00	3055.00
	Group 5 (10% Contamination)	5	2880.0000	297.08585	132.86083	2511.1192	3248.8808	2545.00	3321.00
	Total	25	2722.8000	252.13918	50.42784	2618.7221	2826.8779	2390.00	3321.00

*. The mean difference is significant at the 0.05 level.