Effect of Green Tea (*Camellia Sinensis*) Leaf Extract on Highly Active Anti-Retroviral Therapy Induced Dyslipidemia and Non Alcoholic Fatty Liver Disease in Albino Wistar Rats

By: Tesaka Wondimnew (DMD)

A thesis submitted to School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements of the Degree of Master of Science in Medical Biochemistry

June, 2016
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School of Graduate Studies

This is to certify that the thesis prepared by Tesaka Wondimnew entitled,"***Effect of Green Tea (Camellia Sinensis) Leaf Extract on Highly Active Anti-Retroviral Therapy (HAART) Induced Dyslipidemia and Non Alcoholic Fatty Liver Disease in Albino Wistar Rats***" and submitted in partial fulfillment of the requirements of Degree of Master of Science in Medical Biochemistry complies with regulations of the University and meets the accepted standards with respect to originality and quality.

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Abstract

Back ground: Tea is the second widely consumed drink in the world next to water and used by people in different parts of the world for treatment of different health ailments; especially, green tea. HAART which is a recently(since 1996) developed form of ART medication to treat HIV/AIDS is now becoming hepatotoxic to patients and found to be more hazardous to the health of HIV patients.

Objective: The aim of this study was to investigate the possible remedial effect of GTE on HAART induced derangement of lipid metabolism in albino Wistar rats.

Materials and Methods: Thirty female albino Wistar rats 10-12 weeks old and weight of 145-200 grams were divided in to five groups of six each randomly. Group-I (normal control group) which were given 1mL of distilled water per day, Group-II (negative control group) which were given HAART only, Group-III (which were given HAART and 100mg/Kg of GTE), Group-IV (which were given HAART and 200mg/Kg of GTE) and Group-V (which were given HAART and 400mg/Kg of GTE) for sixty days. On the 60^{th} day of the experiment rats was fasted overnight and sacrificed by cervical dislocation after giving di-ethyl ether general anesthesia. Then blood was taken by cardiac puncture for lipid profile investigation and for liver function test. Liver was also taken from each experimental rat for histopathological evaluation and triglyceride extraction.

Results: The results of our experiment showed that, the body weight change showed a significant decrement ($p<0.05$) in group-II and more decrement in group-IV and group-V rats ($p<0.01$). AST and ALT level in group-V showed a significant decrement ($p<0.05$) but no significant change on serum ALP level though there is reduction. Serum lipid profile TC, TG and LDL-c showed a significant decrement ($p<0.05$) in group-V but no significant change in HDL-c in group-V. TTG (Tissue triglycerides) also showed a significant decrement ($p<0.05$) in group-V as compared to group-II. As demonstrated by NAS, most of group II rats developed steatosis followed by group-III rats and the histopathological observation showed the better remedial effect of GTE on liver especially with the higher dose (400mg/Kg).

Conclusion: The results of this study demonstrated that the green tea leaf extract has a good protective effect against HAART induced hepatotoxicity.Hence,it prevents dyslipidemia and NAFLD which might be due to its antioxidant property.

KeyWords;Highly Active Anti-Retroviral Therapy, Hepatotoxicity, Green Tea leaf Extract, Dyslipidemia, Non Alcoholic Fatty Liver Disease.

Acknowledgment

First and foremost I would like to thank my creator, shepherd almighty God who is always on the side of me in ups and downs in life and his mother St' Virgin Mary who is my intercessor. Then, I would like to thank my advisors Dr. Solomon Genet & Dr.N.Gnanasekaran from department of Medical Biochemistry for their relentless support and their valuable constructive ideas and comments. Dr.Mahlet Areyaselassie from department of pathology for her help in reading and interpreting histopathology slides carefully by giving more time from her busy time and to Ato Yeshiwas Abtie and his colleagues from pathology department who helped me in processing liver tissue to make it ready for microscopic investigation.

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Abbreviations and Acronyms

3TC = Lamivudine

ABC = Abacavir

AIDS = Acquired immune deficiency syndrome

ALP = Alkaline phosphatase

ALT = Alanine transaminase

ART = Antiretroviral therapy

AST = Aspartate transaminase

ATV = Atazanavir

bHLH = Basic helix-loop-helix

CRABP1 = Cytoplasmic retinoic acid-binding protein type 1

CVD = Cardiovascular disease

CYP450 = Cytochrome P450

D4T = Stavudine

dATP = Deoxyadenosine Triphosphate

dCTP = Deoxycytidine Triphosphate

dGTP = Deoxyguanine Triphosphate

ddC = Zalcitabine

ddI = Didanosine

dNTP = Deoxy nucleotide Triphosphate

DMEs = Drug metabolizing enzymes

ECG = Epicatechin-3-gallate

EFV = Efavirenz

EGC = Epigallocatechin

EGCG = Epigallocatechin gallate

ER = Endoplasmic reticulum

FATP2/5 = Fatty acid transporter protein 2/5

FMO = Flavin monooxygenase

FMOH = Federal ministry of health

GTE = Green tea extract

GLUT 2 = Glucose transporter 2

GLUT 4 = Glucose transporter 4

HAART = Highly active antiretroviral therapy

HDL-c = High density lipoprotein cholesterol

HIV = Human immune deficiency Virus

IDV = Indinavir

LDL-c = Low density lipoprotein cholesterol

LFT = Liver function test

MRC = Mitochondrial respiratory chain complex

mtDNA = mitochondrial DNA

NAFLD = Nonalcoholic fatty liver disease

NAS = Non alcoholic fatty liver disease Activity Score

NASH = Nonalcoholic steatohepatitis

NNRTIs = Non-nucleoside reverse transcriptase inhibitors

NRTIs = Nucleoside reverse transcriptase inhibitors

NtRTIs= Nucleotide reverse transcriptase inhibitors

NVP = Nevirapine

PIs = Protease inhibitors

PPAR = Peroxisome proliferator-activated receptor

ROS = Reactive oxygen species

RTV = Ritonavir

S1P = Site 1 protease

S2P = Site 2 protease

SCAP = SREBP cleavage-activating protein

SQV = Squinavir

SREBPs = Sterol regulatory element binding proteins

TC = Total cholesterol

TG = Triglycerides

VLDL = Very low density lipoprotein

ZDV = Zidovudine

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1. INTRODUCTION

1.1. Back Ground

Liver is one of the largest organs in our body in which hapatocytes account 80% and it is divided into segments called lobes. These lobes are surrounded by a fibrous connective tissue which gives protection to the liver called the Gilsson's capsule. This organ has a direct venous blood supply from the gut and toxins after absorption from the intestine via portal vein which is one way of blood entry to this organ and the other is hepatic artery (carries the oxygenated blood) and blood leaves this organ via hepatic vein(Duncan and Stephen, 2000).

The liver cells (hepatocytes) which carry out the main function of the liver are organized into plates and are separated by sinusoidal capillaries (sinusoids) which are linked by endothelial cells and Kuppfer cells and separated from hepatocytes by space of dissie a space where vitamin A is stored, fibroblasts and lipocytes/fat cells found.This close proximity of sinusoids and basal surface of hepatocytes in turn helps to facilitate exchange of substances between the blood and the liver cells (Mutter *et al.,* 2010).

Liver plays a vital role in metabolism of carbohydrate, fat, lipid and protein, urea, processing of xenobiotis, synthesis and excretion of bile, synthesis of clotting factors, detoxification of drugs and many more which make it the busiest and vulnerable organ to damages related to metabolism and intoxication because it is the first organ to encounter toxins and highly reactive metabolites than any other organs in our body (Duncan and Stephen, 2000).

1.2 The Role of Liver in Drug Metabolism

Drug metabolism is a chemical process in which drugs are oxidized, reduced or hydrolyzed usually to more polar metabolites so that they exhibit increased water solubility.This polarity in turn, facilitates easy elimination in urine or bile and allow increased access to excretory transporters. Liver, as described above, is the most important site of drug metabolism both quantitatively and qualitatively though drugs are also being metabolized out of the liver like in the gastrointestinal mucosa and by circulating enzymes such as esterases in the blood tissue (Handschin and Meyer, 2005).

Liver is also known for its unique feature of metabolizing pathways which are characterized by their ability to cope with endless array of drug substrates which enhance the liver to metabolize drugs successfully and clear them irreversibly even drugs that are newer and fully synthetic and have no structural similarity in nature. This broad substrate recognition is achieved by the presence of multiple drug metabolizing enzymes(DMEs) in hepatocytes (Shen *et al*., 1997) which are responsible for metabolizing a vast array of drugs including xenobiotic compounds like, environmental pollutants and endogenous compounds such as steroids and prostaglandins(Guengerich,1995).

Drug metabolizing enzymes include some members of the cytochrome P450 (CYP450) and flavin monooxygenase (FMO) families which are known to metabolize more than 50 structurally diverse compounds (Handschin and Meyer, 2005).These DMEs are not evenly distributed in lobular architecture of the liver to avoid massive damage to the liver during metabolism by highly reactive(toxic) intermediates/metabolites. Hence, for example; CYP3A4 which is responsible for metabolizing large number of drugs is localized in zone 3(around the central vein) of hepatocyte to limit the damage caused by these drugs (Yokose *et al*., 1999).

1.3 Highly Active Anti-retroviral Therapy (HAART)

HIV which is genetically related to a member of *Lentivirus* genus and *Retroviridae* family has persistent viral replication power and known to cause infection that is chronic with latent clinical features that involve central nervous system (Emanuele *et al*., 2010).The genome of these retroviruses composed of two identical single stranded RNA molecules which possesses three structural genes called *gag,pol and env* (Luciw,1996).

These structural genes in HIV are responsible for encoding various proteins that are needed for the replication of this virus. Thus, the *gag* gene encodes the structural proteins of the core (p24, p7, and p6) and matrix (p17), the *env* gene encodes the viral envelope glycoproteins gp120 and gp41, which recognize cell surface receptors and the *pol* gene encodes reverse transcriptase that converts viral RNA into DNA, the integrase that incorporates the viral DNA into host chromosomal DNA and the protease that cleaves large Gag and Pol protein precursors into their components (Emanuele *et al*., 2010).

Different highly active antiretroviral therapy (HAART) regimens are currently being in use and have been found effective in increasing life expectancy and immune status of HIV positive patients in the world since 1996(Rouleau *et al*., 2011). These HAART regimens typically include a combination of at least three drugs, such as different association of protease inhibitors (PIs), non-nucleoside reverse transcriptase inhibitors (NNRTI) and nucleoside/tide reverse transcriptase inhibitors (N/NtRTI) (Gsponer *et al.,* 2012; Young, 2005).

Thus, the introduction of HAART has led to a marked reduction in AIDS-related morbidity and mortality because of the diversity of these drugs and their interference at different sites in the life cycle of the virus at the same time enhance them to disrupt the progress of viral proliferation in the body of the patient aggressively (Enanoria *et al*., 2004).

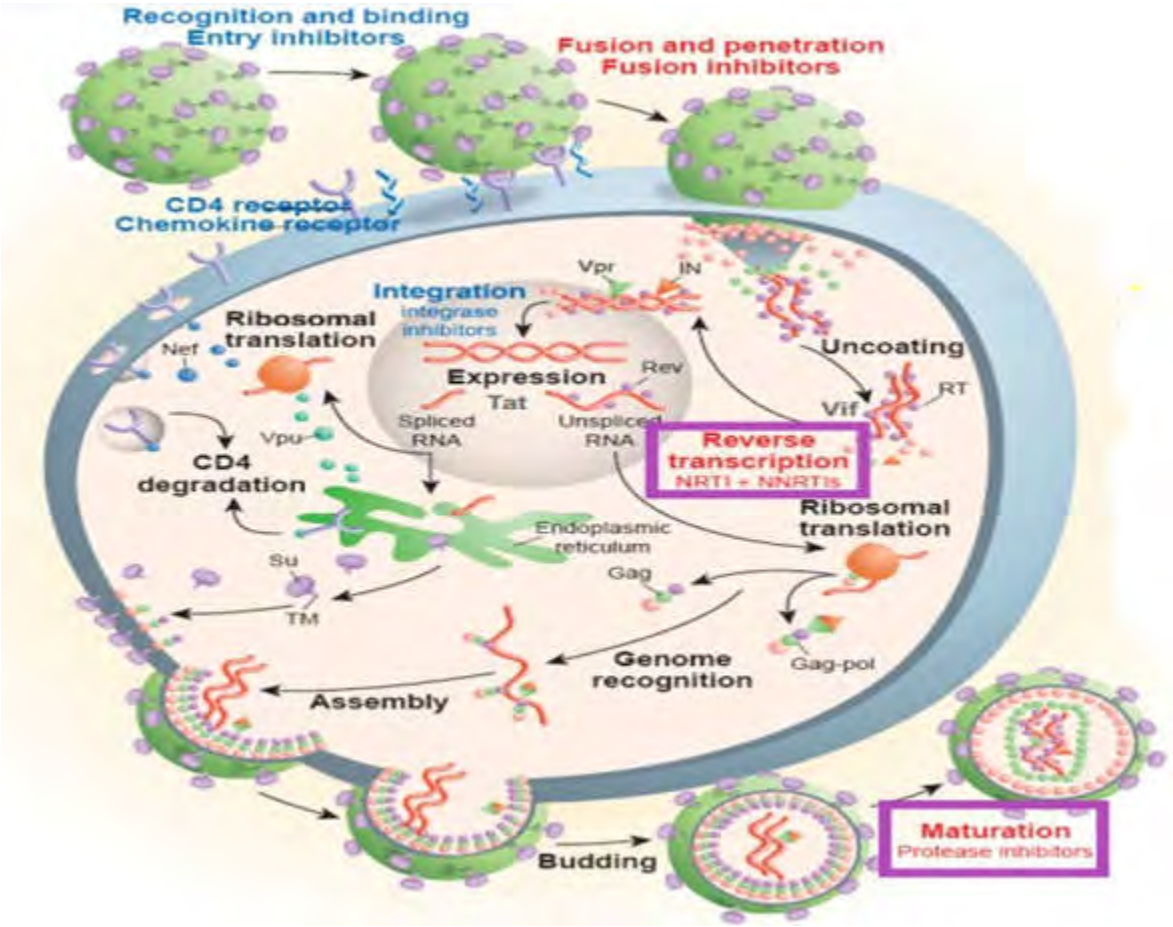


Figure1.1: Targets of highly active antiretroviral therapy (HAART) drugs at different stages of HIV life cycle (*http*://www.science.oregonstate.edu/bio/classes/bi420/BI420).

However, maintaining patients on long term HAART may be restricted by different unexpected metabolic abnormalities, including deregulation of glucose and lipid metabolism (Gallagher, 2007). Hence, use of HAART has been linked to hyperglycemia, dyslipidemia and increased risk of cardiovascular disease (CVD) following these metabolic deregulations in one way or the other in HIV infected patients (Buchacz *et al*., 2008) mainly due to mitochondrial damage induced by these drugs (Apostolova *et al*., 2011).

1.3.1Highly Active Anti-Retroviral Therapy (HAART) Induced Hepatotoxicity

Although HAART has offered a great relieve for the HIV patients by decreasing the morbidity and mortality from opportunistic infections and malignancies which are secondary to immunity deterioration, it also carries negative side effects, including metabolic derangements like, lipodystrophy syndrome, cardio vascular disease (CVD) and hepatotoxicity which impede the management of HIV infected patients and increase burden of the patient to survive (Farrugia *et al.*, 2009).From HAART, the PI's full dose ritonavir (RTV) has been found to be severely hepatotoxic (Bonfanti *et al*., 2001; Pai *et al* .,2000) and several cases of liver toxicity have also been reported associated with the use of Idinavir (IDV) and squinavir (SQV)(Sulkowski,2003). Many NRTIs induce mitochondrial damage and thus, have also been associated with hepatotoxicity and severe hepatic injury has been reported by the use of zidovudine,didanosine and stavudine(Gisolf *et al*., 2000).The hepatotoxic effect of NNRTIs is also been invesitigated and the incidence of liver toxicity is similar to other antiretrovirals (Palmon *et al*., 2002).

Mechanism of hepatotoxicity by HAART is mainly described by mitochondrial damage which is a base line for liver damage. NRTIs are designed to have structural resemblance with natural substrates of viral reverse transcriptase(RT) like,zidovudine (ZDV),which is also called 3'-azido-3'deoxythymidine (AZT), is an analogue to thymidine except replacement of 3'hydroxyl by another group and it competes for the active site of reverse transcriptase(RT) after being phosphorylated by cellular kinases and incorporating into viral DNA to act as a chain terminator in synthesis of HIV proviral DNA (Warnke *et al*., 2007).

Other NRTIs like; didanosine(ddI),stavudine(d4T), zalcitabine (ddC),lamivudine (3TC) and abacavir were developed in 90's for patients who are intolerant to ZDV which is characterized by decrease in CD4+ count,lymphocytes and increase viremia.These drugs after being

phosphorylated to active triphosphate metabolite ,they compete to the active site i.e., ddI converted to dideoxyadenosine and compete with dATP, Zalcitabine is metabolized within cells to dideoxycytidine (ddC) in an active triphosphate form and compete with dCTP. Abacavir, unlike the other NRTI's, is a guanine analogue that, when converted to the active form carbovir triphosphate, competes with the natural substrate dGTP (Thompson *et al*., 2010). NRTIs which are designed to inhibit reverse transcriptase that convert single stranded viral RNA to double strand viral DNA in host cells are becoming the main factor for cellular mitochondrial damage which in turn causes mitochondrial depletion by interfering with polymerase-γ (enzyme for replication of mitochondrial DNA)(Benbrik *et al*., 1997).

Since, NRTIs/NtRTIs act by competing with dNTPs for the catalytic site of reverse transcriptase, when they are in surplus they occupy this site and induce termination of viral DNA elongation. At the same time, these drugs intercalate with mtDNA poly-γ during mtDNA replication and terminate the normal progressing pathway and compromise the number of cellular mitochondria (Benbrik *et al*., 1997). This depletion of mitochondrial DNA impairs the cellular respiratory chain and inhibits pyruvate and fatty acid oxidation pathways because proteins that are needed for the relay of electrons in the mitochondrial matrix are translated from a transcript from mtDNA called mtRNA. This in turn leads to disrupted electron transport through the MRC (mitochondrial respiratory chain complexes) and a concomitant reduction in proton efflux and reducing the membrane potential and ATP production by the mitochondrion. This disturbed mitochondrial function result in increased reactive oxygen species (ROS)production and morphological changes which in turn aggravates further mitochondrial damage (Lewis *et al*., 2006).

Non nucleoside reverse transcriptase inhibitors (NNRTIs) are developed to treat HIV infection by acting directly without being phosphorylated by cellular kinases and non competitively to the active site by binding to allosteric site closer to the active site which causes conformational change and disrupt the routine function of reverse transcriptase of the virus(Warnke *et al*., 2007).

Protease inhibitors (PIs) are synthetic analogue of HIV protein and act by blocking the action of HIV-protease and act in the last and key step in the transformation of the immature viral particles

to mature infectious virion. Hence, these drugs disrupt the processing of large HIV precursor proteins, such as p55 and p40 encoded by the *gag* and *gag-pol* genes of HIV into smaller structural proteins p17, p24, and p7 of the viral core which is performed via proteolytic cleavage by an HIV-encoded aspartic protease (Wensing *et al*., 2010).

1.3.2 Highly Active Anti-Retroviral Therapy (HAART) and Lipid Metabolism

Although the substantial benefits that results from the use of HAART regimens, laboratory and clinical experience have shown that HAART can induce considerable side effects on lipid metabolism which is characterized by lipodystrophy, central adiposity, dyslipidemia, increased risk of CVD and atherosclerosis (Sprinz *et al*., 2010).

HAART associated dyslipidemia which is characterized by hypertriglyeridemia hypercholesterolemia, decreased serum levels of HDL-c either accompanied or not by increased levels of LDL-c and involves hormonal and genetic predisposition; is one of complex lipid metabolism derangement(Fisher *et al*., 2006).HAART also affects the hydrolysis of triglyceride rich lipoproteins and tissue lipase, disrupts normal postprandial free fatty acid and lipoprotein catabolism and interferes with peripheral fatty acid trapping. All of these effects could be due to the interaction of these fatty acids with the master transcriptional regulator sterol regulatory element binding protein1 (SREBP1) (Miserez *et al*., 2002).

The sterol regulatory element binding proteins (SREBPs) are transcription factors that are encoded by 2 genes, SREBP1 &2 and wraps out from the ER membrane. Their activation as transcription factor is tightly controlled by the levels of cholesterol present in the membrane. Hence, they are important regulators of cholesterol metabolism (Horton *et al*., 2002) most notably with respect to cholesterol biosynthesis; but also have an important role in fatty acid metabolism (Edwards *et al*., 2000). SREBP-2 stimulates the transcription of genes encoding enzymes which are needed in cholesterol synthesis.SREBP-1c which is one isoform of SREBP-1 activate genes encoding enzymes that are needed for fatty acid synthesis like acetylcoA carboxylase and fatty acid synthase which in turn contribute for synthesis of TG and phospholipids.SREBP-1c and SREBP-2 positively regulate the three enzymes, mallic enzyme, glucose-6-phosphodehydrogenase & 6-phosphogluconate dehydrogenase which participate in cholesterol and fat synthesis (Beatrice *et al*., 2006).

The NRTI-based HAART, zidovudine, stavudine or lamivudine, have become associated with the occurrence of dyslipidemia; however, lipid metabolism disorders are most evident in individuals who are in use of PI-based therapy (Abebe *et al*., 2014).Protease inhibitors promote dyslipidemia (hypertriglyceridemia, increase in total cholesterol and LDL-C and decrease in HDL-C) even in short-term studies (≤ 4 weeks) with RTV (Purnell *et al*., 2000), and LPV/RTV (Lee *et al*., 2005) in HIV-seronegative men.

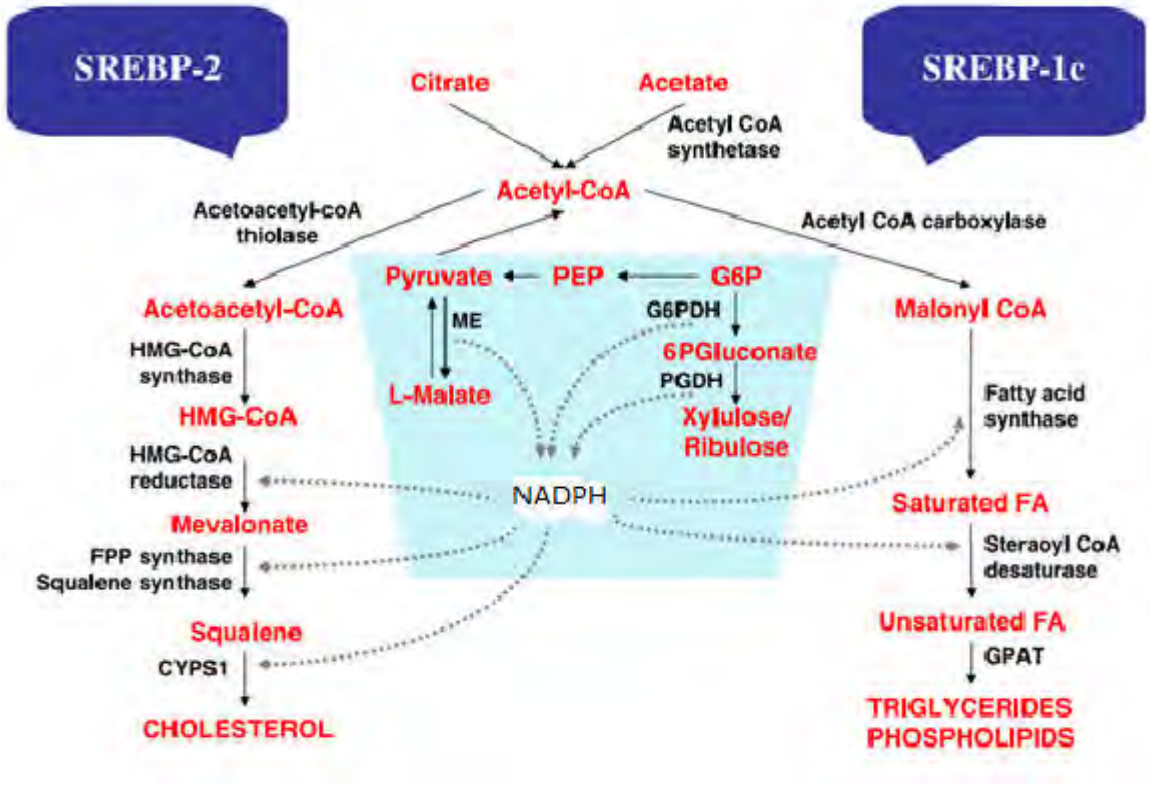


Figure 1.2: Role of Sterol regulatory element binding proteins in cholesterol and fatty acid metabolism (Beatrice *et al*., 2006).FPP = Farnesyl-pyrophophate synthase, G6P = glucose-6-phosphate, G6PDH = glucose-6-phosphodehydrogenase, HMG-CoA = 3-hyroxy-methyl glutaryl CoA, GPAT = glycerol-3-phosphate acyltransferase, ME = Malic enzyme, PEP = Phospho-enoyl pyruvate, PGDH = 6-phosphogluconate dehydrogenase.

Protease inhibitors stimulate hepatic lipid synthesis by maintaining the nuclear activity of the SREBP (Huan *et al*., 2003). GLUT2 which is the major glucose transporter in hepatocytes is unaffected by PIs unlike GLUT 4 in adipocytes in which PIs bind to GLUT 4 and prevent glucose transport to the adipocytes and muscle cells (Flint *et al*., 2005). There is also no concrete

evidence that PIs affect the transport of fatty acids by FATP2 or FATP5. Instead, PIs affect hepatocyte metabolism by extending the activity of transcription factors involved in regulating lipid synthesis (Parker *et al*., 2005).

In resting state, these SREBPs remain bound to the membrane of endoplasmic reticulum in close association with SCAP (SREBP cleavage-activating protein) the sterol-sensing escort protein (Horton *et al*., 2002). But when sterols are depleted, the SREBP-SCAP complex moves from the endoplasmic reticulum into the Golgi, where Site 1 and 2 proteases (S1P, S2P) clip the basic helix-loop-helix (bHLH) domain/N-terminal domain of SREBP out of the membrane for export to the nucleus (Brown *et al*., 2000). Then SREBP enters the nucleus and binds to response elements of lipogenic genes, inducing their transcription and ultimately increasing the synthesis of cholesterol and triglycerides. SREBP half-life in the nucleus is determined by its rate of degradation by nuclear proteasomes proteolysis (Hirano *et al*., 2001) but PIs inhibit this proteolysis, extending the half-life of SREBP, thus further increase the synthesis of hepatic lipids (Parker *et al*., 2005).

1.3.3 HAART and Non Alcoholic Fatty Liver Disease (NAFLD)

Nonalcoholic fatty liver disease (NAFLD) refers to fat deposition in hepatocytes (>5%) in individuals with little (less than 20 gram/day) and with no infection of HCV (Perez-Matute *et al*., 2013) or no alcohol use. When it is accompanied by inflammation and fibrosis, it is referred to as nonalcoholic steatohepatitis (NASH) (Farrell and Larter, 2006).

In HAART-induced metabolic derangements, many features are similar to those observed in NAFLD in obese peoples though HAART-induced NAFLD in HIV patients do not always seem to be correlated with diet. Although HAART-related NAFLD has no its own unique clinical feature, many patients taking PIs may have NAFLD with no symptoms, like the overweight population does (Akhtar *et al*., 2008). However, some patients develop nonalcoholic steatohepatitis (NASH), which is accompanied with excessive inflammation and scarring, potentially causing severe damage to the liver and as previous studies demonstrated, NASH found in more than 50% of HAART-treated patients who were undergoing liver histopathological assessments (Akhtar *et al*., 2008; Ingiliz *et al*., 2009).

HAART has been clearly shown to alter lipid and carbohydrate metabolism pathways which contribute to the underlying mechanism of NAFLD development (Rector *et al*., 2008).There is a strong association between insulin resistance and NAFLD and this fat that accumulates in the liver may come from adipose tissue, *de novo* lipogenesssis (DNL) and from high fat diet though Donnelly and his colleagues demonstrated that the majority of hepatic lipids in patients with NAFLD came from peripheral non esterified fatty acids(NEFA) and DNL, not from the diet (Donnelly *et al*. 2005).

Although the development of NAFLD (steatosis) and NASH (steatohepatitis) have no a clear mechanism, earlier studies suggested that steatosis may be mediated by insulin resistance which is caused by HAART specially by PIs (Flint *et al*., 2005).There is also a strong association between NAFLD and DM, hyperlipidemia & obesity which strengthen that insulin resistance as a possible mediator of NAFLD development. Liver metabolizes, free fatty acids by lipase partly and these free fatty acids oxidized in mitochondrion to produce energy and to the synthesis of cholesterol and triglycerides. Normally, insulin inhibits lipases and persons with IR have more insulin in their serum which enhances more inhibitory effect on lipases. This causes more delivery of free fatty acids to the liver than oxidation which in turn leads for more accumulation of triglycerides and fat in the liver (Reid, 2001).

Protease inhibitors which are in the front line for the derangement of hepatic metabolism, has been frequently accompanied by the development of western diet-induced like NAFLD by inducing ER stress(Erickson, 2009) and this induction has been linked to various human diseases including NAFLD/NASH (Wu *et al*., 2010).

1.4 Green tea (*Camellia Sinensis*)

Tea plant is an evergreen shrub or small tree of the *theaceae* family*, Camelia* genus, native to Japan, China, India, Southeastern Asia, with dark green shiny leaves and white flowers and used for preparation of the second largely consumed drink in the world. It was discovered accidentally by a Chinese emperor Shen Nung in 1273 B.C and when some leaves fell in a pot of boiling drinking water producing a special taste and fragrance .When the emperor tasted the infusion, he was pleasantly surprised and the tea become a famous drink from that time on ward (Wheeler and Wheeler, 2004) now become the second largest drink in the world next to water. Processing

of tea leaves allows the production of various types of tea like, white, oolong, green and black tea depending on the extent of the oxidation/fermentation (Zhang *et al.*, 2012).

There are four main steps in tea leaf processing. The first step is withering and wilting which is done usually in sun light and cold room with plenty of air movement to remove water from the leaf which in turn enhance breakdown of tea proteins by peptidases to free amino acids (Hampton, 1992) and high level of caffeine and polyphenol oxidase are released as tea leaf cells are more permeable following this peptidase effect(Bondarovich *et al*., 1967).The next step is leaf bruising in which the wilted tea leaves are bruised to break down the leaf structure that allows mixing of enzymes and polyphenols for more oxidation to take place. For better bruising, all of the moisture should be removed at first step. The third step is oxidation/fermentation; the key step that determines the major chemical constituents of tea leaf. At this step, the tea leaves are left in climate controlled room for oxidation or fermentation to proceed for a desired period of time (Bondarovich *et al*., 1967).The last step is drying in which the tea leaves are dried by pan fry traditionally and other techniques like sunning and oven drying (Graham, 1992).The use of heat at this stage limits further oxidation/fermentation by inactivating polyphenol oxidase and removes remaining moisture (Bondarovich *et al*., 1967).

From the total tea production of the world, black tea accounts 78%, green tea 20% and other tea types account ~ 2% only(Cooper, 2011). Green tea and oolong tea, previously peculiar to Asia are also now becoming popular in Western world and this increased popularity of green tea in Western countries resulting from a number of studies which link its consumption with beneficial health effects like anticancer (Link *et al* ., 2010), antimicrobial (Ikigai *et al*., 1993),cardio-protective (Dreger *et al*., 2008) and anti-diabetic properties (Chen *et al*., 2010) associated mainly with its antioxidant properties that comes from its highly enrichment with polyphenols (Cooper, 2011).

The term polyphenol is used to describe a chemical structure that is built around two or more phenol groups. Most of these polyphenols in tea are flavanols commonly called catechins (Balentine *et al*., 1997) and these flavanols are also a sub class of flavonoids. Green tea is one of the most abundant food sources of well preserved catechins (Łuczaj and Skrzydlewska, 2005).

The main catechins in green tea are epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG). Catechin and epicatechin both are monomeric flavanol, epicatechin has an ortho-dihydroxyl group in the B-ring at carbons 3' and 4' and a hydroxyl group at carbon 3' on the C-ring. EGC has trihydroxyl group at carbons 3', 4', and 5' on the B-ring. ECG has gallate moiety esterified at carbon 3 of the C-ring. However, EGCG has both trihydroxyl groups at carbons 3', 4', and 5' on the B-ring and a gallate moiety esterified at carbon 3' on the C-ring (Yilmaz, 2006).

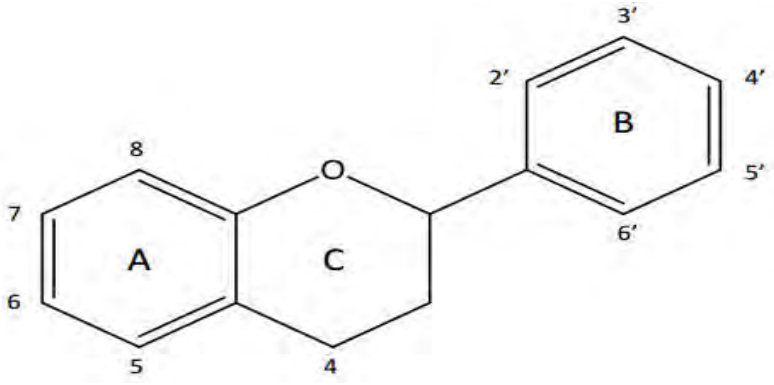


Figure 1.3: The chemical backbone of flavonoids (Wang *et al*., 2000).

These catechins account 30-42% of the dry weight of green tea and EGCG accounts 50-80% of the total catechins (Arshad *et al*., 2015).It was reported that radical-scavenging ability of EGCG was higher than that of other catechins due to the presence of a gallate moiety in the C ring (Xu *et al.*, 2004). Catechins are ordered based on their antioxidant activity as indicated by a number of studies: EGC ~ EGCG >> ECG = EC > catechins (Łuczaj and Skrzydlewska, 2005)and the number of hydroxyl groups is the major factor contributing to antioxidant potential of catechins of green tea (Rice-Evans *et al.*, 1997).

1.5 Literature Review

HIV-1-infected patients on HAART frequently develop a metabolic syndrome; in particular lipodystrophy syndrome, which is characterized by peripheral lipoatrophy(failure of adipocyte differentiation caused by NRTIs induced mitochondrial depletion) and visceral fat redistribution and is associated with metabolic alterations including dyslipidaemia, insulin resistance and cardiovascular disease and lactic acidosis(Kosmiski *et al*., 2003).

Although dyslipidemia can occur in children not treated with antiretroviral drugs, on those who use especially the PIs, favors the development of dyslipidemia (Tassiopoulos *et al*., 2008) and ritonavir (RTV) is found to be most commonly associated with dyslipidemia (Chantry *et al*., 2008). In another study, on HIV-infected children there was an association between the use of PIs and increased levels of cholesterol and TG and the prevalence of elevated total cholesterol ranged from 15 to 68%, while the prevalence of elevated TG ranged from 11 to 79% when PIs are substituted for Efavirenze (McComsey *et al*., 2003).A study done by Shafran and his colleagues in seronegative volunteers by giving RTV 100 mg bid for 14 days, the result showed that there was a significant increase in TC,TG,LDL-c and a significant decrease in HDL-c level(Shafran *et al*., 2005) and work of Francis and his colleague on HAART taking patients also showed a significant increase in TC,TG,LDL-c, but HDL-c did not show a significant change(Francis and Onyinye, 2011).

In a study done in Nigeria on HIV infected HAART users for 1 year, there was no significant weight gain (Olatunji *et al*., 2012) and another study on those who were taking HAART for 6 months showed a significant body weight decrement; thus, those who were obese before they start the medication became in normal body weight range after the treatment (Adeolu *et al*., 2014).But the work of Denue and his colleagues, which was done prospectively on 120 patients who were taking HAART,83.1% patients gained weight,4.7% had no weight change, and12.2% lost weight(Denue *et al*., 2014). As Jibu and his colleague Gavin demonstrated in their work, the crude extract of green tea was found to be antiobese, antilipidemic and its administration showed significant decrease in the body weight in dose related fashion in rats i.e., the higher the dose, the more the weight loss(Jibu and Gavin ,2013).

As a number of studies revealed, patients on HAART also showed a significant elevation in serum AST and ALT level (Robert *et al*., 2015;Shakirat *et al*., 2014;Vijay *et al*., 2016) ALP and γ-glutamyl transpeptidase level also showed a significant elevation(Robert *et al*., 2015).

In Ethiopia, a study done on the effects of HAART on lipid metabolism by Abebe and his colleagues at Burayu Health Center showed an increased prevalence of hyperglycemia, hypercholesterolemia and hypertriglyceridemia in patients receiving antiretroviral drugs than non-HAART taking study participants (Abebe *et al*., 2014).Also, in another study in Defense Hospital in Addis Ababa, among all study participants involved in the study, 73.68% on HAART and 53.51% HAART naive persons had at least one lipid profile abnormality and as revealed by this study, the prevalence of dyslipidemia on HAART taking patients, TC, LDL-c & TG were greater than HAART naive patients (Habtamu *et al*., 2014). In Cameroon, on a study done in one Hospital on patients taking HAART, the prevalence of raised TG on HAART group was 39 % (Manuthu *et al*., 2008).

As demonstrated by the work of Ratziu and his colleagues, the prevalence of NAFLD in the general population ranges from 25-46%, increasing to 70% in diabetics and 90% among obese (Ratziu *et al*., 2011) and its prevalence is higher in individuals with HIV infection (30–40%) than in the general population (14 – 31%) (Angulo *et a l*., 2007). In one cohort study on HIV-infected patients, hepatic related mortality accounted for 7.1% of deaths of which 56% were associated with hepatitis(usually HCV infection) (Lemoine *et al*., 2012). Although most studies have been carried out in co-infected patients, one study found that 31% of HIV patients without HCV co-infection had NAFLD as revealed by ultrasound evaluation (Crum-Cianflone *et al*., 2009).

The effects of green tea on obesity and diabetes have received increasing attention and its catechins, especially EGCG, appear to have anti-obesity and anti-diabetic effects (Kao *et al*., 2006) and it was also found effective in decreasing diet induced obesity in mice (Klaus *et al*., 2005) and also the work of El-Sayed and his colleague Eslam, which was done on rats, GTE was found to reduce the body weight gain significantly as compared with GTE untreated normal

control group(El-Sayed and Eslam,2014).Moreover,green tea extract and its isolated constituents were also found to be effective in preventing oxidative stress (Babu *et al*., 2006).

One recently conducted study on humans showed that the consumption of green tea and green tea extracts may help to reduce body weight, mainly body fat, by increasing postprandial thermo genesis and fat oxidation (Sabu *et al*., 2010).Another epidemiological study done by Mennen and his colleagues in 2003 with 3000 subjects showed that increased tea drinking is significantly associated with lower levels of serum glucose and triglycerides(Mennen *et al*., 2003); and green tea has been reported to reduce body weight, body mass index and body fat (Tsuchida *et al*., 2002) and serum cholesterol level (Imai and Nakachi, 1995).

Also, as shown by the study of Shereen and his colleague, administration of crude GTE for rats for 14 weeks showed a significant decrease in serum level of ALT, ALP, AST and malondialdehyde and a highly significant decrease in TG,TC as compared with control group(Shereen and Doaa,2013) and work of Nada and his colleagues demonstrated that administration of green tea reduced serum TG,TC and LDL-c significantly in the heart of rats(Nada *et al*., 2015).Other studies done by Tehassein and his colleagues and El-Sayed and his colleague Eslam on rats showed that administration of GTE lowered the serum level of ALP,ALT and AST significantly(El-Sayedand Eslam,2014;Tehassein *et al*., 2011).

In addition, Babu and his colleagues showed that green tea consumption reduce blood glucose as well as total cholesterol level and body fat, also they suggested that green tea may be effective in preventing the development of not only diabetes but also cardiovascular diseases (Babu *et al*., 2007). Green tea given to normal rats at a high dose decreased plasma glucose, and triglycerides (Wu *et al*., 2004).Tokunagag and his colleagues also observed that, green tea consumption has a lowering effect on plasma cholesterol and triglycerides levels (Tokunagag *et al*., 2002).Also, Erba and his colleagues studied the effect of moderate intake of green tea on human health and they found a significant decrease in LDL-cholesterol (Erba *et al*., 2005). Catechins extracted from green tea have been shown to normalize plasma cholesterol concentration without affecting HDL-cholesterol concentration, growth rate and food efficiency in rats (Muramatso *et al*., 1986).

1.6 Statement of the Problem

In 2010 there were about 34 million people who were affected by HIV globally from this 1.1 million were from USA(Nishijima *et al*., 2011).In Ethiopia there were an estimated 793,700-893,200 living with HIV/AIDs in 2013 in which most of them are user of ART according to 2014 country progress report on the HIV response (FMOH, 2014). According to the 2005 sixth report of federal ministry of health, AIDS was the leading cause of mortality in 15-49 years of age groups and accounted 43% of adult death country wide and 66% in urban. Although ARTs changed HIV/AIDS from acutely lethal disease to chronic disease, they affect vital organs of the patients who are taking these medications for the rest of their life. Improvements in highly active antiretroviral therapy (HAART) has shifted the mortality related to acquired immune deficiency syndrome to liver-related diseases, which have become a leading cause of hospitalization and death in HIV infected patients. Among these vital organs liver is in the frontline to be damaged by these drugs and others because it is the main center of drug metabolism than any other organs. These damages to the liver may range from minor hypersensitivity to liver failure secondary to drug induced intoxication. As many studies revealed that HAART which are a combination of three or more ART drugs, are found to be associated with liver problems, due to oxidative damage of the mitochondria of the liver.

Among these liver associated problems which are developed secondary to oxidative mitochondrial damage, dyslipidemia (abnormal lipid profile of the blood) and Non Alcoholic Fatty Liver Disease (NAFLD) are now becoming frequent liver disorders. In studies done in Ethiopia, one in Defense Hospital in Adddis Ababa (73.68%) (Habtamu *et al*., 2014), Burayu Health Center (51%) and in Jimma (32.6 %) (Birhanie *et al*., 2012) of patients who were taking HAART developed dyslipidemia.This aberrant lipid metabolism in turn leads to the development of other systemic problems like atherosclerosis, stroke, hypertension, hepatic steatosis, steatohepatitis and even hepatic failure.

Medicinal plants are used by a large number of populations in the world to treat different health ailments especially in developing countries. These plants are used as traditional medicines by the order of traditional medicine practitioner. Among these medicinal plants, *Camellia Sinensis,* which is the most common beverage in Far East countries like China, Japan and Western world

is one of the traditional healers for a number of health ailments. But, in Ethiopia the awareness of the society about the health benefits of this plant (*Camellia Sinensis*) is at a very low level even among in health professionals.

1.7 Significance of the study

Steatosis like NAFLD and deranged lipid plasma profiles are now becoming the most common side effects of HAART which are inturn a predisposing factor for other systemic diseases as revealed by many studies .Due to these side effects, liver related morbidity and mortality is increasing in patients who are taking HAART. For this reason, HAART is likely to be on the brink of being out of market or scientists will be obliged to design other effective drugs with minimal side effects. Hence, this study tries to reveal these HAART related problems of the liver and other systemic diseases associated with these side effects and the remedial effect of green tea leaf extract from the perspective of its antioxidant property if especially the results are in line with the hypothesis of this study. Also, this study serves as an eye opening for researchers and local pharmaceutical companies to conduct more researches on Ethiopian high land green tea since; the culture of consuming green tea is at lowest level in Ethiopia. On top of these benefits this study will create substantial awareness for our society about the health benefits of green tea consumption especially to HIV patients.

1.8 Hypothesis of the study

The extract of green tea (*Camellia Sinensis)* leaf protects rats from HAART induced dyslipidemia and Non Alcoholic Fatty Liver Disease (NAFLD).

2. OBJECTIVES

2.1 General objective

To investigate the potential effect of green tea leaf extract on HAART induced dyslipidemia and Non Alcoholic Fatty Liver Disease (NAFLD) in albino Wistar rats.

2.1.1 Specific objectives

- To evaluate the influence of HAART and the various doses of *Camellia sinensis* leaf extract on body weight of the rats.
- To assess the effect of HAART and counter effect of green tea leaf extract on serum lipid profile of rats.
- To assess the effect of HAART and reversing effect of GTE on liver tissue.
- To examine the effect of HAART and green tea leaf extract on total lipid of liver of rats.
- To examine the hepatotoxic effect of HAART and remedial effect of green tea leaf extract histologically.

3. MATERIALS AND METHODS

3.1 Study Area and Period

The study was conducted from November, 2015 to May, 2016, at the department of Medical Biochemistry, School of Medicine, College of Health Sciences, Addis Ababa University.

3.2 Reagents and Chemicals

In this study the following reagents and chemicals were used; HAART drugs(Zidovudine, Lamivudine,Lopinavir and Ritonavir),ethanol (70-95%),methanol, distilled water, chloroform,LFT kits, kits for lipid profile, Triglyceride kit(Human),1,1-Diphenyl-2-Picrylhydrazil(DPPH),10%formalin,Paraffinwax,xylenesolution,0.04%$Cacl_2$,0.034%$Mgcl_2$,0.58 % Nacl,0.9%Nacl,TritonX-100,benzene, di-ethyl ether, tert-butyl alcohol, ascorbic acid(BDH chemicals Ltd poole, England).

3.3 Equipments

In this study, Erlenmeyer flasks (2L,5L,0.5L,0.25L),volumetric flask (2000ml), Buchner funnels, Whatman filter paper No 1,filtering cloth, lyophlizer(OPR-FDU-5012,Korea),Triple bimbalance, rotary evaporator (Heizbad Hei-VAP, Germany), stainless steel and plastic cage, full chemistry analyzer (BS-200,China),light microscope (LEICA DM750),centrifuge(PLC-012E,Taiwan),dessiccator, high definition colored camera(PC1732,China), rat oral gavage, test tubes, nun tubes(Serum vials), beaker (1000ml),metallic spatula, deep freezer(-80^{o}C),Micropipettes(HumanPipette)(20µm, 20-200µm, 1000µm),TLC plate, Alumunium foil, Incubator shaker(G25,USA),Polypropylene centrifuge tubes(15ml,50ml), tissue homogenizer (RZR 2100,Germany),scissors, mortar and pestle, electrical analytical balance (KERN ALJ220-4,Germany), vortex mixer (XH-D), Microtome, Spectroflourophotometer (CM-Solar 2203, Ukraine),Spectrophotometer(UV-1600PC,Germany) and tissue cassettes were used.

3.4 Plant Material Collection

The packed coarse powder of green tea was purchased from the local tea processing and packing factory called Ethio Agri-CEFT private limited company which is a sister company of MIDROC technology group in Ethiopia.

3.5 Green Tea Crude Extraction

The coarse powder of green tea were weighed and 918 gram of *C.Sinesis* powder was macerated in 80% methanol (V/V) with a ratio of 1:10(weight of plant material powder in grams to extraction solvent in milliliter) for three days(72 hours) with mechanical shaking twice a day. This process was repeated twice until the residue of the plant material gave no coloration to maximize the percentage yield. Then the extract was filtered first using filtering cloth to separate coarse powder and then by Whatman filter paper No.1 to separate fine powders from the filtrate and then the filtrate was evaporated to dryness by rotary evaporator and was further concentrated by water bath at 40^{O}C.Then the filtrate was taken to thermostatic oven at 40^{o}C for overnight to evaporate the remaining methanol. After that, the final gummy extract which is almost free of methanol was taken to deep freezer to have a solid consistency and dried by freeze dryer (lyophilizer).This freezed and solidified extract was lyophilized repeatedly until the water was completely removed and the final dry extract was obtained. Then the final extract was weighed after packing in glass to avoid any contamination from the atmospheric air. After that, percentage yield of the extraction was calculated as follows:

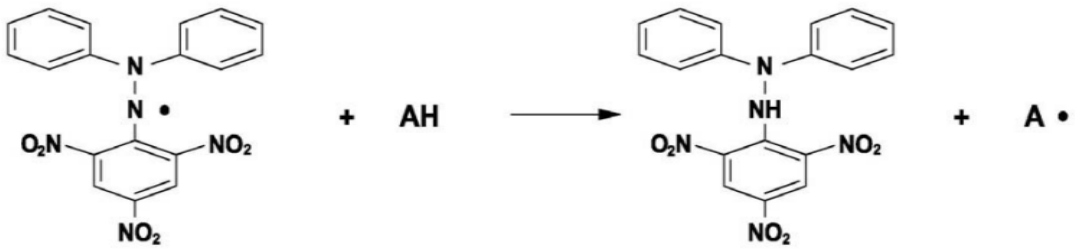
$$\% \text{ yield} = \frac{\text{Weight of the dry extract Percentage yield}}{\text{Weight of coarse powder}} \times 100\%$$

Then the extract was kept in a refrigerator at 4^{O}C until it was used for experimentation.

3.5.1 *In-vitro* antioxidant activity determination of green tea leaf extract

3.5.1.1 Thin Layer Chromatography technique

DPPH assay is one of the most commonly used methods for determining the antioxidant activity of various extracts of medicinal plants (Blois, 1958).In this procedure, a stable free radical $DPPH^{\cdot}$(deep violet), after accepting electron or hydrogen radical, is converted into stable DPPH-H(pale yellow) (Ebrahimzadeh *et al*., 2009;Muthusamy *et al.,* 2009).



DPPH (Free radical) (Violet) Antioxidant DPPH-H (faint yellow)

Figure 3.1: Principle of reaction of DPPH with antioxidants (Aurelia *et al*., 2009).

Accordingly, the antioxidant activity of GTE was determined by using DPPH as free radical and scavenging activity of the extract was determined according to the modified method described by Yamaguchi *et al*. (1998).The hydro-methanolic extract of green tea was dissolved in 80% methanol and the preparation was 100mg/100mL, 200mg /100mL and 400mg/100ml and 0.1mM of DPPH solution was prepared in 100% methanol. Then, this adequate amount of DPPH solution was poured to a wide Petri dish for easy immersion of the TLC plates. After these preparations, 2µL of GTE with different concentration were loaded on individual TLC plates and waited for 30 minutes until the GTE loaded on the plates completely dried. Then the TLC plates were stained with 0.1mM DPPH solution for few seconds. Then the mixture was allowed to stand at room temperature for 30 minutes. After that, the antioxidant activity of GTE at various doses was analyzed and interpreted and presented in pictures.

3.5.1.2 Spectrophotometric method

The antioxidant activity of green tea leaf extract was also determined quantitatively by using DPPH (2, 2-diphenyl-1-picrylhydrazyl) as a stable free radical which has an absorption band at 517nm.Hence, the free radical scavenging activity of GTE was assessed according to the method developed by Tekao and his colleagues (Tekao *et al* ., 1994). According to this method DPPH was prepared with a concentration of 0.008% (8mg/100ml of methanol) and the extract of green tea leaf was prepared with a concentration of 1mg/ml, 500µg/ml, 250µg/ml, 125 µg/ml and 62.5µg/ml in methanol. Ascorbic acid was also prepared in the same concentration with that of GTE (1mg/ml, 500µg/ml, 250µg/ml, 125 µg/ml and 62.5µg/ml) in methanol serially as a standard. Then 1ml of the extract from each concentration was mixed with 1ml of 0.008% DPPH, and then incubated for 30 minutes at room temperature in darkness.Finally, the absorbance was measured at 517nm by using DPPH as a control and methanol as a blank. Then

the concentration versus absorbance curve of GTE and ascorbic acid was drawn to extrapolate IC50 of GTE and ascorbic acid. Median inhibitory concentration (IC50) is the concentration of the extract that decreases the absorbance of DPPH by half (50%) (Williams *et al*., 2004).

3.6 Experimental Animals

Thirty female albino rats were obtained from Pharmacology Department, School of Medicine College of Health Sciences, Addis Ababa University. The rats were weighing 145-200 gram initially after acclimatization period and were of age 10-12 weeks. Then, the rats were grouped into five groups randomly in which each group contained six rats. All experimental animals were allowed to share the same environmental condition and they had exposure of 12 hr light/dark cycle. Also, the animals had free access of tap water and standard chow/pellet 24/7(*ad libithum*) until the last day of the experiment.

3.6.1 HAART dose extrapolation to experimental animals

The human's dose of HAART drug was extrapolated to animals by the formula as follows as developed by Chen Qi; Dose for rats = (Xmg/kg x 70 kg x 0.018) / 0.2 kg = 6.3 Xmg/kg, where X = the effective dose for man; 70 kg = the standard weight of adult human; 0.018 = ratio of the equivalent dose between man and rat based on body surface area; 0.2 kg = the standard weight of a rat (Chen, 2006).According to this formula the HAART was given for rats for 60 days. The HAART which were given during the study period were in combination of ZDV/3TC+LPV/RTV with adult dose of 300/150 mg Po bid and 200/50 mg 2 tablets Po bid respectively. Then dose for rats were extrapolated as follows; ZDV/3TC (6.3x900/70 = 81mg/kg) and LPV/RTV (250x4= 1000mg which is normal adult dose/day). Hence, dose for rats was calculated as 6.3x1000/70 = 90mg / Kg) per day. Then, rats in group II, III, IV and V were given the appropriate doses for sixty days based on their body weight.

3.6.2 Animal Grouping

The experimental animals were grouped as follows; after the LD50 of crude GTE was reviewed from a number of literatures and its toxicity was tested starting from 100 mg/kg up to 5000mg/kg on mice and found to be safe to this level (Jyoti Gupta and Mohammad Afzal, 2015).

Group I, normal control group in which the rats were given 1mL of distilled water IP.

Group II, negative control group in which the rats were given HAART only by oral gavage.

Group III, a group in which the rats were given 100mg/kg of GTE plus HAART by oral gavage.

Group IV, a group in which the rats were given 200mg/kg of GTE plus HAART by oral gavage.

Group V, a group in which the rats were given 400mg/kg of GTE plus HAART by oral gavage.

3.6.3 Body Weight

The weight of the rats was recorded at the interval of a week to see body weight change in all groups of experimental animals to evaluate mean body weight change from the start of the study week to the end of the study week and to adjust doses for each experimental rat on weekly basis.

3.7 Sample Collection

On the 60^{th} day of the experiment after an overnight fast, all rats were sacrificed by cervical dislocation after giving di-ethyl ether general anesthesia. Then blood was collected from each rat by cardiac puncture for LFT and lipid profile analysis from serum. To do this, the blood was collected into the serum vials without any anticoagulant for easy serum separation by simply waiting for 30 minutes. Then, the blood sample was centrifuged at a rate of 3000 rpm for 10 minutes for separation of serum from formed elements of blood and the serum was kept in deep freezer(-80^{o}C) until the laboratory investigation was done. In addition, the liver from each experimental animal were excised carefully and part of it was put in 0.9% normal saline in deep freezer(-80^{o}C)for triglyceride determination from the liver and the remaining part was fixed in 10% formalin for histopathological examination of each experimental rat.

3.8 Biochemical Analysis

From serum, the liver damage markers like ALT, AST, ALP and lipid profile (HDL-c, LDL-c, Total cholesterol and Triglyceride) were determined using standard principles and procedures by using chemistry analyzer (BS-200, China). Also, liver lipid was extracted by using Folch *et al* (1957) method from the liver of each experimental rat and measured by using Hiroshi's and his

colleagues' simple enzymatic quantitative analysis of tissue triglyceride(Hiroshi *et al*.,1992) using spectroflourophotometer (CM-Solar 2203, Ukraine).

3.8.1Alanine transaminase(ALT)/Serum Glutamate Pyruvate Transaminase(SGPT)

This enzyme which is predominantly expressed in the liver and catalyzes the transfer of amino group from L-alanin to α-ketoglutarate in the presence of pyridoxal phosphate (PLP) to form pyruvate and L-glutamate. In the presence of NADH+H, lactate is also formed from pyruvate with the enzyme lactate dehydrogenase (LDH) by oxidizing NADH+H to NAD^+. When the liver is damaged by internal and external factors commonly known as hepatotoxins, the serum concentration of this enzyme (ALT) elevates due to leakage from the liver.ALT is also considered as a better index of liver damage because of its more specificity to the liver than AST (Paraskevas *et al*., 2011;Pradumna *et al*., 2009).

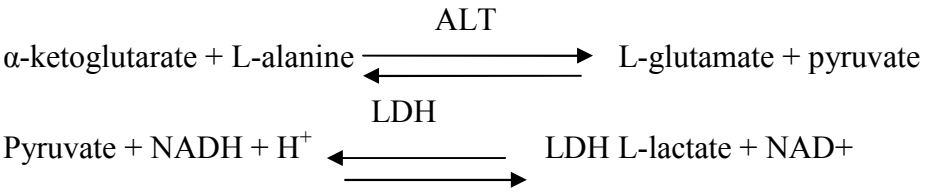


Figure 3.2: Scheme of reaction catalyzed by ALT

3.8.2Aspartate transaminase(AST)/Serum Glutamate Oxaloacetate Transaminase (SGOT)

This enzyme which is also called Aspartate aminotransferase, is one of the key enzymes for transamination reaction in the liver. It catalyzes the transfer of amino group from L-aspartate to α-ketoglutarate to form L-glutamate and oxaloacetate in the presence of a co-enzyme PLP.Though this enzyme is not a typical exclusive marker of liver damage and less sensitive than ALT to liver damage because it is also synthesized in muscle and cardiac tissue and its concentration elevation in the serum following liver damage (Nyblom *et al*., 2006).

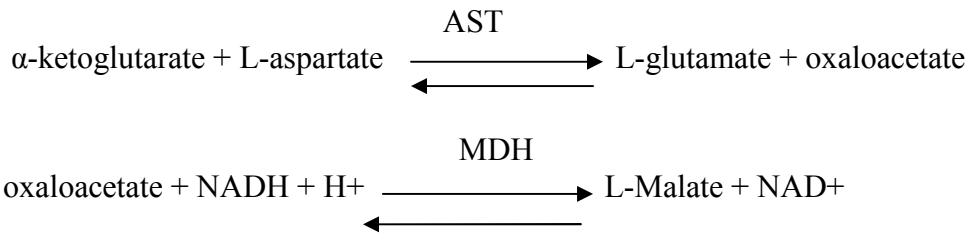


Figure 3.3: Scheme of reaction catalyzed by AST

3.8.3 Alkaline phosphatase (ALP)

ALP is found in liver, bile ducts and bone tissue in high concentration. It catalyzes the hydrolysis of 4-Nitrophenyl phosphate to 4-Nitrophenolate and phosphate in alkaline P^H and its concentration in serum elevates when there is bile duct damage,inflammation,cirrhosis (advanced liver damage) or bile duct obstruction due to stones and alcoholic hepatitis (Nyblom *et al*., 2006).

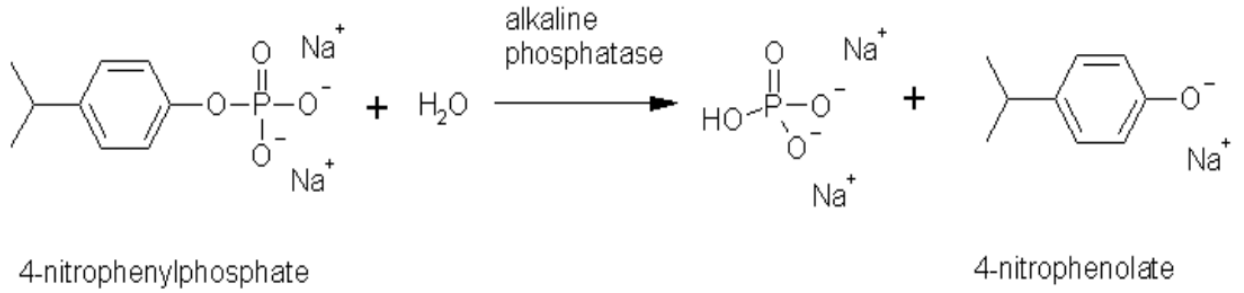


Figure 3.4: Scheme of a reaction catalyzed by ALP (http://www.public.asu.edu).

3.8.4 Tissue Triglyceride (TTG) Extraction procedure

To isolate total tissue lipid from liver, the liver tissue was homogenized by using method described by Folch *et al* (1957)as follows; reagent grade chloroform and methanol were prepared. Then chloroform, methanol and water were mixed with 8:4:3 ratio and 0.04%(40mg) of aqueous CaCl2,0.034%(34mg)of aqueous MgCl2,0.58% (580mg) aqueous NaCl and 0.74%(740mg) of aqueous KCl were added and shaked to minimize the interface between the two phases (upper and lower phase).The expected proportion of chloroform: methanol: water would be 3:48:47 in the upper phase and 86:14:1in lower phase by volume. After this preparation is completed, the tissue was taken out of the refrigerator and thawed for some time until the ice is removed completely from the tissue and homogenized in the above solvent to a final volume of 20 times the volume of the tissue sample (0.5 g tissue sample in 10 ml of solvent mixture) by using tissue homogenizer (RZR 2100, Germany). After dispersion, the whole mixture was agitated for 20 min in a shaker at room temperature. Then the homogenate was filtered by centrifuging to recover the liquid phase at 3000 rpm for 10 minutes and this crude extract was mixed thoroughly with 0.2 volumes (2 ml for 10 mL of filtrate) of 0.9% NaCl solution.

After that, this mixture was again centrifuged to separate 2 phases and the upper phase was removed by siphoning. The lower phase was further diluted by adding 2:1 chloroform to methanol to a final volume of 10mL then centrifuged for 20 minute at 2400rpm to get again 2

phases and the upper phase was removed carefully by pippeting and the lower phase which was the total pure lipid extract was left in the tube. This purified lipid was evaporated by an overnight incubation at 37^{o}C then redissolved in benzene and again evaporated by incubation for about 3hours. The TG level in the liver was determined by using triglycerides standard with a known concentration (2mg/ml, 1mg/ml, 0.5mg/ ml, 0.25mg/ml, 0.125mg/ml,0.0625mg/ml, and 0.03125mg/ml) by using spectroflourophotometer (CM-Solar 2203, Ukraine) and a reagent blank which is expected to have zero absorbance. After the removal of benzene by evaporation, the liver sample and standard were redissolved by adding 30µl of tert-butyl alcohol and 20µl of Triton-X100/methyl alcohol (1:1) mixture. Then 1ml of triglyceride enzymatic reagent (Human) was added to each test tube and mixed carefully followed by incubation of the standard and sample for 18 minute at 37^{o}C and the absorbance was measured at 505 nm (Hiroshi *et al*., 1992).

3.9 Histopathological Study of rat liver

To see the effect of HAART and counter effect of GTE, histopathological examination of liver was performed; to do this, the liver tissue was taken from each rat carefully after the rats were sacrificed and dissection was done from the neck to the pubis and the peritoneum was opened by using sterile scissor and part of the liver was excised and taken by blunt forceps to preserving tissue can which contain 10% of formalin. Then the tissue was taken out from the preservative and washed by ethanol subsequently with different concentration starting from 70% to 95% and xylene solution was used to remove ethanol from the tissue and replace this ethanol with fluid that readily miscible with paraffin wax which enhance the tissue to embed easily with the wax to form tissue blocks. Then after, the tissue block was sectioned by microtome and that section was immersed in a water bath at 40^{o}C and the unfolded section were taken and dried by putting in an oven at 56^{o}C for 15 minutes (Yahya *et al*., 2013).

Then this dried section was stained with hematoxylin-eosin stain after removal of paraffin wax by using ethanol with a descending concentration and examined by using light microscope under different magnification (20X,200X) photo was taken by using microscope camera and pictures were read & interpreted by pathologist.

After these subsequent procedures, the tissue was examined under microscope to see the effect of HAART and counter effect of green tea extract on liver of rats and to do NAS (Non alcoholic

fatty liver disease activity score) the method of Brunt his colleagues was used (Brunt *et al*., 1999).

3.10 Statistical Analysis

All data are expressed as mean ± standard deviation (SD) and statistical significance was carried out using one way analysis of variance (ANOVA) followed by Post hoc-Tukey test where $P < 0.05$ was considered as statistically significant, employing SPSS statistical soft ware package version 22.0.

3.11 Ethical Approval

The study was conducted after experimental protocols were approved by the departmental research and ethical review committee (DRERC), meeting number DRERC 03/15, and by protocol number MSc.Thesis 05/15, on 04 September, 2015. All rules applying to animal handling, safety and care were as per the guide line set by the national academies press, Washington, D.C, USA.

4. RESULTS

To observe the effect of Green tea *(C.sinensis)* leaf extract against HAART induced dyslipidemia and NAFLD in albino Wistar rats, the *in-vitro* antioxidant activity of GTE was studied qualitatively and quantitatively first and its effect on the above mentioned derangements were biochemically and histopathologically investigated in all experimental groups and presented below in tables and figures.

4.1 Percentage Yield of *C.Sinensis* leaf Extract

The amount of crude extract which was obtained from 918 gram of *C.Sinensis* leaf coarse powder was 146 gram. Therefore, the percentage yield of this extraction by using 80% methanol was calculated and given as:

%yield = (146/918) x100 = 15.9 %(w/w)

4.2 *In-vitro* Antioxidant Activity GTE by using DPPH as free radical

As shown below in Figure 4.1, the diameter of reduced zone increased in direct proportion to dosing i.e., the higher the concentration, the larger is the diameter. The yellowish pale color shows a reduced zone that masks the color of DPPH and the unreduced region or the region where the antioxidant can't reach remains violet which is the color of DPPH.

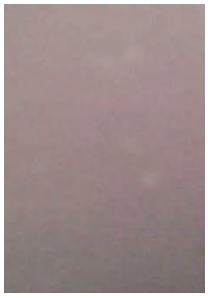
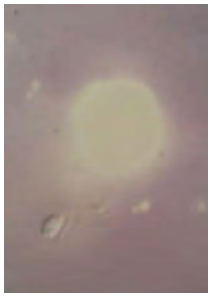
a)  b)  c)  d) 

Figure 4.1: Qualitative *In-vitro* antioxidant activity test of 80% hydro-methanolic green tea leaf extract (GTE). a) TLC plate which is immersed in 0.1mM DPPH, b) 2µL of 100mg/100ml of GTE, c) 2µL of 200mg/100ml of GTE, d) 2µL of 400mg/100ml of GTE.

As shown below in Figure 4.2, the IC50 of the crude green tea extract (GTE) is 260µg/ml (0.26mg/ml) whereas that of Ascorbic acid is ~160µg/ml (0.16mg/ml).This result shows that the crude green tea extract has a closer antioxidant activity with ascorbic acid and also both ascorbic

acid and GTE showed a decrease in the absorbance of DPPH in dose dependent manner i.e., their inhibitory effect increase as their concentration increase. The absorbance of 0.008% DPPH was 0.98 which was used as a control and the absorbance of methanol was 0.0017 which was taken as a blank at 517 nm.

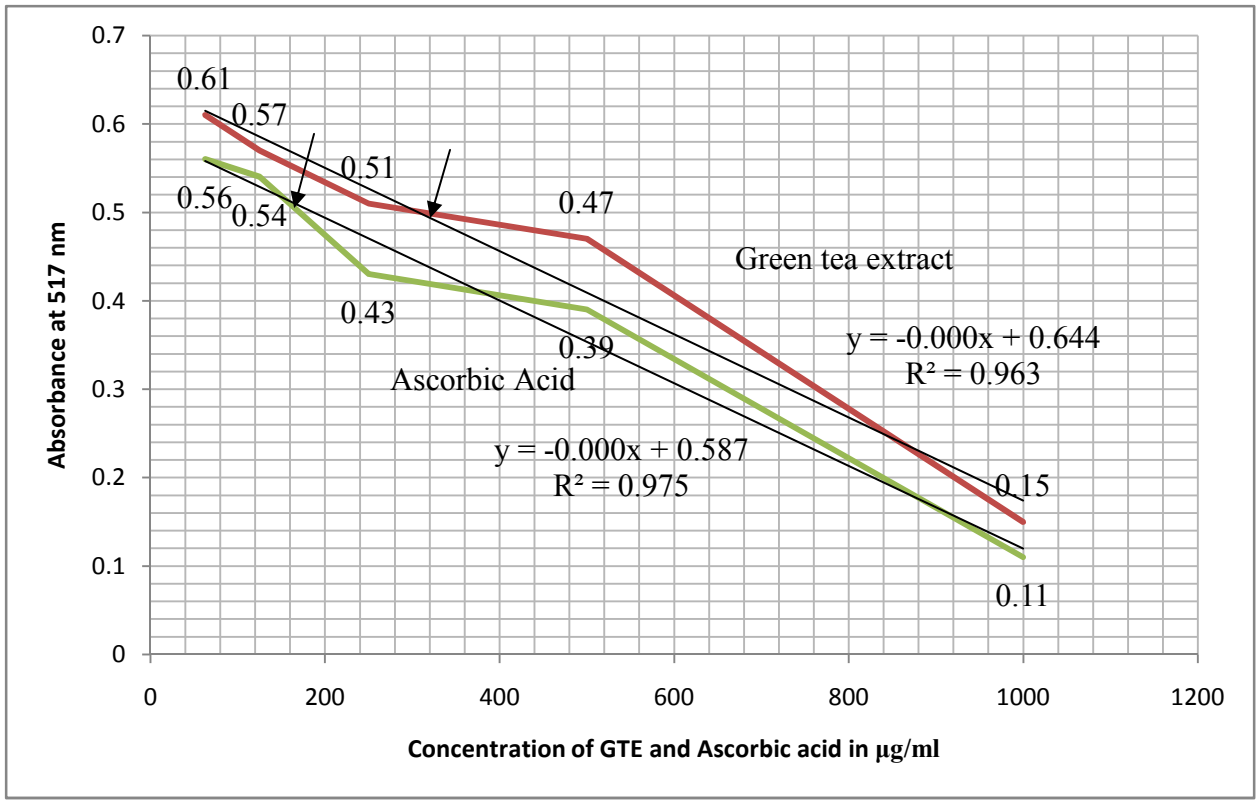


Figure 4.2: Curve of GTE and ascorbic acid concentration versus absorbance at 517 nm.

4.3 Body Weight Change

As shown below in Figure 4.3, group-II (162.0000±5.529150) showed significant decrement ($p<0.01$) in their mean body weight as compared to group-I (181.1000±5.81550).But, when group-II is compared with group-IV and group-V the decrement is not significant ($p>0.05$). All GTE treated groups showed significant decrease in their body weight as compared to normal control group in dependent of dose. The more body weight decrease was observed in group-IV (160.6667±3.50238) and group-V(159.5000±4.50555) with $p< 0.01$ for both groups as compared to group-I.

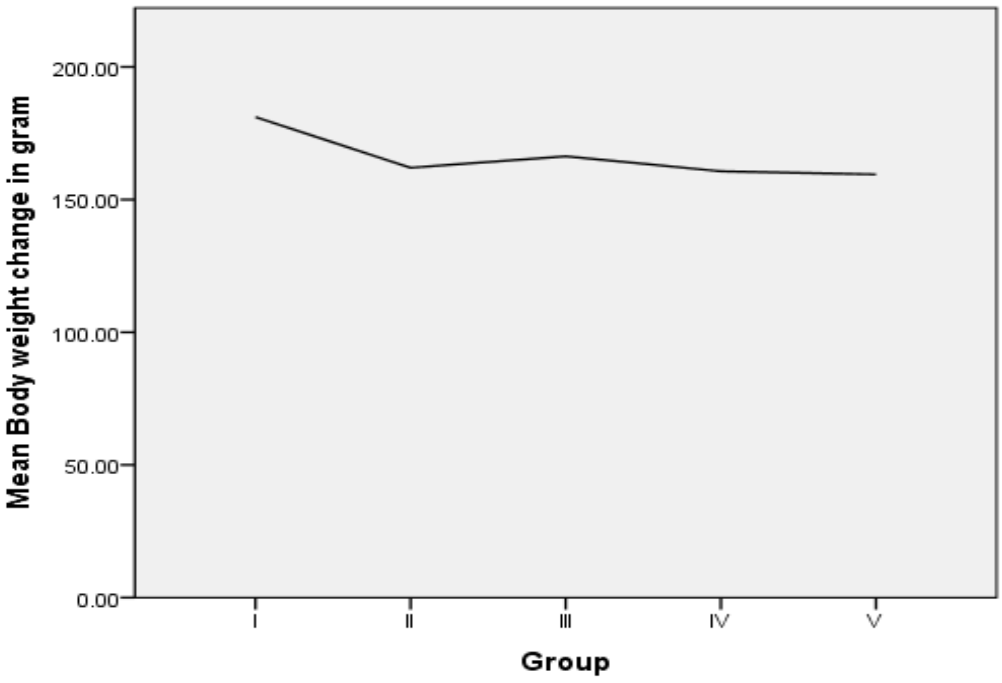


Figure 4.3: Mean body weight change of experimental rats after 60 days of HAART and GTE treatment.

4.4 Effect of green tea leaf extract (Camellia Sinensis) on serum lipid profile

4.4.1 Serum Total Cholesterol (TC)

As shown below in Figure 4.4, the serum total cholesterol in group-II (66.1667±12.9215) elevated significantly ($p<0.01$) as compared to group-I(41.0000±8.07465).Group-III also showed a significant increment ($p<0.05$) as compared to group-I whereas group-IV showed a reduction in TC level though not significant as compared to group-II. But group-V(47.5000±4.92950) showed a significant decrement in total cholesterol (TC) ($p<0.05$) when compared with group-II.

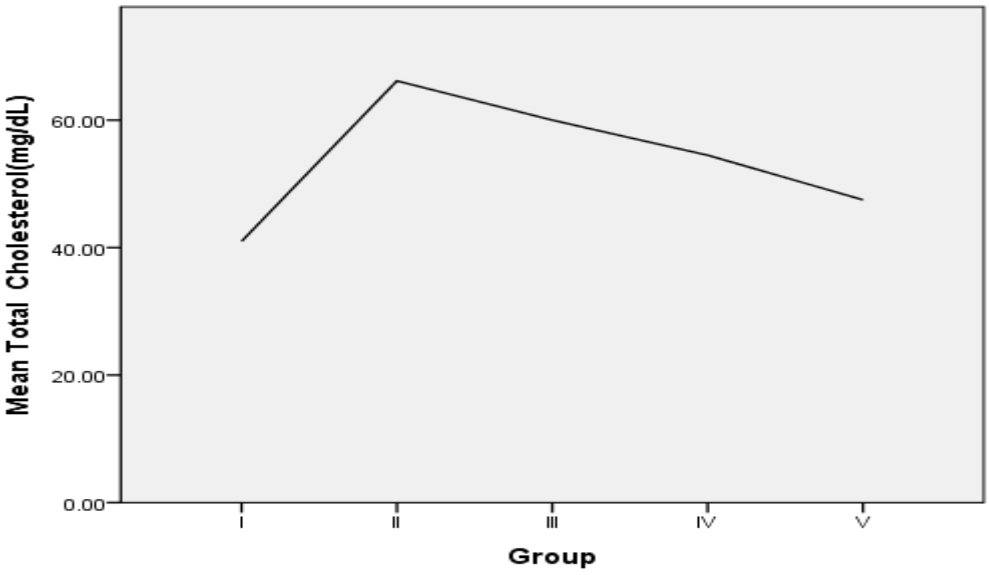


Figure 4.4: Serum total cholesterol level after 60 days of HAART and various doses of GTE administration.

4.4.2 Serum Triglycerides (TG)

Group-II showed significant increment ($p<0.05$) in the serum TG level (69.3333±14.32015) as compared to group-I(47.5000±8.59651).Group-III, IV and V rats showed a significant ($p<0.05$) decrement in their serum level of triglyceride as compared to group-II with more reduction in group-V (48.8333±8.18332) as shown in Figure 4.5.

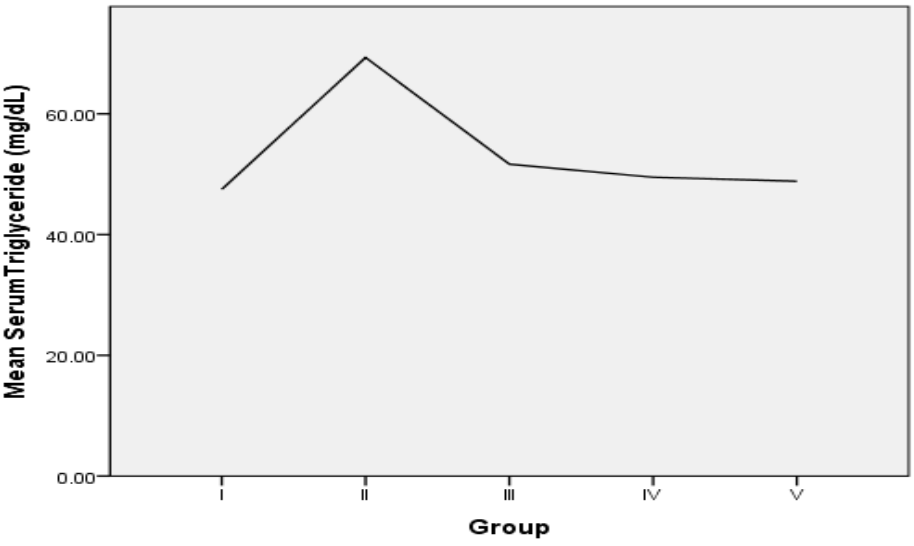


Figure 4.5: Serum Triglyceride level after 60 days of HAART and GTE treatment.

4.4.3 Serum LDL-cholesterol

As shown below in Figure4.6, the serum level of LDL-cholesterol in group-II (14.6667±5.88784) increased significantly ($p<0.05$) as compared to normal control group-I (7.3333±2.16025). Although there is a reduction in the serum level of all GTE treated group (III, IV, V), the significant reduction ($p<0.05$) is observed in group-V (8.0000±2.28035) which were given 400mg/kg GTE as compared to group-II rats.

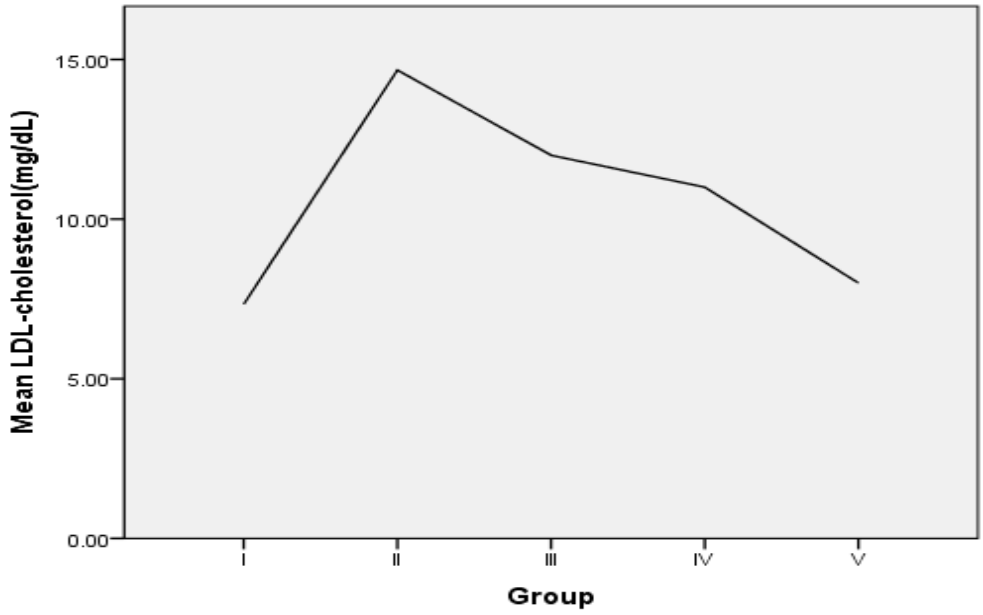


Figure 4.6: Serum LDL-cholesterol level after 60 days of HAART and GTE administration.

4.4.4 Serum HDL-cholesterol

Although the HAART only treated group-II (27.6667±9.02589) showed a slight decrement in the serum level of HDL-cholesterol, it was statistically insignificant ($p>0.05$) when compared to normal control group-I (35.0000±7.01427).In group-III, the decrement in the serum level of HDL-c is almost the same to group-II. But group-IV and group-V showed a slight increment in the serum level of HDL-c though it is statistically non significant ($p>0.05$) as compared to group-II as shown in Figure 4.7.

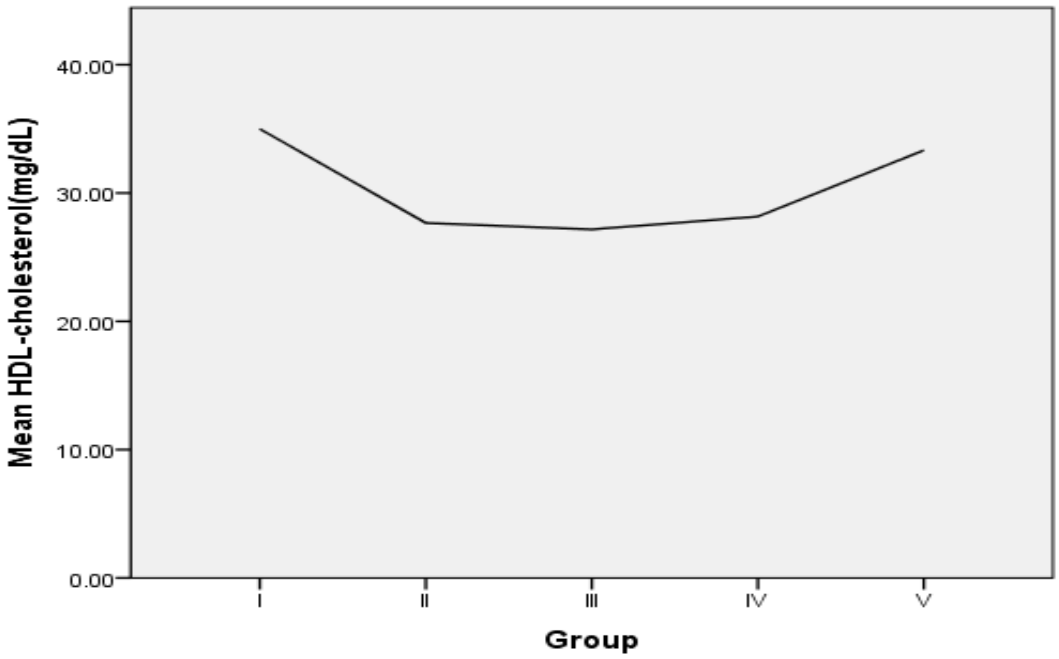


Figure 4.7: Serum HDL-cholesterol level after 60 days of HAART and GTE administration.

4.5 Liver Tissue Triglyceride (TTG)

Tissue TG is an indispensible marker of fat accumulation on the respective organ. Hence, liver tissue is used in this study to rule out whether there is a development of NAFLD or not. A graph with y=0.063x-0.000 with $R^2 = 0.997$ was drawn automatically as shown in Figure 4.8.

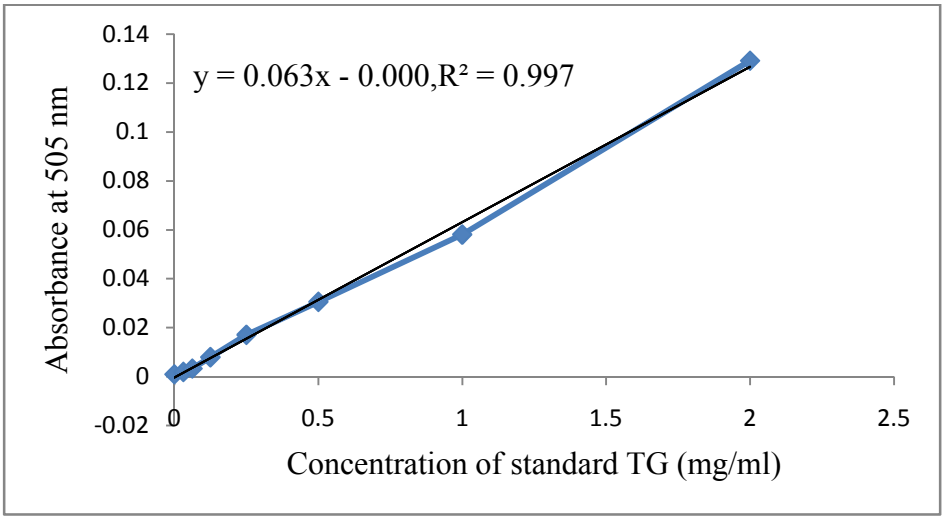


Figure 4.8: A standard triglyceride (TG) curve at 505 nm.

The amount of tissue triglyceride (TTG) in group-II (62.5517±18.26940) rats showed a significant increase ($p<0.05$) as compared to group-I (32.2450±12.50539). Groups which were given GTE at various doses in addition to HAART showed a decrement though significant decrement ($p<0.05$) is observed only in group-V (33.9267±13.71276) when compared with group-II as shown below in Figure 4.9.

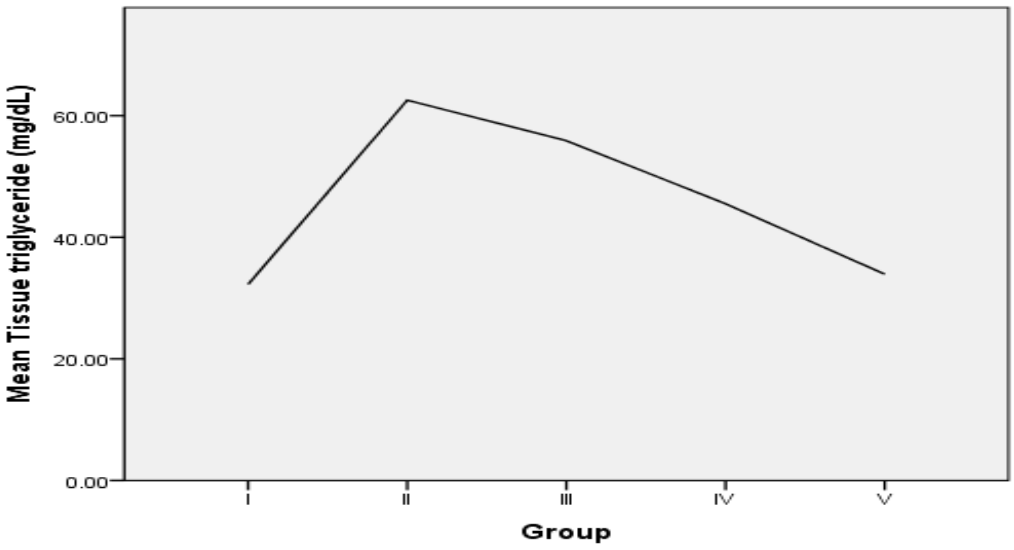


Figure 4.9: Liver tissue triglyceride level after 60 days of HAART and GTE administration.

4.6 Liver damage markers

4.6.1 Serum Aspartate Transaminase (AST)

As shown in Figure 4.10, the serum AST level in group-II (115.0000±11.76435) increased significantly ($p<0.01$) as compared to group-I (70.0000±14.24079). The GTE treated groups responded differently; hence, group-III and group-IV rats which were treated with 100mg/kg of GTE+HAART and 200mg/kg of GTE +HAART showed decrement but not statistically significant. In group-V, the serum AST level showed a significant decrease ($p<0.05$) when compared with group-II (76.3333±31.08483).

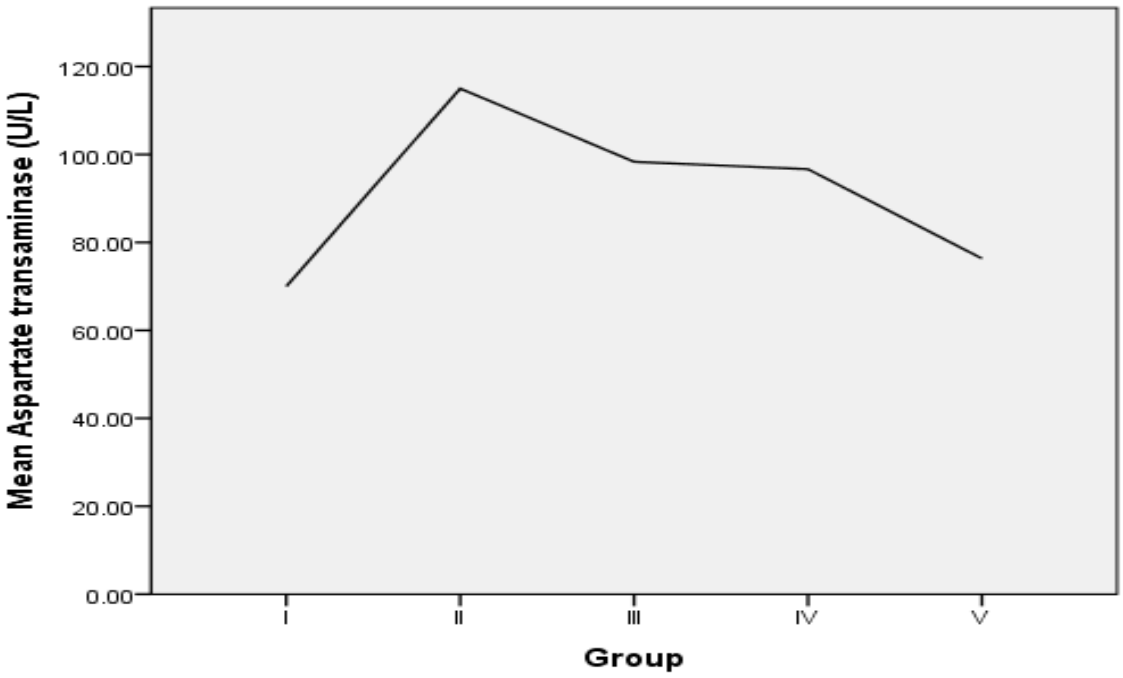


Figure 4.10: Effect of GTE on serum AST after 60 days of treatment.

4.6.2 Serum Alanine Transaminase (ALT)

As shown in Figure 4.11 below, the serum ALT level in HAART only treated group-II (88.6667±24.97732) showed a significant increment ($p<0.05$) as compared to normal control group-I. Group-III and group-IV rats did not show a significant decrement as that of group-V (52.3333±10.80123) which were treated with 400mg/kg of GTE. Group-V that showed a significant decrement in serum level of ALT ($p<0.05$) when compared with group-II.

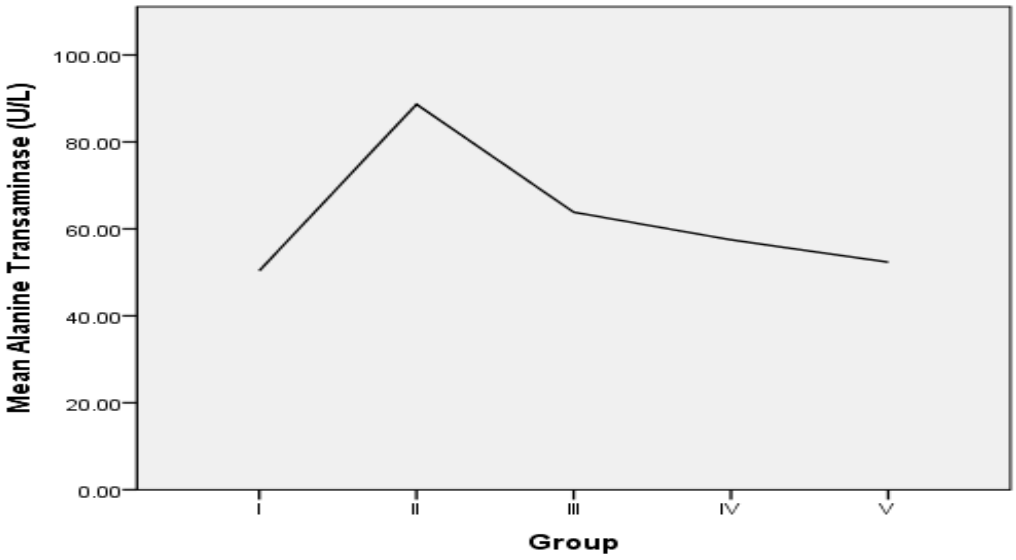


Figure 4.11: Effect of GTE on serum ALT after 60 days of treatment.

4.6.3 Serum Alkaline phosphatase (ALP)

The serum level of ALP in group-II (98.6667±22.61563) did not show a significant increase ($p>0.05$) as compared to group-I (71.5000±16.31870) though there was a slight increment. The GTE treated groups (III, IV and V) with HAART did not show a significant decrement as compared with group-II rats even though they showed relatively decreased serum level of ALP when compared with group-II as shown in Figure 4.12 below.

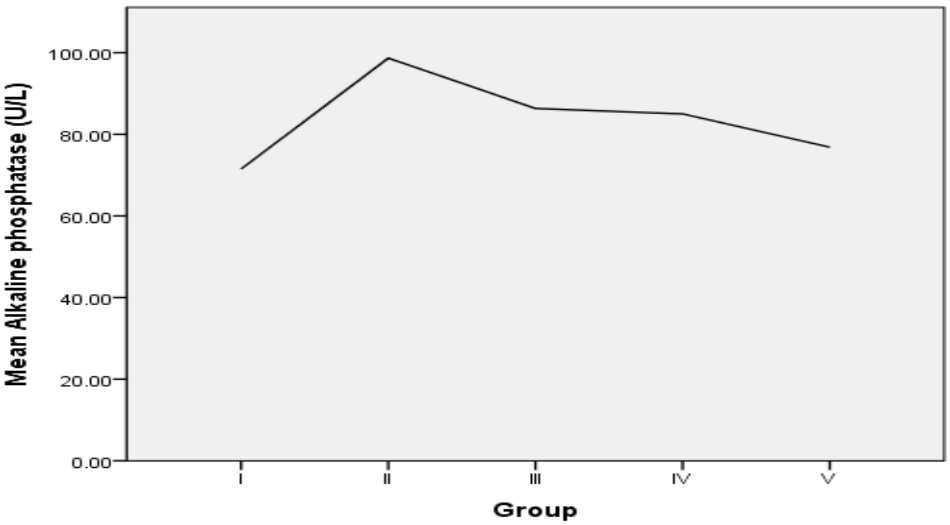


Figure 4.12: Effect of GTE on serum ALP after 60 days of treatment.

4.7 Histopathological Evaluation of rat liver

4.7.1 NAFLD Activity Score (NAS)

According to Brunt and his colleagues (Brunt *et al*., 1999) developed criteria of evaluating development of NAFLD, the liver tissue of all experimental rat was invesitigated and histopathological changes that can be detected easily by Hematoxylin and Eosin stain (H&E) and under light microscope with a magnification size of 20X and 200X were investigated and the findings are given below in Table 4.1.

Table 4.1: Non Alcoholic Fatty Liver Disease Activity Score (NAS).

Evaluation Criteria	Total Number of rats	G-I	G-II	G-III	G-IV	G- V
Steatosis Grade						
- <5%	17(56.67%)	5	1	2	4	5
- 5%-33%	9(30%)	1	3	3	1	1
- ≥33-66%	4(13.33%)	-	2	1	1	-
- ≥66%	0	-	-	-	-	-
Microvesicular steatosis	2(6.7%)	-	1	1	-	-
Inflammation (200X)						
- No foci	24(80%)	5	4	5	5	5
- <2 foci	5(16.67%)	1	1	1	1	1
- 2-4 foci	1(3.33%)	-	1	-	-	-
- >4 foci	-	-	-	-	-	-
Liver cell injury (Ballooning)						
-None to rare	27(90%)	6	4	5	6	6
- Many	3(10%)	-	2	1	-	-

As shown above in the Table 4.1, 13 (33.3%) of rats developed steatosis from which 9 of them developed grade-I steatosis(5-33% fat deposition in liver tissue) and 4 of them developed grade-II steatosis(33-66% fat deposition in liver tissue). From those rats which developed steatosis, group-II rats took a larger proportion followed by group-III rats. From a total of 30 rats in this study, only 2(6.7%) developed microvesicular steatosis; 1 from group-II and 1 from group-III and only 6(20%) rats developed inflammation most of them from group-II rats. From among the rats which developed inflammation, 3 rats i.e., 2 from group-II and 1 from group-III developed advanced liver cell injury (ballooning).

4.7.2 Photomicrographs showing histopathological changes of liver of experimental rats.

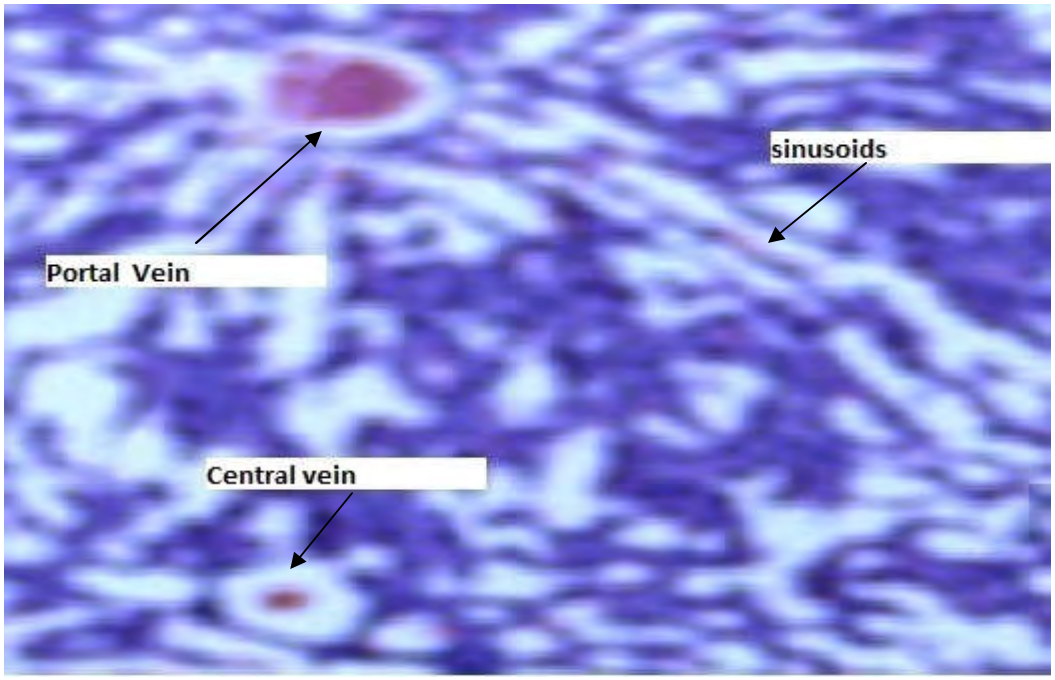


Figure 4.13: Photomicrograph of liver of normal control group rat (20X, H&E stain).

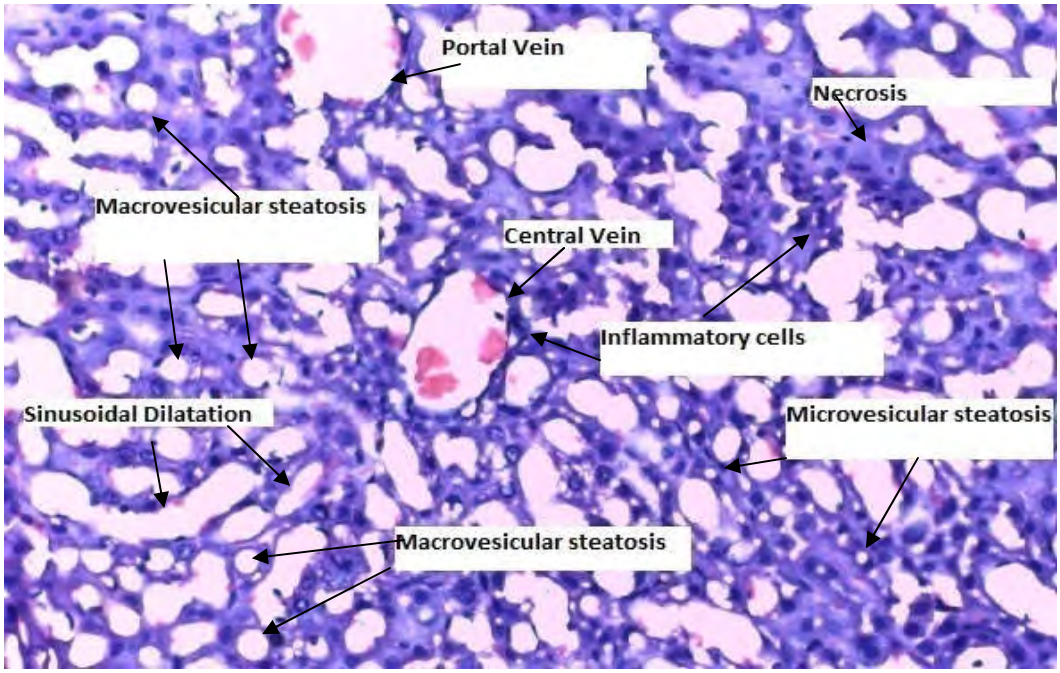


Figure 4.14: Photomicrograph of HAART only treated group-II rat for 60 days (20X and H& E stain).

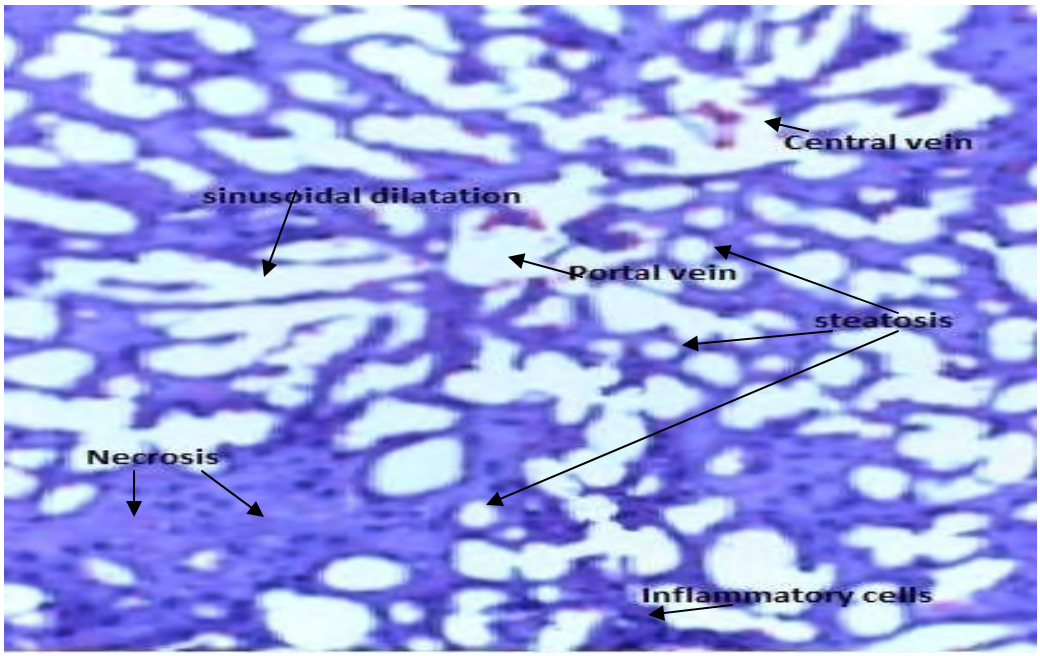


Figure 4.15: Photomicrograph of HAART with 100mg/kg of GTE treated group-III rat for 60 days (20X, H&E stain).

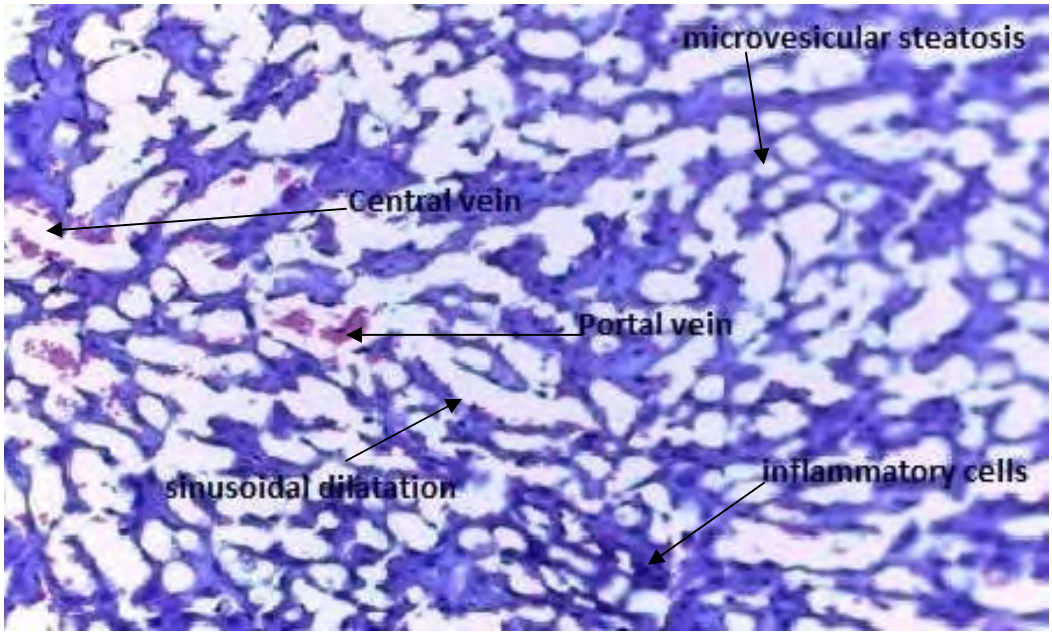


Figure 4.16: Photomicrograph of HAART with 200mg/kg of GTE treated group-IV rat for 60 days (20X, H&E stain).

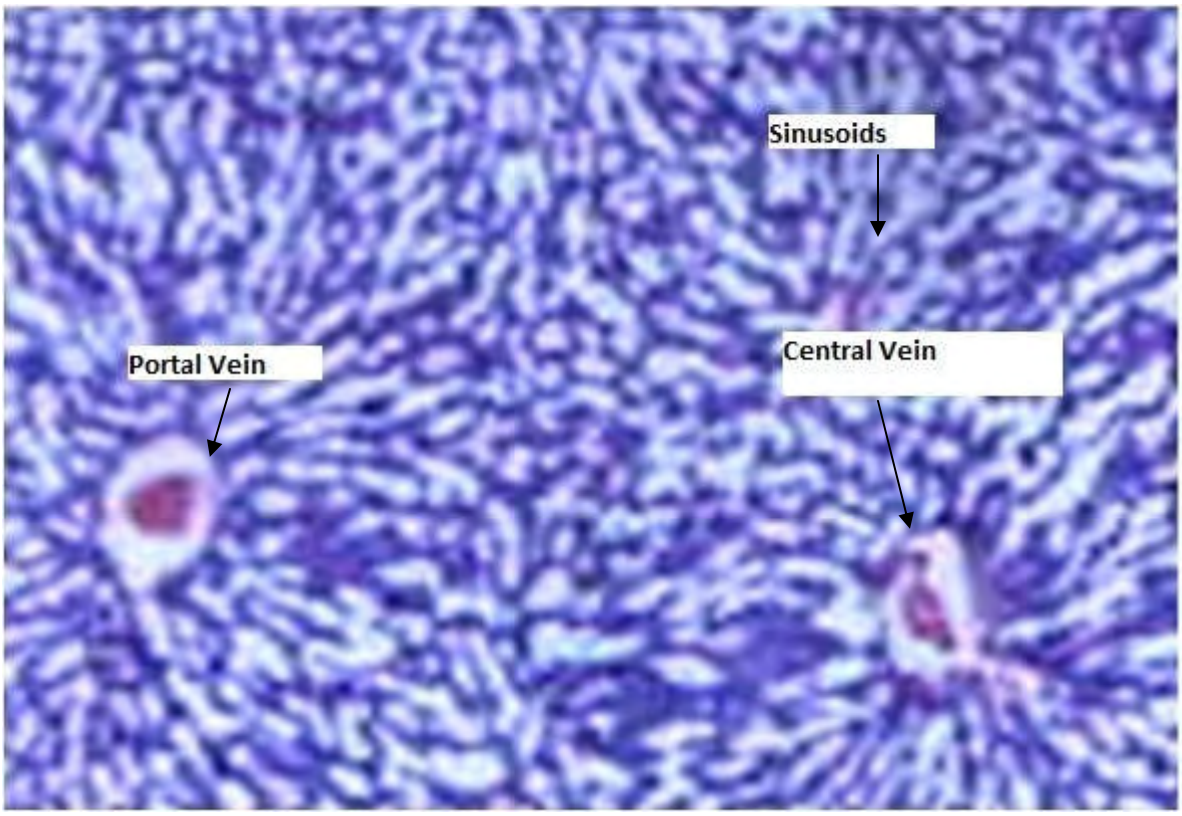


Figure 4.17: Photomicrograph of HAART with 400mg/kg of GTE treated group-V rat for 60 days (20X, H&E stain).No region of necrosis, no more compressed hepatocytes, no clearly visible inflammatory cells around the central vein, no macrovesicular steatosis.

5. DISCUSSION

Although the habit of using green tea as a beverage and as traditional medicine was originated and widely used in Far East countries like China and Japan, it is now becoming common in Western countries. This popularity of green tea around the world may be due to its healing effect for different health ailments like obesity, diabetes and even cancer. Though this plant is being studied extensively nowadays, peoples of by gone times use it simply as routine drink or as a traditional medicine. But now this plant is becoming the front line medicinal plant because of its well endowment with high concentration of polyphenols known as catechins which are unaltered and well preserved in green tea. These catechins play a central role for green tea to become famous throughout the world by enhancing green tea to be one of the strongest antioxidant. This antioxidant effect of green tea in turn enhances it to prevent a number of metabolic derangements and health ailments which are developed following these metabolic problems.

Even though the antioxidant property of green tea was studied and being studied extensively in different parts of the world(Zaveri,2006), the potency of this property varies from country to country even place to place in the same country because the content of its active constituents are affected by type of soil, climate, season of collection and age of the plant(Rani *et al*., 2014).So, green tea which is processed from Ethiopian high land tea plant *in-vitro* antioxidant activity was investigated prior to giving to rats of the experiment.

I*n-vitro* antioxidant activity of green tea leaf extract was done by using thin layer chromatography technique qualitatively and by using spectrophotometer quantitatively, and DPPH as free radical. As shown in Figure 4.1 the reaction of green tea leaf extract with DPPH formed pale yellowish circles with a diameter proportional to dosing i.e., the higher the dose the larger the diameter. The development of this pale yellowish color shows a reduction of DPPH (violet color) to DPPH-H is consistent with the postulate of Muthusamy and his colleagues (Muthusamy *et al.,* 2009) and work of Ebrahimzadeh and his colleagues which is believed to be due to the transfer of electrons from the reducing agent (antioxidant) to DPPH (Ebrahimzadeh *et al*., 2009).

HAART which is an effective medication to treat HIV infection also became a potent hepatotoxin causing devastating health problems associated with drug induced liver injury and metabolic derangement.This is because liver is the main organ that performs a number of activities in our body like metabolism, detoxification of drugs and other toxins which makes it the primary target for chemical and/or drug induced toxicity (Alcolado *et al*., 2007). Due to this and such alike damage, normal functions of the liver are impaired and result in dyslipidemia, hyperglycemia,steatosis,steatohepatitis even liver failure which are manifested by change in body weight, abnormal serum lipid profile, a decrease or an increase in the level of proteins synthesized by the liver in the plasma including major liver enzymes like AST, ALT and ALP (Robert *et al*., 2015; Shafran *et al*., 2005 ; Shakirat *et al*., 2014; Vijay *et al*., 2016).

In this study as shown in Figure 4.3, HAART only treated group showed a significant decrease in body weight as compared to normal control group which is in agreement with other previously done study on humans (Adeolu *et al*., 2014) but not in line with the study of Denue and his colleagues on humans in which most of the patients gained weight significantly (Denue *et al*., 2014). Group-IV and group-V rats showed even a more decrease body weight than group-II though it was not statistically significant.But when they are compared to group-I the decrease in body weight was statistically significant. So, GTE showed a synergistic effect on reducing the body weight which is in line with previously done works on mice (Klaus *et al*., 2005) and other studies on rats showed significant body weight decrease as compared to normal control group(El-Sayed and Eslam, 2014; Jibu and Gavin, 2013).

This decrease in the body weight of the rats which received GTE may be due to its enhancing effect of postprandial thermogenesis and fat oxidation (Sabu *et al*., 2010) that decrease the accumulation of fat in the body of the rats. In this study, although all groups of *C.Sinensis* leaf extract treated rats showed a significant body weight decrement, the rats treated with the maximum dose of GTE (400mg/kg) showed the highest body weight decrement which indicates that, an increase in concentration of GTE is directly related to decreasing the body weight. This decrease in body weight may be due to green tea catechins especially, EGCG which lower food intake by increasing the production and release of cholycystokinin which has hunger suppressive effects (Moon *et al*., 2007). EGCG also decrease lipid digestion and absorption by suppressing

activities of enzymes that involve indigestion and emulsification of dietary lipids which might inhibit lipid absorption from meals (Wang *et al*., 2006).

Though dyslipidemia is seen sometimes in individuals who are not taking HAART, it is usually associated with HAART taking (Chantry *et al*., 2008; Shafran *et al*., 2005; Tassiopoulos *et al*., 2008). This study showed that, HAART only treated group and HAART plus lowest dose (100mg/kg) of GTE treated group-III showed a significant increase ($p<0.05$) in the serum level of TC as compared to normal control group. But, group-V which were treated with 400mg/kg and HAART at the same time showed a significant decrease in the serum level of TC as compared to group-II. Group-II also showed a significant increment ($p<0.05$) in the serum level of triglyceride (TG) and low density lipoprotein cholesterol (LDL-c) as compared to normal control group (G-I) which is in line with previous studies done on HAART taking patients (Francis and Onyinye,2011).Group-V rats showed a significantly low ($p<0.05$) serum level of TG and LDL-c as compared to group-II rats which is in agreement with previous study done in rats(Nada *et al*., 2015) and also studies done on humans, consumption of green tea showed a significant decrease on serum level of TC,LDL-c and TG (Erba *et al*., 2005;Nada *et al*., 2015;Tokunagag *et al*., 2002).

This decrease in serum TC level might be due to the green tea catechins potential to increase fecal excretion of cholesterol, bile acid (Yang and Koo, 2011) and preventing reabsorption of bile acids from the small bowel through disruption of micelle formation of bile acid. This increase in bile acid and cholesterol excretion in turn activates cholesterol 7α-hydroxylase that catalyses the conversion of liver cholesterol to bile acid for restocking the loss which further decrease cholesterol (Goto *et al*., 2012).Another proposed mechanism for decreased cholesterol may be due to crude green tea catechins up regulating potential of LDL receptor which in turn increase the uptake of LDL-cholesterol from the blood circulation (Bursill *et al*., 2007).

The decrease in serum TG, might be due to the suppressing effect of green tea on expression of stearoyl-CoA desaturase (SCD 1) gene which determine hepatic triglycerides synthesis by involving in the biosynthesis of oleate and palmiteolate which are the main monounsaturated fatty acids of triglycerides (Rabia *et al*., 2015).

In this study, the serum level of high density lipoprotein cholesterol (HDL-c) which is also known as good cholesterol in HAART only treated group showed a minimal and statistically insignificant decrease ($p>0.05$) as compared to group-I. But, GTE treated groups showed a slight increment though it was insignificant change($p>0.05$) as compared to group-II except group-III which showed the same decrease as that of HAART only treated group. This insignificant change of HDL-c is supported by a previous study done by Francis and his colleague on HAART taking patients, there was a significant increase in the serum level of TG, TC, LDL-c and insignificant change in the level of HDL-c(Francis and Onyinye, 2011) but inconsistent with the study of Shafran and his colleagues which was done on HIV seronegative volunteers, RTV given for 14 days showed a significant increment in the serum level of TC, TG &LDL-c and a significant decrease in the serum level of HDL-c (Shafran *et al*., 2005).

When we see the counter effect of various doses of GTE on the serum level of HDL-c of experimental rats, the minimum dose of GTE (100mg/kg) did not show any change in their serum level of HDL-c but group-IV and group-V rats showed a relative increment than group-II though not significant. This finding is in agreement with a previous study done by Muramatso and his colleagues on rats by giving catechins extract from green tea normalizes serum level of total cholesterol but no significant change in the serum level of HDL-c (Muramatso *et al*., 1986).

This study also tried to quantify the amount of fat that accumulate in the liver of rats and group-II rats found to have a significantly increased TG level as compared to normal control group-I ($p<0.05$) and rats which were given GTE with different doses in addition to HAART showed a decrease in liver tissue TG level though significant decrement was observed in group-V rats as compared to group-II. This study complements the green tea inhibitory effect of oxidative stress which is a predisposing cause of NAFLD in HAART using HIV patients (Babu *et al*., 2006).

AST and ALT are the two main enzymes in the liver that catalyze the transamination reaction by transferring amino-group from one amino acid to the carbon skeleton of another amino acid. In normal conditions, these enzymes exist in plasma in a very low concentration but when the liver is injured, these enzymes leaked out from the liver tissue and exist in blood plasma in high

concentrations. Specially, the elevation of ALT marks the definite damage or injury to liver because this enzyme is specific to liver (Pradumna *et al*., 2009) though the significant elevation of AST indicates a marked damage to the liver, it might not be always guiding because of non specificity of this enzyme to the liver tissue(Paraskevas *et al*., 2011).

In this study, group-II showed a significantly elevated serum level of ALT and AST which is in agreement with previous studies done on HAART taking patients that showed a significant increase in these transaminases(Robert *et al*., 2015; Shakirat *et al*., 2014; Vijay *et al*., 2016). ALP which is also one indicator of liver damage though this enzyme is predominantly expressed in bone tissue it also found in sinusoids of liver tissue; when the liver is damaged it leaks out from liver and its concentration increase in serum especially when there is advanced stage of liver damage like hepatitis, bile duct damage, obstruction of bile duct and cirrhosis (Nyblom *et al*., 2006).As shown in Figure 4.12, ALP level in group-II did not show a significant increment and GTE treated groups though they showed a slight decrease in the serum level of ALP ,it was insignificant statistically($p>0.05$). The ALP's insignificant increase in group-II is not in consistent with the study of Robert and his colleagues in which patients who were taking HAART showed a significantly elevated level of gamma glutamyl transferase, ALP, AST and ALT (Robert *et al*., 2015) and its insignificant decrement by various doses of GTE is also inconsistent with the studies which are done previously on rats in which GTE treated rats showed a significantly decreased serum level of AST,ALT and ALP(El-Sayed and Eslam,2014;Tehassein *et al*., 2011).

The significant decrease in serum level of aminotransferases in GTE treated rats might be due to the antioxidant property of green tea extract catechins that decrease oxidative stress and increase plasma level of antioxidants like superoxide dismutase, catalase and glutathione (El-Bishbishy, 2005). Another study also reaffirm that green tea catechins have scavenging activity of reactive oxygen species and reactive nitrogen species (Paquay *et al*., 2000) which contribute for the decrease in serum level of these aminotransferases by keeping the integrity of the cell membrane of liver cells from these free radicals.

As histopathological investigation of this study revealed, most of group-II rats developed steatosis as shown in Table 4.1with inflammatory cells infiltration, lipid vacuolation which is caused by the accumulation of water and lipids; This may be due to loss of cell membrane integrity and lipid metabolism derangement caused by HAART and compression of hepatocytes following this vacoulation which led to extensive macrovesicular and microvesicular steatosis (Figure 4.14) as compared to normal control group-I rats and group-V rats which were treated with 400mg/kg of GTE. Group-III(Figure 4.15) rats also developed macrovesicular and microvesicular steatosis though not extensive like that of group-II rats, distorted architecture of hepatocytes though not intensified like that of group-II rats.

In group-IV rats, there are no clear markers of liver cells damage like that of group-II and group-III rats but the sinusoids are dilated, few inflammatory cells infiltration and no region of necrosis as shown Figure 4.16.Group-V rats which were given HAART and maximum dose of *C.Sinensis* leaf extract, showed more or less similar cellular architecture like that of normal control group rat, no compressed cells(Figure 4.17), there is no clearly visible infiltration of inflammatory cells and fat, no prominent vacuolation and no region of cell necrosis. This clearly shows that the GTE has efficient hepatoprotective property especially at higher doses; that might be due to its preventive role of oxidative stress.

6. CONCLUSION

As demonstrated by this study, GTE has shown a profound effect in restoring serum lipid profiles which is in direct proportion to dose increment of GTE.GTE effect on the body weight change of the rats was found to be dose independent i.e., there was a significant body weight reduction on all groups of rats which were taking GTE; this supports its importance on body weight reduction which might protect obesity. But, on serum lipid profiles (HDL-c, LDL-c, TC, TG) and tissue TG, the response was in dose dependent manner.

The damaging effect on liver was indicated through increased serum level of ALT and AST. However, GTE showed a readjustment of these enzymes in a dose dependent manner. But when we see the ALP level, though there was reduction in dose dependent manner the reduction was not significant. This insignificant change of ALP might be associated with the absence of advanced liver damage like cirrhosis which may in turn associated with relatively short period of HAART treatment. Histopathologically too, GTE showed a significant hepatoprotective effect in group-V rats which were given a maximum dose of GTE (400mg/kg).This hepatoprotective effect may be due to its high content of polyphenols known as catechins which have free radical scavenging antioxidant activity and its weight reducing effect may be due to its enhancing effect of fat oxidation and thermogenesis and by decreasing the appetite as demonstrated by other studies. Based on the findings of this study the minimum effective dose of green tea extract is 400mg/kg because most of the significant responses are observed in rats which were given this dose. So, it is possible to deduce that the higher the dose of the green tea extract, the better would be the response.

In general, this study demonstrated that the green tea leaf extract has anti-dyslipidemic and preventive effect on development of NAFLD with a satisfactory response in dose wise fashion.However, a further more elaborated study shall be done in a form of cohort study on humans to reach to a dependable and acceptable conclusion.

7. LIMITATIONS

- The study was unable to include group which were given green tea extract only to see the effect of the extract on lipid metabolism and liver tissue.
- The study was also unable to include the effect of HAART and green tea extract on total bilirubin of the experimental animals.

8. RECOMMENDATIONS

As the principal investigator of this study, I recommend the following things to be done on green tea.

- Similar study should be done on both HIV negative and HIVpositive humans for further elaboration of GTE effect on lipid metabolism and liver.
- Extensive phytochemical study should be done on green tea leaves which are collected in different seasons and from different places, to compare the respective responses.
- Further study on the active ingredients of green tea leaf should be done after fractional extraction.

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