EFFECTS OF AQUEOUS GARLIC EXTRACT INTERVENTION ON PLASMA GLUCOSE, LIPID PROFILE AND ALKALINE PHOSPHATASE CONCENTRATIONS IN ATAZANAVIR TREATED *RATTUS NORVEGICUS ALBINUS*

BY

ARISI JEPHTER

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DEDICATION

This work is dedicated to people living in difficult conditions in informal settlements that are a characteristic of many developing countries.

ABSTRACT

Patients on Highly Active Antiretroviral therapy (Protease inhibitors) are prone to complications including abnormalities in glucose metabolism, lipid derangement, and increased risk of cardiovascular disease. Studies have shown that Garlic can improve glycemic status and changes in lipid profiles. The objective of this study was to evaluate the effect of aqueous garlic extract intervention on the plasma glucose, lipid profiles and alkaline phosphatase concentration in atazanavir treated *Rattus norvegicus albinus*. The specific objectives were to determine the effect of aqueous garlic extract intervention on plasma fasting blood glucose, triglycerides, high density lipoproteins cholesterol and low-density lipoproteins cholesterol and alkaline phosphatase concentrations in atazanavir treated male *Rattus norvegicus albinus*. This was a randomized experimental study which lasted fourteen weeks.The study used thirty-nine (39) male *Rattus norvegicus*that were randomly divided into three (3) groups, (control, treat 1 and treat 2) consisting of 13 animals each. Treat 1 rats received a daily atazanavir treatment at 10mg/Kg. Treat 2 rats received daily atazanavir treatment as in treat 1 above plus garlic extract 250mg/kg body weight as from the third week. The control rats received 2ml of normal daily throughout the study period. The plasma total triglycerides, high density lipoproteins cholesterol, low-density lipoproteins cholesterol and alkaline phosphatase concentrations were done using automated analyzer for lipid profile and liver enzymes respectively. The differences between meanconcentrations of fasting blood glucose, total triglycerides, high density lipoproteins cholesterol and low-density lipoproteins cholesterol were analyzed using one- way ANOVA. Test of significance was set at $P < 0.05$. Treat 1 showed a significant ($P=0.000$) increase in the mean concentration of fasting blood glucose of 5.4mmol/L at the end of the study while treat 2 rats showed an increase to 4.7mmol/L as at the eight week with a significant decline at end of the study to 4.4mmol/L compared to the controls whose FBG levelranged between4.0mmol/Land 4.3mmol/Lduring the study period. Treat 1 animal exhibited significant ($P=0.00$) increase in total tryglycerides to 64mg/dL while treat 2 also recorded an increase to 63mg/dL followed by a decline to 47mg/dL by the end of the study. The mean LDLc increased significantly ($P=0.000$) to about 74mg/dl by the end of the study in treat 1 whereas in treat 2 this increased to 72mg/dL by the sixth week before declining to 62mg/dL. Treat 1 exhibited a significant ($P=0.000$) decline of HDL from…..to 47mg/dL during the fourth week and down to 41mg/dL by the end of the study while Treat 2 showed an initial decline to 42mg/dL at six weeks before significantly($p=?$) rising to 52mg/dL at the end of the study. Treat 1 showed a significant ($P=0.000$) elevation of the mean plasma ALP in the fourth week (65U/L. Treat 2 on the other hand showed a significant increase to 63U/L by 4th week before declining to 56U/L by the end of the study. The findings of this study show that atazanavir caused elevation of FBG, TTGLD, LDLc and ALP and a decline in HDLc concentration. Aqueous garlic extract reversed all the atazanavir treatment effects hence can be considered as an adjunct therapy in patients on PI based regimen. These findings suggest that garlic can be used as a supplement for intervening the metabolic effects of atazanavir.

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LIST OF ABBREVIATIONS

ADA	:	American diabetes association
AGE	:	Advanced glycation products
ALP	:	Alkaline phosphatase
ALT	:	Alanine transaminase
ANOVA	:	Analysis of variance
ART	:	Antiretroviral therapy
AST	:	Aspartate aminotransferase
APO	:	Alipoprotein
BMI	:	Body mass index
CAD	:	Coronary artery disease
CETP	:	Cholesterol ester transfer protein
CHD	:	Congestive heart disease
CHREPB	:	Carbohydrate response element binding protein
CR	:	Caloric restriction
CRABP1	:	Cellular retinoic acid binding protein
CRP	:	C- reactive protein
CVD	:	Cardiovascular disease
C/EBP	:	C/Enhancer binding protein
D.M	:	Diabetes mellitus
ER	:	Endoplasmic reticulum
ERK1	:	Extracellular regulated kinase 1
FFA	:	Free fatty acids
GGTT	:	Gamma Glutamyl amino transferase
GH	:	Growth hormone
GHS	:	Growth hormone secretagogue
GLP1	:	Glucagon like Peptide 1

GLUT	:	Glucose transporter
HART	:	Highly active antiretroviral therapy
HDL	:	High density lipoproteins
HDL-C	:	High density lipoprotein cholesterol
HIV	:	Human immunodeficiency virus
HK	:	Hexokinase
HMT	:	Health management team
IDF	:	International diabetes federation
IDFAR	:	International diabetes federation for African region
IFG	:	Impaired fasting glycaemia
IGT	:	Impaired glucose tolerance
IL	:	Interleukin
INF Y	:	Interferon gamma
IRSI	:	International research services
KENPHIA	:	Kenya population based HIV impact assessment
LDL	:	Low density lipoproteins
LDL-C	:	Low density lipoproteins cholesterol
LPL	:	Lipoprotein lipase
MAPK	:	Mitogen activated protein kinase
MI	:	Myocardial infarction
NACCK	:	National Aids control council of Kenya
NAFLD	:	Non alcoholic fatty liver disease
NCD	:	Non communicable disease
NGT	:	Normal glucose tolerance
NLRP3	:	Node like receptor protein 3
NIDD	:	National institute of diabetes, digestive and kidney disease
NNRTI	:	Non nucleoside reverse transcriptase inhibitor

NSREBP	:	Nuclear sterol regulatory element binding protein
PAD	:	Peripheral artery disease
PI	:	Protease inhibitors
PLWHIV	:	People living with HIV
PON	:	Paraoxinases
PPAR *Y*	:	Peroxisomal proliferative activator receptor gamma
RAAS	:	Renin aldosterone angiotensin system
SREBP-1c	:	Sterol regulatory element-binding protein 1c
TC	:	Total cholesterol
TG	:	Total glycerol
tHcy	:	Total hyperhomocysteine
TNF	:	Tumour necrosis factor
UPR	:	Unfolded protein response
VLDL-C	:	Very low density lipoprotein cholesterol
WHO	:	World health organization

OPERATIONAL DEFINITIONS

Adipogenesis: the process by which fat laden cells (adipocytes) develop and accumulate at various sites in the body.

Alipoproteins: The protein component of plasma lipoproteins, such as chylomicrons, low density lipoproteins and high-density lipoproteins.

Atherosclerosis: Any of a group of diseases characterized by thickening and hardening of the artery wall and narrowing of its lumen.

Euglycemia: A state of having a normal glucose level.

Fasting blood sugar: Blood sugar taken without caloric intake 8 hours prior.

Glucolysis: The first step in cellular respiration in which a glucose molecule gets broken down into 2 molecules of pyruvate.

Gluconeogenesis: The formation of glucose from non-carbohydrate molecules, such as amino acids, lactic acid, and glycerol.

Glycogenesis: Formation of glycogen.

Glycogenolysis: Glycogen breakdown to glucose.

Glycolysis: The metabolic pathway that converts glucose to pyruvic acid.

Glycosylation – is the process by which a carbohydrate is covalently attached to a protein or a lipid.

Homeostasis: The dynamic constancy of the internal environment, the maintenance of which is the principal function of physiological regulatory mechanisms.

Hypercholesterolemia: Excessive cholesterol in blood**.**

Hyperglycemia: High blood glucose (sugar) concentration**.**

Hyperinsulinemia: Excessive insulin in blood**.**

Hyperlipidemia: An excess of fat in the blood, characterizing a group of metabolic disorders.

Hypertriglycidemia: Excessive tryglyicerides in blood.

Hypoglycemia: Low blood glucose (sugar) concentration.

Ketoacidosis: A type of metabolic acidosis resulting from the excessive production of ketone bodies, as in diabetes mellitus.

Ketogenesis: Theproduction of ketone bodies.

Lipid profile: Blood tests for evaluation of lipid abnormalities**.**

Lypodystrophy: A condition in which amount /or distribution of adipose tissue in the body is not normal.

Lipolysis: The hydrolysis of triglycerides into free fatty acids and glycerol.

Phosphorylation: a process by which a phosphate is added to a molecule.

Prediabetes: When blood sugars are higher than normal but not yet high to be called diabetes.

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CHAPTER ONE
INTRODUCTION

1.1 HIV-AIDS Treatment

Treatment of HIV is life long embracing frequent clinical evaluation and follow up during which an individual may experience treatment associated adverse events or drug toxicity (Tadesse et al., 2022). Atherosclerosis and the development of high-risk plaque are promoted by protease inhibitors (PIs), which are HAART components, and have been linked to irregularities in glucose metabolism, lipid derangement, and alterations in body fat deposition hazards, raising the risk of CVDs (Fathallah et al., 2015; Karla et al., 2011).

The increased use of HAART and the high levels of viral suppression it provides have resulted in sizable reductions in HIV mortality and the risk of HIV transmission, which are likely factors in a potential decline in HIV incidence. Reductions in HIV prevalence on a population level are therefore possible if there are sharp drops in new HIV infections or high rates of HIV-related death in a population(Kimanga etal.,2014; Bershteyn et al., 2018; Kim et al., 2021).Additionally, expedited ART initiation, such as beginning ART the day after HIV diagnosis, can improve clinical outcomes by increasing the proportion of persons who begin and continue taking ART, which is crucial for those with very low CD4 cell counts, for whom the risk of mortality is high (Ford et al., 2018). These data show that anti-retroviral medication reduces the danger of viral transmission while also providing advantages for individual patients and the general population (Lundgrenet al., 2015).

Major cardiovascular disease risk factors like hypertension, diabetes, and abnormal blood lipid levels (high total cholesterol, high serum triglycerides, low high density lipoprotein cholesterol, and high low density lipoprotein cholesterol) are becoming more prevalent in patients receiving HART in low- and middle-income countries, which raises the possibility that ART may be linked to these risk factors. Examining the link between ART and CVD risk factors has become much more challenging due to the current epidemiological trend towards higher incidence and prevalence of non-communicable illnesses (Dimala et al., 2018).

According to studies, the use of HIV protease inhibitors may interfere with the normal stimulation effect of insulin on glucose and fat storage. HIV infection-related chronic

inflammation and the use of protease inhibitors have been shown to negatively affect intermediary metabolism and result in decreased total adipocyte storage capacity as well as increased resistance to fat storage from remaining adipocytes. This procedure produces pro-atherogenic lipids (Erdembilbeg et al., 2010). A decrease in glucose disposal was observed in relation to the mechanisms/sites of insulin resistance brought on by protease inhibitor treatment, but glucose synthesis appeared to be properly lowered for the current plasma insulin levels. (Woerle et al., 2003).

Recent studies have reported a higher incidence of diabetes mellitus in PLWHIV compared to HIV negative counterparts with patients who develop DM having a greater proportion of followup time and exposure to PI's (Samad et al., 2017). Diabetes is a progressive disease and in most patients intensified treatment over time is required to attain and maintain glycemic control with poor glycemic control associated with microvascular and macrovascular complications (Blonde et al., 2019).

Short term exposure to atazanavir significantly increases serum antioxidant capacity, lowers total and low density lipoprotein levels, and may reduce mean arterial pressure but both endothelial dependent vasodilation and endothelial independent vasodilation remains unchanged after treatment (Milan et al., 2015 however, Atazanavir has shown minimal inhibitory effects on insulin regulated glucose transporter (GLUT _4) (Mejide et al., 2010).

Elevated liver enzymes are a typical issue for HAART users. Antiretroviral (ARV) medications harm liver cells either directly or via their active metabolites. An indication of the etiology of liver damage may be found in the length of treatment and the beginning of liver disease. Predictable liver damage and toxicity may be attributed to the drug's dosage having been greatly raised by ALP. The observed considerable variation in ALP may reflect an ARV medication adverse effect that causes the liver to produce moreisoenzyme for ALP (Tesfa*et al., 2019).*

Garlic reduces the production of cholesterol, blocks enzymes that regulate lipid synthesis and angiotensin-converting enzymes, and reduces other factors including the rate at which low-density lipoprotein (LDL) is oxidized, all of which assist diabetics better manage their blood glucose levels. Additionally, it helps regulate high/low density lipoprotein and total cholesterol (Wang et al., 2017).

This study therefore evaluated the effect of aqueous garlic extract intervention on plasma glucose, lipid profile and alkaline phophatase concentrations in atazanavir treated *Rattus norvegicus albinus.*

1.2 Statement of the Problem

The development of combination active antiretroviral therapy (ART) for Human Immunodeficiency Virus (HIV) infection has significantly extended life expectancy and decreased morbidity and mortality from AIDS-related causes. Clinical concerns about the use of HAART have been raised regardingthe metabolic abnormalities linked to its use. Heart disease may result from certain of these metabolic abnormalities, such as dyslipidemia (abnormally high triglycerides and cholesterol) (Ogundahunsi et al., 2008).

There are 27.5 million people living with HIV who are receiving HAART equaling to global ART coverage rate of 73% in 2020 (WHO report on HIV AIDS, 2021). Alterations in liver enzymes and lipoprotein metabolism have also been recognized as separate risk factors for the emergence of cardiovascular disease (CVD) (Sangappa, 2017). The most potent risk factor for developing the metabolic syndrome is serum ALP, and increased liver enzymes may be an indication of obesity, fatty liver, hepatosteatosis, or inflammation, all of which affect insulin signaling both locally and systemically. In response to oxidative stress, there may be an increase in ALP activity, which allows for a greater uptake of GSH precursors into cells (Rahul et al., 2020).

To lower morbidity and mortality in PLWHIV, control measures must be taken to lessen the increasing metabolic abnormalities and the consequences they cause. According to studies, both the aqueous and methanol extracts of garlic significantly lower the blood sugar levels of diabetic rats. (Faroughi, et al., 2018). Therefore, this study evaluated the effect of aqueous garlic extract intervention on plasma glucose, lipid profile and alkaline phosphatase concentrations in atazanavir treated*Rattus norvegicus albinus* and the finding provided evidence for recommending garlic use as a supplement in routine management of HIV.

1.3 Research Objectives

1.3.1 General Objective of the Study

To evaluate the effects of aqueous garlic extract intervention on plasma glucose, lipid profile and alkaline phosphatase concentrations in atazanavir treated *Rattus norvegicus albinus.*

1.3.2 Specific objectives

i. To determine the effect of aqueous garlic extract intervention on plasma concentrations of fasting glucose in atazanavir treated *Rattus norvegicus albinus.*

ii. To determine the effect of aqueous garlic extract intervention on plasma concentrations of total triglycerides, high density lipoproteins cholesterol and low-density lipoproteins in atazanavir treated *Rattus norvegicus albinus.*

iii. To determine the effect of aqueous garlic extract intervention on liver alkaline phosphatase levels in atazanavir treated *Rattus norvegicus albinus.*

1.4 Hypothesis

1.4.1 Null hypothesis

H_0: There is no significant difference in the means of the blood glucose, lipid profile levels and alkaline phosphatase levels among the three treatment groups.

H_0: $\mu 1 = \mu 2 = \mu 3$

1.5 Justification of the Study

HIV is now a chronic, treatable condition because of anti-retroviral therapy (ART), which has decreased morbidity and death (Jammy et al., 2016). However, people living with HIV and are on protease inhibitors are prone to complications including hyperglycemia, dyslipidemia in a considerable proportion of patients (Grace et al., 2005). HIV has taken a toll on individuals, societies and economies especially in Kenya a country already burdened with communicable diseases as HIV/AIDS affects the most productive age group in the society resulting in reduced quality of life, increased morbidity and mortality in Kenya as of 2020, 86% of PLWHIV were on HAART (UNAIDS, 2021). It takes work to combat these negative impacts without creating additional problems and negative effects. The goal of this research was to see if administering garlic extract together with protease inhibitors may lessen or perhaps reverse these negative effects.

Shosh and Akter's investigation, which solely examined glucose, found that garlic significantly improved glycemic control by reducing fasting and postprandial blood glucose levels. (Shoshi and Akter, 2017). Clinical studies using different kinds of garlic preparations have shown contradictory results in hypercholesterolemia patients, and it was previously believed that these discrepancies may have been brought on by differences in the content of garlic preparations and the possible responses they may elicit (Qidwai and Ashfaq, 2013). This study evaluated plasma fasting glucose, lipid profiles and liver enzymes.

Studies have so far been conducted in isolation, focusing either on the hyperglycemic effects of HIV protease inhibitors, lipid abnormalities, liver enzyme alterations, or the antihyperglycemic benefits of garlic on diabetic people. For instance, a research by Nzuza et al. observed that metabolic problems such as insulin resistance, glucose intolerance, and overt diabetes are known to be linked to long-term use of HIV protease inhibitors (Nzuza et al., 2017). Another study by Muya noted that PI's are significantly associated with lipid derangements (Muya, 2019). The curent study focused on effect of PI's on blood glucose, lipid profile and liver ezymes alkaline phosphatase. Four primary types of hepatotoxicity are linked to protease inhibitors. The first is the mild-to-moderate elevations in serum aminotransferase and alkaline phosphatase levels that a large proportion of patients on antiretroviral regimens that include protease inhibitors suffer (Liver tox, 2012).

The presence of this knowledge gap forms the basis for the justification of this study. The surge of these life-threatening complications in PLWHIV on protease inhibitors justifies the need to evaluate the extent of the effect of blood glucose, serum lipid levels and alkaline phosphatse levels in these patients. Good management and control of both hyperglycemia and dyslipidemia can prevent cardiovascular complications as such these changes in lipid parameters due to increased free fatty acids flux secondary to insulin resistance (Sulaiman,20 14). There exists a relationship between lipid levels and liver enzymes especially alkaline phosphatase. A study by Monica et al noted that only ALP is independently associated with obesity and central adiposity, whereas only high serum triglycerides are associated with raised levels of each of the four liver enzymes (Monica et al., 2005).In hyperglycemic conditions, increased glycogen synthesis causes intracellular glycogen to accumulate in hepatocytes, leading to the usual biochemical results of mild to moderately raised aminotransferases, normal liver synthesis, with or without minor

elevations of alkaline phosphatase. With proper glycemic management, all of these biochemical abnormalities are reversible (Han et al., 2012).

Few studies have been undertaken on the effects of garlic and have shown to have hypoglycemic effects, lipid lowering effects and liver enzymes the presence of this knowledge gap justifies this study. This study therefore evaluated the effect of aqueous garlic extract administration on plasma levels of fasting glucose, lipids and alkaline phosphatase hormone in atazavir treated hyperglycemic *Rattus norvegicus albinus*

1.6 Significance of the study
The present study has provided evidence that garlic can reduce blood glucose, lipid levels and liver enzymes levels in patients with diabetes dyslipidemia, elevated liver eznymes that are always associated with atazanavir medication. These findings are therefore very significant in the formulation of HIV-AIDS treatment. Garlic can form part of the supplements that can help in diverting the possible cardiovascular disease in HAART treated HIV patients.

1.7 Scope of the Study
The scope of this study was limited to withdrawal of blood and assessment of the effect of aqueous garlic extract administration on fasting blood glucose concentration, lipid profile, and alkaline phosphatase levels of atazanavir treated winstar rats.

CHAPTER TWO

LITERATURE REVIEW

2.1 Highly Active Antiretroviral Therapy (HAART)

Since the advent of highly active retroviral therapy (HAART), the outlook for those living with HIV has greatly improved (Johnsen, 2007). All HIV-infected people with detectable plasma virus should begin therapy, with recommended first regimens consisting of NNRTI + 2 NRTIs when administered properly. Antiretroviral medications remain the cornerstone of HIV treatment and prophylaxis. (Günthard et al., 2016). It's the most important intervention that delays and prevents the progression of HIV to AIDS (Ankrah et al., 2017).

Table 2.1: Classes of HART (Adapted from Zdanowicz, 2006)

Drug Class	Examples	Mechanism
Nucleoside, Nucleotide Analogues	Reverse Zidovudine Didanosine Zalcitabine Lamivudine Abacavir Stavudine Emtricitabine Tenofovir	Competitive inhibitors of HIV
Non- Nucleosides	Nevirapine Efavirenz Delavirdine	Non-competitive antagonists that directly bind and inhibit HIV reverse transcriptase.
Protease Inhibitors	Ritonavir Saquinavir Amprenavir Indinavir Nelfinavir Atazanavir Tipranavir Fosamprenavir	Inhibit HIV protease enzymes that are responsible for viral maturation
Fusion Inhibitors	Enfuvirtide (T-20)	Prevents fusion of HIV with host cells
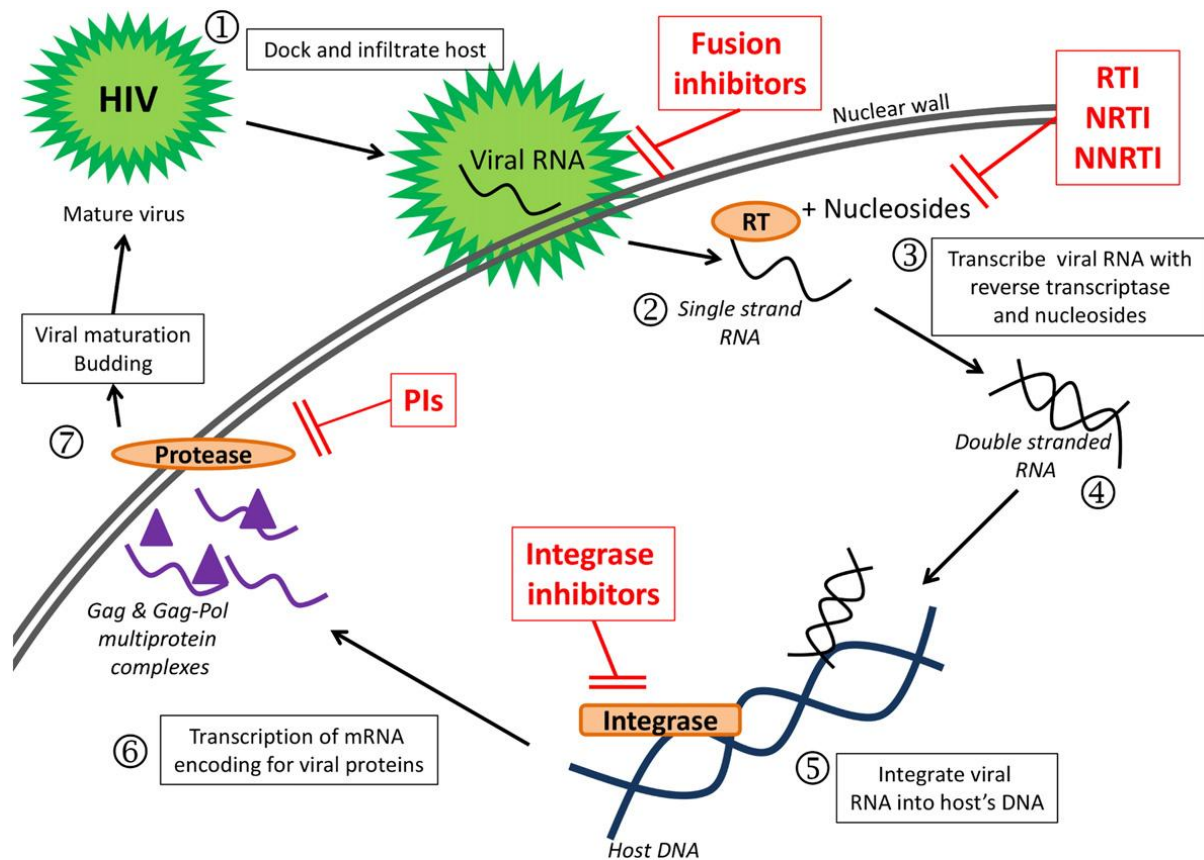
Figure 2.1: HIV lifecycle and antiretroviral drug targets

*Source:*Adapted from***Badley, (2005)***

As a first-in-class integrase inhibitor for the treatment of HIV-1 infection, raltegravir stops the circularization of linear viral DNA by host DNA repair enzymes, which leads to the formation of episomes with two copies of the viral long-terminal repeat or recombination, which results in the formation of a 1-LTR circle. Because of this, episomal DNA is more abundant when integrase inhibitors halt active replication (Buzón et al., 2010).

According to studies, one benefit of greater HAART accessibility is the management of antiretroviral drug-related toxicities, which is now a crucial part of HIV care in impoverished nations. For a number of reasons, the range of HAART's negative effects may differ across industrialized and poor nations. First, financial limitations, prohibitive laboratory monitoring costs, co-occurring diseases like anemia and malnutrition that are more common in areas with limited resources, the use of herbal medications, and host genetics may all have an impact on the frequency of adverse effects. (Subbaraman et al., 2007).

A child's time on ART is linked to a lower likelihood of developing sequential processing disorder, a lower incidence of HIV-related encephalopathy, and fewer HIV-positive cells invading the central nervous system. One intriguing aspect of ART is its potential to considerably lessen and even reverse the signs and symptoms of HIV dementia/encephalopathy as well as neurologic impairments brought on by a reversible malfunction of neuronal metabolism brought on by soluble neurotoxins. Although viral suppression is attainable with moderate adherence to HAART, studies have shown that lower adherence raises the likelihood of the development of resistant strains and death (Ortegoa, 2011).

2.2 Protease Inhibitors

Proteolytic enzymes, sometimes referred to as peptidases or proteases, are very significant enzymes that make up just about 2% of the genes in humans, infectious organisms, and other forms of life. They control the activation, synthesis, and turnover of all proteins to regulate the majority of physiological activities. The equilibrium of protease-catalyzed human physiology can be upset by genetic and environmental variables, which can result in aberrant growth, ill health, disease, and death. Additionally, proteases are necessary for the reproduction and spread of bacteria, parasites, and viruses that cause infectious illness in animals (Abbenante and Fairlie, 2005). Among the different kinds of HAART treatment regimens, protease inhibitors are acknowledged to be extensively used as a primary component and shown to be effective in treating HIV/AIDS (Chndrashektar, 2019). The construction of a fully developed and infectious viral particle requires the cleavage of the Gag and Gag-Pol polyproteins into their final functional protein products, which is accomplished by the HIV protease (Clavel and Mammano, 2010). The HIV aspartyle protease is the target of the PI class of antiretroviral drugs, which prevents the cleavage of viral polyproteins and the consequent production of individual viral proteins. Although these medications do not stop upstream infection activities including entrance reverse transcription and integration, they do impede the generation of functional virions (Badley, 2005).

Studies have demonstrated that protease inhibitors, particularly when taken in combination therapy regimens, have a powerful prolonged suppression of HIV replication and have already established themselves as a vital addition to the arsenal of medications available to treat HIV infection. (Hoetelmans et al., 1997). There are now nine HIV protease inhibitors that have been

given the green light for clinical use in people (Saquinavir, Indinavir, Nelfinavir, Lopinavir, Amprenavir, Atazanavir, Tipranavir, Ritonavir, and Fosamprenavir). . In animal models of sepsis, hepatitis, and stroke, protease inhibitors reduce apoptosis and illness because their anti-apoptotic actions have been extended to non-HIV, non-immune cells (Rizza and Badley, 2008). HIV PIs, despite PI resistance, prevent CD4+ T cell death and boost CD4+ T cell survival (Vlahakis et al., 2007. Protease inhibitor use is linked to a significant improvement in the mental health of HIV-positive individuals in addition to their spectacular clinical effects (Low-beer et al., 2000). There is a link between high interferon levels and hypertriglyceridemia in AIDS patients. As a result of reduced levels of lipoprotein lipase, interferon may cause hypertriglyceridemia. Additionally, patients with AIDS have a 2.5 times higher prevalence of the lipoprotein B phenotype, which is linked to hypertriglyceridemia (Kaul et al., 1999).

It has been shown that ritonavir increases plasma lipids to a degree equivalent to that required to maximize the effects of protease inhibitors. Other pathways have also been put up as contributors to the negative effects of ritonavir-boosted protease inhibitors on cardiovascular health, including endothelial dysfunction, insulin, and cholesterol accumulation in macrophages. Although first-generation protease inhibitors were able to produce endothelial dysfunction via a variety of mechanisms, modern protease inhibitors like lopinavir/ritonavir or atazanavir have not been demonstrated to do so in healthy volunteers or HIV-infected patients. Because HIV-infected patients consistently have higher levels of endothelium biomarkers than uninfected people do, despite good antiretroviral treatment (Llibre et al., 2012). The dysmetabolic syndrome connected to HAART has also been related to the inhibitory effect of one of the PIs on the glucose transporter 4 (Glut-4) on adipocytes and rat muscle. PIs also inhibit the expression of the PPAR, or proteasome proliferation activator receptor, in cultured adipocytes. The 11 beta hydroxysteroid dehydrogenase type-1 enzyme is more active in visceral fat, which would speed up the retrograde conversion of inactive cortisone to active cortisol, increase lipolysis and the levels of free fatty acids in the portal system, and ultimately cause insulin resistance and hyperlipidemia (Salehian et al., 2005).

2.3 Atazanavir

Due to its once-daily dosage schedule, excellent metabolic profile, and infrequent side effects, atazanavir is one of the most often recommended protease inhibitors. It is a cytochrome P450 (CYP) system substrate and strong inhibitor. This aza-dipeptide analog has potent anti-HIV activity and contains a large phenylpyridyl P1 group that is asymmetrical to its benzyl P1 group. (Mastan and Kumar, 2009). Insulin resistance in HIV protease inhibitor-treated individuals is connected to the blockage of a glucose transporter. The insulin-regulated glucose transporter-4 may be directly inhibited by a number of HIV protease inhibitors. It has a strong genetic barrier to resistance, a good adherence profile, and no impact on lipid and glucose metabolism. Protease inhibitors only inhibit the isoform of glucose transporter-4 and do not significantly inhibit glucose transporter-1.

Individuals with hepatic impairment should be treated with care because atazanavir concentrations may increase. Atazanavir is mostly metabolized by the liver. ALP is found on the sinusoidal surface of hepatocytes, according to studies on liver function tests, and pregnant rats treated with ATV/r exhibited higher levels of ALT, AST, ALP, LDH, and GGT in their blood and livers in a dose-dependent way. Individuals with underlying hepatitis B/C virus infections or severe transaminase elevations before to therapy may thus have a greater risk of having later transaminase elevations, including hepatic breakdown (Wood, 2008).

Due to its quick absorption and Cmax arriving within just 2.5 hours, ATV has demonstrated its effectiveness in both treatment-experienced and treatment-naive individuals. ATV is 86% linked to alpha-1-acid glycoprotein and albumin in human serum proteins, respectively. P-glycoprotein is a substrate for the medication, which restricts its distribution and absorption. Similar to other PIs, hepatic cytochrome P450, particularly the CYP3A4/CYP3A5 isoenzymes, extensively metabolizes ATV. ATV metabolites are excreted in the bile and urine, respectively, for 79% and 13% of the dosage. RTV boosting boosts ATV's ability to diffuse into the cerebral fluid (CSF) or semen, which it hardly does on its own. As evidenced by the fact that only 5–10% of patients stopped using ATV because of side effects, it is generally well tolerated (Fernández-Montero et al., 2009).

Atazanavir alone was shown to be less effective than a regimen of lopinavir/ritonavir in treatment-experienced subjects who had previously failed antiretroviral therapy; however, atazanavir boosted with ritonavir was found to be more tolerable and to have equivalent efficacy to lopinavir/ritonavir (Aruksakunwong et al., 2007).

Atazanavir/RTV is linked to notable reductions in fasting triglycerides and total cholesterol. Hyperlipidemia, lipodystrophy, and impaired glucose metabolism are examples of chronic consequences that have been described and are categorized as metabolic syndrome. Cardiovascular problems such diabetes, hypertension, abdominal obesity, low HDL levels, hypercholesterolemia, and hypertriglyceridemia are caused by the latter (Gontran et al., 2009). Unboosted atazanavir treatment, according to certain research, significantly improves fasting lipidemic markers, insulin sensitivity, and glucose tolerance (Guffanti et al., 2007)

In contrast to other PIs, particularly when combined with RTV, a second trial found no evidence of an association between ATV and clinically significant dyslipidemia or insulin resistance. These results have been validated by other large-scale phase 3 trials, which also showed noninferiority between ATV and efavirenz, an NNRTI used in combination with two NRTIs (combivir), or between ATV/RTV and LPV/RTV. ATV differs from other PIs in its resistance profile, with susceptibility being retained against 86% of isolates that are resistant to 1-2 PIs (Anderson et al., 2007).

2.4 Effect of HAART on Glucose Metabolism

As glucose metabolism provides intermediates for glycosylation, a post translational modification that modifies the function of proteins and lipids, information regarding normal hepatic glucose metabolism may aid in understanding the pathogenic mechanisms behind diabetes mellitus (Maria et al., 2016). The three-step process of glucose delivery to the muscle, GLUT4 transport of the glucose into the muscle and hexokinase phosphorylation of the glucose within the muscle is what defines the sites involved in the regulation of muscle glucose uptake, which is complex physiologically (David et al., 2011). The small range of plasma glucose between 4 and 7 mmols reflects the equilibrium between (i) the release of glucose into the circulation through either absorption from the intestine or breakdown of stored glycogen in the

liver and (ii) the uptake and metabolism of blood glucose by peripheral tissues (Tsimihodimis and Florentin, 2015).

According to studies, diabetes mellitus begins with insulin resistance, is followed by activation of the pancreatic beta cell to maintain homeostasis, and results in greater plasma levels when the body's need for insulin cannot be satisfied. Because diabetes mellitus has a chronic course, many people dismiss its signs and symptoms and do not view them as severe issues (Ramachandrian 2014). As a result, complications might arise years before symptoms become noticeable. The medium time to achieve optimal glycemic control in diabetic patients is long, suggesting that patients are exposed to a higher risk of complications and death. Poorly controlled diabetes mellitus leads to major consequences and even death (Tigist, 2019).

Microvascular complications are primarily caused by hyperglycemia, whereas macrovascular complications are primarily caused by atherosclerosis (Daisuke, 2018). By way of a non-enzymatic reaction involving the carboxyl group of the reducing sugar and a free amino acid, glucose forms covalent bonds with proteins. Advanced glycation end products are produced as a consequence, which alter tissue functions and molecular conformation, leading to the development of atherosclerosis and protein anchoring sites (Saberzadeh-Ardestani et al., 2018). Plasma glucose concentration depends on the ratio between the rate at which glucose enters the bloodstream to the pace at which it is removed. The glucoregulatory hormones, which include insulin and amylin, glucagon, glucose dependent insulinotropic peptide, cortisol, and growth hormones, keep the level of blood glucose within a relatively small range (Stephen et al., 2004). The exact synchronization of endogenous glucose synthesis or dietary glucose delivery is necessary to maintain a normal plasma glucose concentration (David et al., 2008).

According to reports, diabetes and its complications have the potential to undo some of the health improvements made in sub-Saharan Africa in recent years. This is because only 10% of people with diabetes receive the necessary medications, and only 50% of those with the disease are aware that they have it (Atun et al., 2017).

The past ten years have seen a significant investment in research on the side effects of HAART. One potential mechanism underlying PI-related insulin resistance in PI-treated individuals is the

suppression of glucose transport mediated by glut-4, the main isoform of glucose transporter that mediates insulin-stimulated cellular absorption of glucose in humans. Although the B cells can initially make up for insulin resistance, as the condition worsens, this ability diminishes and postprandial hyperglycemia of IGT manifests (Dube, 2000).

According to studies, the risk of developing diabetes is dose- and time-dependent and that PIs can cause a variety of levels of insulin resistance. The downregulation of GLUT-4, suppression of peroxisome proliferator-activated receptor activity, and decreased beta cell function are a few hypothesized pathways (Dosunmu et al., 2016). HIV-associated resistance typically manifests as dyslipidemia and a relative absence of peripheral adipose tissue storage. HIV-related chronic inflammation and protease inhibitor therapy both set off cellular homeostatic stress responses that have negative effects on glucose metabolism. A pathologic cycle of insulin resistance results from a reduction in the total amount of energy stored by adipocytes and a resistance to additional energy storage on the part of the remaining adipocytes (Noor, 2007).

According to a study by Ergun-Longmire et al. (2006), whether or not HIV-infected young individuals are taking PIs has no meaningful impact on their insulin resistance, whether they have lipodystrophy or not. According to in-vitro testing, peptides with an aromatic core and hydrophobic ends block glucose transporter-4 and glucose transporter-2 moderately but not transporters-1 and 3 acutely (Zhengtong, 2015). Contradictory studies examining the effects of short-term exposure to protease inhibitors on glucose transport have suggested that protease inhibitors may result in decreased glucose transport in adipocytes and other cell types, but the increased basal lipolysis induced by protease inhibitors does not appear to be connected to these changes in GLUT4-mediated glucose uptake. According to research, catecholamine stimulation has been shown to promote lipolysis in adipocytes by activating the MAPK pathway through the _3-receptor and subsequently phosphorylating ERK1/2. This increased lipolysis may represent a sizable portion of the total lipolysis brought on by iso stimulation (Adler-wales et al., 2005).

According to earlier reports, there hasn't been any evidence of islet cell autoimmunity or beta cell destruction in HIV patients; however, more recent reports suggest that it might manifest in some HIV-infected patients after immune function is restored during HAART because the restoration of immune function raises the risk of autoimmune disease. Cellular retinoic acid-binding protein

type 1 (CRABP 1) and the peroxisome proliferator-activated receptor (PPAR) g interact, but PIs prevent this interaction. Inhibition of PPAR-g promotes insulin resistance, free fatty acid release, and adipocyte inflammation. Almost of patients see their hyperglycemia resolve after PIs are discontinued (Kalra et al., 2011).

By lowering the expression of the genes for IRS1, phosphoinositide 3-kinase and GLUT4, high amounts of cytokines block adipocyte insulin signaling. In PLWH with peripheral lipoatrophy using a PI-based ART regimen, there was increased apoptosis, fibrosis, and macrophage infiltration, decreased expression of the genes encoding adiponectin and leptin, and higher levels of IL-6 and TNF. There was also a marked decrease in the gene and protein expression of pro-adipogenic transcription factors, such as PPAR, C/EBP, and SREBP1, as Defective adipogenesis may also be the cause of the reduced expression of adiponectin and leptin, indicators of mature adipocytes. Additionally, it has been shown that lipoatrophic SAT from PLWH displays persistent low-grade inflammation, which is indicated by increased gene and protein expression of proinflammatory markers (such as IL-6 and TNF). These proinflammatory signals, which are connected to increased fibrosis, TGF expression, and adipocyte mortality, are released by invading immune cells and stressed adipocytes (Koethe et al., 2020).

NCDs, in particular from HIV infection itself, from ART's detrimental metabolic side effects, and from the risk associated with aging. Additionally, socioeconomic status, ethnicity, and gender have been connected to the risk of getting NCDs. The links between ART exposure and NCDs are still not entirely proven since some research have shown a link between exposure and the diseases, while others have not (Rajagopaul and Naidoo, 2021). Patients with HIV frequently experience insulin resistance, which causes dyslipidemia and impairs glucose metabolism. Chronic inflammation is the outcome of immune system activation, which may be more pronounced in patients who are receiving treatment or may lessen in people who are not receiving treatment. Proinflammatory cytokines are reduced by ARV treatment, but insulin status persists because of virus production, deterioration of lymphoid structure, and loss of SREBP-1, a protein that controls the expression of genes involved in lipid synthesis. In addition, glucose transporter 4 (GLUT4) can be decreased by inducing endoplasmic reticulum stress. Insulin resistance may result from a decrease in GLUT4. The nuclear sterol regulatory element-binding proteins (nSREBP) are suppressed from breaking down in adipose tissue by PIs. Inhibiting

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nSREBP breakdown causes the liver to produce more fatty acids and cholesterol, which causes lipodystrophy and insulin resistance in adipose tissue (Marin et al., 2021).

Antiretroviral-induced lipodystrophy in HIV-1-infected patients is related with altered peripheral adipocyte differentiation status and significantly decreased SREBP1c expression. Since protease inhibitors quickly target the differentiation factor SREBP1 in vitro, it has been hypothesized that SREBP1c may play a key role in mediating peripheral lipodystrophy, which results in metabolic changes including insulin resistance (Bastard et al., 2002).

Due to its influence on the metabolic processes involved in converting excess glucose to triglycerides, increased fasting blood sugar can considerably result in elevated serum levels of triglycerides. Increased TNF alpha and IL6 expression may be the main factors reducing these changes in adipocyte differentiation and adipocytosis in adipose tissue from patients with HIV-related lipodystrophy. The observed whole-body insulin resistance may be caused by decreased adipocyte differentiation, which may then lead to decreased adiponectin and leptin expression (Cerrera et al., 2004).

FFA is triggered by hyperinsulinemia and hyperglycemia and is mediated by the proteins sterol regulatory element binding protein 1c (SREBP-1c) and carbohydrate response element binding protein (ChREBP). In contrast to the largely inert or even protective nature of hepatic triglycerides contained in lipid droplets, FFA may contribute to insulin resistance by promoting lysosomal instability, cathepsin B leakage, and stimulation of the NF-B TNF. Through the activation of protein kinase C, diacylglycerol encourages insulin resistance (Gulab and Neeraj, 2018).

2.5 Effect of HAART on Lipid Metabolism

Inflammation, dysregulation of lipid metabolism, and HIV PIs all contribute significantly to cardiovascular problems, according to recent studies. The homeostatic stress response that PIs cause in cells has negative consequences on intermediate metabolism. However, as not all PIs cause dyslipidemia in HIV-negative or -positive patients, the impact of PIs on serum lipid levels appears to be independent of their HIV-inhibitory actions. Under euglycemic, hyperinsulinemic

clamp conditions, rats treated with ATV (atazanavir) did not exhibit any inhibition in comparison to controls at therapeutic concentrations (Flint et al., 2009).

One of the primary risk factors for cardiovascular disease is dyslipidemia, which also dramatically speeds up the development of atherosclerosis, the pathology that underlies CVD (Sign and Kumar, 2012; Subrahadeb et al., 2018). Increased risk for heart disease and atherosclerosis may be characterized by changes in antioxidant enzymes, such as the family of paraoxonases (PONs), which may partially explain some of the mechanisms underlying HAART-associated dyslipidemia. The pathogenesis of atherosclerosis is thought to begin with oxidative alterations to LDL, which are linked to oxidative stress mechanisms brought on by substances including superoxide, nitric oxide, and hydrogen peroxide that convert LDL into oxLDL. OxLDL builds up in the arterial intimal layer, causing the vascular endothelium to become cytotoxic. This is followed by inflammation and the transformation of monocytes into macrophages, which phagocytose oxLDL particles to form foam cells that build up in the intima and cause atheromatous plaques to form (World Journal of Virology, 2015). The HIV infection itself, the negative metabolic side-effects of ART, and the risk associated with advancing age all put PLWHIV at risk for developing NCDs. The risk of developing NCDs has also been linked to gender, ethnicity, and socioeconomic level. As some studies have shown a relationship between exposure and NCDs, while others have not, the relationships between ART exposure and NCDs are still not fully established (Rajagopaul and Naidoo, 2021).

According to studies, HIV-associated lipodystrophy syndrome typically manifests as an increase in visceral fat linked to insulin resistance and dyslipidemia along with a relative absence of peripheral adipose tissue storage (Anderson, 2016). Apoptosis, endoplasmatic reticulum stress activation following PI therapy, and a significant rise in intracellular oxidative stress in cardiac myocytes and hepatocytes were all observed in several investigations (Tricarico et al., 2016). According to several writers, oxidative stress is a major factor in the negative effects of PIs. In both in vitro and in vivo animal investigations, the activation of ER stress, oxidative stress, and an increase in the production of inflammatory cytokines by a variety of cell types, such as macrophages, hepatocytes, intestinal epithelial cells, and adipocytes, have all been connected to HIV PIs. LDLs are cholesterol-rich particles that macrophages, especially in the liver or adipose tissue, pathologically internalize through apoB-100. The dyslipidemia brought on by HAART

seems to have an influence on several aspects of this pathway. Despite the fact that ongoing therapy impacts hepatic glucose production, few clinical studies have shown that HIV PIs really work to reduce peripheral glucose absorption initially (Zha et al., 2014).

Observations show that non-transmissible diseases associated with metabolic disorders, such as dyslipidemia, which causes atherosclerosis and is a known risk factor for cardiovascular events alone or in combination with the metabolic syndrome, are becoming more common in PLWHIV (Moukaila et al., 2019). Previous studies have shown that PIs alter the sterol and fatty acid metabolism in primary rat hepatocytes by increasing the levels of activated sterol regulatory element-binding proteins (SREBPs) and decreasing the expression of the mRNA for the enzyme cholesterol 7-hydroxylase (CYP7A1). Numerous studies have also shown that dyslipidemia may potentially be influenced by the disruption of proteasome activity caused by HIV PIs. Similar to other HIV PIs, atazanavir may also cause lipodystrophy, insulin resistance, and hyperglycemia, which are individual risk factors for cardiovascular disease. HIV PI-induced endoplasmic reticulum (ER) stress may activate the unfolded protein response (UPR), which may be a crucial cell signaling pathway for HIV PI-induced metabolic problems. They promote the growth of foam cells in macrophages, trigger apoptosis, activate the unfolded protein response (UPR), deplete the calcium reserves in the ER, and increase the accumulation of free cholesterol (Zou et al., 2006).

According to a different study looking at IMT in HIV patients taking antiretroviral medications, a PI-containing regimen increases endothelial injury through the beginning of intimal proliferation of smooth muscle cells, lipid accumulation, and monocyte infiltration, which results in the development of an early atherosclerotic lesion. Patients with underlying vascular disease may also develop lesions more quickly as a result of ongoing antiviral exposure. According to Jiang et al. (2010), hyperlipidemia is associated with endothelial dysfunction/activation, direct endothelial injury, and antiretroviral-mediated vasomotor functional impairment. In the general population, it has been shown that central obesity, low HDL levels, various forms of dyslipidemia, modifications in glucose metabolism, and low HDL levels are all important independent risk factors for cardiovascular morbidity and death. The elevation in triglycerides caused by PI usage is alarming since there is mounting evidence that they represent a separate risk factor for cardiovascular disease. An estimated 20% rise in lipoproteins and triglycerides

was seen in HIV-infected individuals one year after beginning PI-based antiretroviral therapy under the circumstances of standard treatment (Levy et al., 2005)

One of the main processes in atherogenesis is the production of lipid-rich macrophages, which is thought to be partly caused by the uncontrolled uptake of modified lipoproteins. Although the precise involvement of CD36 in the development of atherosclerotic lesions is unknown, a human CD36 gene natural polymorphism suggests that CD36 is probably a crucial receptor for the in vivo metabolism of lipoprotein. In comparison to control individuals, monocytes and macrophages from patients with a CD36 polymorphism bound and accumulated less oxidized LDL and sterol. (Dressman et al., 2003).

The host acute-phase response to infection and inflammation is mediated by cytokines, particularly TNF, interleukin-1, and interleukin-6. It is now well accepted that changes in lipid metabolism are also mediated by these cytokines. Through their impact on lipid and glucose metabolism, chronic infections may actively contribute to the atherosclerotic process by producing cytokines continuously at low levels, resulting in an atherogenic lipid profile, raising triglyceride and total cholesterol levels, and lowering HDL concentrations. In the adipose tissue, immune cells are functionally active and create a variety of cytokines and other regulatory substances that affect the balance of lipids, the control of steroid hormones, prostaglandin, and fat-soluble vitamins. These variables also regulate the storage of extra lipids and triglycerides (either healthy or unhealthy fatty acids) found in the blood. Adipocytes are severely impacted by many infectious agents, including HIV-1, which causes them to become dysfunctional and unable to store the majority of lipids (Melzi et al., 2010).

There is proof that ART is linked to dyslipidemia, which is defined by low levels of high-density lipoprotein (HDL-c) and high levels of low density lipoprotein (LDL-c), very low density lipoprotein (VLDL), apolipoprotein B (apoB), and elevated serum concentrations of total cholesterol, triglycerides, LDL-c, and VLDL. Within three months of starting ART, these lipid alterations start to occur and plateau six to nine months later. 70% to 80% of patients using protease inhibitor-containing ART had elevated plasma lipid concentrations. Long-term protease inhibitor users usually have hypertriglycemia, increased LDL-c levels, decreased HDL-c levels,

and an accumulation of apolipoprotein E and apolipoprotein CIII (a NpoCIII). However, an increase in serum HDL-c has been linked to a decrease in HIV-1 viral load (Souzaa et al., 2013).

A few of the numerous variables influencing metabolic alterations in HIV-infected patients taking ARV therapy include HIV infection, direct and indirect effects of ARVs on substrate metabolism, genetics, cytokines, diet, physical inactivity, behavior, gender, and age. The hallmarks of dyslipidaemia associated with combined ART therapy include elevated blood total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), and triglyceride levels as well as a decrease in high-density lipoprotein cholesterol (HDL-c). The risk of cardiovascular illness in persons with HIV/AIDS may also increase due to lipid abnormalities connected to ART medication, according to certain research (Fiseha et al., 2021).

A decrease in the degradation of very low-density lipoprotein (VLDL) and an increase in VLDL synthesis brought on by PIs have both been found to cause lipid changes. Reduced hydrolysis of TG-rich lipoprotein, reduced catabolism of free fatty acid (FFA), and decreased FFA trapping are additional processes that may contribute to dyslipidemia. Through expression of essential enzymes involved in the manufacture of TG, protease inhibitors may also boost hepatic TG synthesis. FFA is hydrolyzed from TG by lipoprotein lipase, which encourages its buildup in adipocytes. Cellular retinoic acid-binding protein type 1 interacts with the peroxisome proliferator-activated receptor (PPAR) g, and LPV/r and ATV/r block this interactionInsulin resistance, free fatty acid release, and adipocyte inflammation are all facilitated by PPAR-g inhibition. When PIs are stopped, hyperglycemia goes away in nearly all patients (Muya and Kamuhabwa 2019).

Inhibiting the breakdown of the nuclear versions of the proteins that bind sterol regulatory elements in the liver and adipose tissue is one of the main effects of protease inhibitors. Increased fatty acid and cholesterol biosynthesis is caused by nSREBP accumulation in the liver, but lipodystrophy, reduced leptin expression, and insulin resistance are caused by nSREBP accumulation in adipose tissue. They also prevent the proteasome from breaking down nascent apolipoprotein B1, which leads to an excess of tryglyceride-rich lipoproteins being produced and secreted (HUI, 2003).

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An essential cell signaling mechanism of HIV PI-induced metabolic disorders is the activation of the unfolded protein response (UPR) in response to endoplasmic reticulum (ER) stress. The ER is a key location for protein synthesis and folding, calcium storage and signaling, cholesterol and other lipid production, and it is extremely sensitive to changes in calcium homeostasis and environmental disturbances. Numerous pharmacological and physiological triggers have the ability to stress the ER, alter its homeostasis, and ultimately cause a buildup of unfolded or misfolded proteins in the ER lumen. To prevent its protein-folding capacity from being exceeded, the ER has evolved extremely specialised signaling pathways known as the UPR. HIV PIs alter regular lipid metabolism through a number of ways, including UPR activation. (Zhou et al., 2006).

According to a study by Zha et al, the changed body morphology, insulin resistance, dyslipidemia, and inflammatory state can all be caused by disruption of the cellular homeostasis of adipocytes. The starving response, which has lately been intimately related to ER stress and the unfolded protein response (UPR) pathways, depends on autophagy, an intracellular protein degradation machinery. In addition to being a crucial regulator of hepatic lipid metabolism, it also controls the storage of adipose lipids and adipocyte development. It has recently been discovered to be a biological target for the dysregulation of lipid metabolism, and by regulating adipocyte differentiation, it governs the accumulation of body fat (Zha et al., 2013).

Although the body needs cholesterol for many different processes, too much of it can be dangerous since it builds up in the blood vessels (Robert, 2013). The main risk factor for the quick development of CAD is cholesterol, which is a primary component of the plaques identified in atherosclerosis (Rajeswari et al., 2014). The pathophysiology of lipoprotein metabolism is determined by their ratio. Individual lipoprotein levels rise and fall as a result of total serum cholesterol levels and vice versa an the ratio between total cholesterol and high-density lipoprotein cholesterol is helpful in interpreting the results of lipid profiles since it detects more dyslipidemia than either of the components of lipid profiles (Mshela et al., 2009).

In human macrophages, HIV affects plasma HDL via affecting the cholesterol-dependent efflux transporter binding cassette protein A1 (ABCA1). This causes an inflammatory response that stimulates endothelial lipase and phospholipase A2 and lowers HDL levels. An increase in

interferon Y levels produced by lymphocytes and macrophages may also be used to describe the inflammatory process. Tryglycedemia and increased IFNy levels are both associated with early infection phases (Cunha et al., 2015).

A 2-3-fold increase in the risk of future myocardial infarction, stroke, and peripheral atherosclerosis has been linked to CRP plasma levels that are slightly above the usual upper limit of normal (1 mg/dL) (Fummilayo et al., 2017). The most significant atherogenic lipoprotein is LDL. More atherogenic than bigger buoyant LDL are smaller, denser LDL. The most harmful lipoprotein is Lp (a), a hereditary variant of LDL-C that has an aberrant protein called apo(a) linked to it (Manjula et al., 2015). According to Bonne et al. (2020), the metabolic syndrome (MS) is a collection of interrelated complicated disorders that include belly fat, hypertension, dyslipidemia, and increased blood sugar (Bune et al., 2020).

Plasma lipoprotein levels are linked to the prevalence of CVS illness. They cause proatherogenic effects via the LDL moiety and prothrombotic effects via plasminogen-like alipoproteins (Jing et al., 2019). It has become clear that different PIs affect metabolism in different ways as a result of analyzing the direct impact on lipid and glucose metabolism.PI has several impacts on the metabolism of lipids. (Grace et al., 2005). HDL levels remain low with protease inhibitor-based therapy, and hypertriglyceridemia may be detected, resulting in a clearly atherogenic lipid profile. Since considerable increases in plasma TG, TC, and LDL-C concentrations have been documented in HIV patients on HAART, fasting plasma lipid profiles should be performed on all HIV-infected individuals before commencing HAART with periodic repetitions after enrolling on HAART. Results from lipid profiles are an excellent indicator of how HIV patients' diseases are progressing and how to manage them HAART produces an increase in TC and low-density lipoprotein (Singha et al., 2014).

2.6 Effects of HAART on Liver Enzymes Levels

The liver is frequently the target of drug-induced harm because it is the organ in charge of the majority of medication metabolism (Otto et al., 2021). Glycosylphosphatidylinositol-anchored ectophosphomonoesterase known as ALP is mostly expressed in the colon, liver, and bone. Adenosine triphosphate, adenosine diphosphate, and uridine diphosphate are just a few examples of the host-derived nucleotides that ALP is capable of hydrolytic phosphatase and

transphosphorylase activity on. According to (Targher and Byrne 2015), ALP is an independent predictor of death, myocardial infarction, and stent thrombosis in patients with CHD. increased baseline After the start of ATV, smaller changes in bilirubin are predicted by ALP and HCV seropositivity. It is reasonable to assume that patients with chronic hepatitis B or C co-infection may be more susceptible to ATV-induced hyperbilirubinemia given that they are more likely to experience drug-induced liver damage and elevated liver enzymes than patients without co-infection (Cotter et al., 2013).

In individuals with underlying liver illness, such as HCV or HBV co-infection, and in patients who have had ART in the past as opposed to those who have not, such liver enzyme elevations are more common (Castro et al., 2016). Major risk factors for severe hepatotoxicity include advanced liver disease, hepatitis co-infection, and increased liver transaminases at the start of therapy (Omonge et al., 2013). Patients on HAART have experienced severe hepatotoxicity in 5–10% of cases. Studies have demonstrated that the fact that many of the drugs used in HAART are known to carry a considerable risk of liver adverse effects makes managing the co-infected patient more challenging. Therefore, it is crucial that liver illness in this patient population is promptly identified, assessed, and treated (Kaspar and Sterling, 2016).

ALP has been linked to numerous cardiovascular diseases, including stroke incidence, coronary artery calcification score, myocardial infarction, stent thrombosis, major adverse cardiac event, and coronary calcification (Cheung et al., 2013). ALP is made up of several alkaline phosphatase isoforms from different tissues, including the liver, bone, and kidney, with a significant portion coming from the latter two. ALP inhibitors may prevent vascular calcification in people with diabetes (Cheung and Cheung 2021). ALP contributes to the development of diabetes by causing vascular calcification, which is connected to insulin resistance. The increased FG levels may partially reflect the aberrant glucose metabolic status as a result of the reduced pancreatic alpha and beta cell function and the resulting impaired insulin secretion. Elevated tHcy and ALP levels might be linked to the same process that leads to the onset of diabetes. Therefore, the negative impacts of greater tHcy levels could mitigate the beneficial association between serum ALP levels and the risk of diabetes (Zhang et al., 2020).

Very few studies have reported the correlation between liver enzymes and lipid parameters in garlic treated diabetes rats in our set up; hence the current study as designed to do so.

2.7 Garlic

Garlic is a member of the Alliaceae family and includes more than one hundred biologically beneficial secondary metabolites, some of which have health benefits like antibacterial, antithrombotic, antioxidant, immunomodulatory, antidiabetic, and drug metabolism modulation. Hepatoprotection, antihelminthics, anti-inflammation, anti-fungal, and wound healing are some other applications of garlic (Divya et al., 2017; Chinedu and Zaaku, 2019). According to their chemical structure, the many chemical compounds known as phytochemicals—which include alkaloids, sulfur-containing phytochemicals, terpenoids, and polyphenols—can be divided into four primary classes that are found in plants naturally (Barbieria et al., 2017). Unique organosulfur chemicals found in garlic are responsible for the majority of its powerful biological action, as well as its distinct flavor and odor. The two water-soluble organosulfur compounds that make up the majority of AGE are S-allylcysteine (SAC) and S-allylmercaptocysteine (SAMC), both of which exhibit strong antioxidant activity (Borek ,2001).

When raw garlic is taken in its crushed form, the allicin that is released breaks down in the stomach's acid to release a number of volatile substances, including diallyl disulfide (DADS) and diallyl sulfide (DAS). The phase II enzyme glutathione catalyzes in vivo methylation to produce the major volatile metabolites, allyl methyl sulfide and allyl methyl disulfide (Rose et al., 2001). More than 82% of the sulfur in garlic is found in the non-volatile -glutamylcysteine peptides and cysteine sulfoxides (such as alliin). Freshly peeled garlic cloves have 62.8 percent moisture, 6.3% protein, 0.1% fat, 1% mineral, 0.8% fiber, 29 percent carbs, and 13.1% vitamin C by weight. Proteins, phosphorus, potassium, calcium, magnesium, and carbohydrates are also abundant in it (Panpatil et al., 2013).

Through their intrinsic metabolic pathways, primary metabolites (innate proteins, lipids, and carbohydrates) are directly engaged in activities such normal growth, development, and reproduction. Contrarily, the phytochemicals found in garlic, also known as its secondary metabolites, are split into four main chemical categories: phenolics, alkaloids, organosulfides, and terpenes. Despite not being involved in the plant's primary metabolic pathways, these

phytochemicals confer a selective advantage to the plant (Batiha et al., 2020). Allicin, alliin, ajoenes, sulfides (diallyl sulfide, diallyl disulfide, and diallyl trisulfide), 2-Vinyl-4H-1,3-dithiin, and allylmethyl sulfide are the principal organosulfides (sulfur-containing compounds) found in garlic (Delgado et al., 2021).

Garlic juice produced by crushing garlic has quite different organosulfur components from those found in intact garlic cloves. With a small amount of S-allyl cysteine (SAC), the intact garlic primarily contains non-volatile g-glutamyl-Salken)yl-L-cysteines, such as g-glutamyl-S-allyl-L-cysteine, g-glutamyl-S-trans-1-propenyl-L-cysteine, and g-glutamyl-S-alk(en When garlic cloves are crushed or chopped, the allinase enzyme that has been hidden in the vacuoles is released. This enzyme then interacts with cytosolic alliin to transform it into a variety of thoisulfinates, the most notable of which is allicin. Dialylsulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), methyl allyl disulfide (MADS), methyl allyl sulfide, ajoene, and vinyl dithiins (2-vinyl-1,3-dithiin, 3-vinyl-1,2-dithiin) are among the numerous sulfides that are produced (Bhatwalkar et al., 2021).

2.8 Effect of Garlic (Allium Sativum) On Blood Glucose and Lipid Metabolism

Numerous studies have found that the organosulfur components, like allicin, glutamylcysteine, and its derivatives, are primarily responsible for these health advantages. In addition to these organosulfur compounds, garlic is high in protein content, dietary fiber, vitamins, ascorbic acid, and polyphenols (Chu, et al. 2013). It is also high in trace elements (zinc, magnesium, copper, selenium, and iodine).   Previous research on garlic photochemicals has typically concentrated on its ability to prevent cancer, but there is little evidence that it has therapeutic potential (Zhang et al., 2020). Garlic acts as an insulin secretagogue in diabetic rats. Furthermore, it guards against the sulphydryl group, which is known to make insulin ineffective. Garlic's allicin may work synergistically with compounds like cysteine to increase blood insulin levels. Garlic is also recognized to lessen the long-term effects of diabetes by decreasing the creation of advanced glycation end products (AGEs), which are recognised contributors to the pathophysiology of aging and diabetic chronic issues (Phil et al., 2011).

The anti-diabetic impact of consuming garlic components in rats implies that consuming S-allylcysteinesulfoxide, S-methylcysteinesulfoxide, and diallyltrisulfide, three potent components

of garlic, may successfully lower blood glucose levels. Garlic can increase the flow of blood sugar to the tissues in the periphery, which boosts insulin sensitivity and improves the conversion of dormant glycogen synthase to its active form, which increases the rate at which blood sugar is converted to glycogen. In addition, garlic may increase the release of bound insulin (Emami et al., 2017).

Garlic has been shown to inhibit lipid synthesis enzymes, reduce platelet aggregation, prevent lipid peroxidation of oxidized erythrocytes and LDL, increase antioxidant status, and inhibit angiotension-converting enzyme by inhibiting human squalene monooxygenase and HMG-CoA reductase, enzymes involved in cholesterol biosynthesis. The more water-soluble sulfur compounds, including diallyl sulfide, have also been shown to be less cytotoxic and more efficient at preventing the formation of cholesterol. S-allylcysteine (SAC), which is present in old garlic extract, is one of these compounds. Garlic has been shown to inhibit the in vitro oxidation of isolated human LDL by scavenging superoxide (ROS) and reducing the production of lipid peroxides (Rahman and Lowe, 2006).

Dipeptidyl peptidase 4 (DPP-4) is a naturally occurring serine protease that is well known to regulate glucose metabolism and is expressed on the surface of cells, according to a research by Kalhotra et al. The therapeutic drug target dipeptidyl peptidase 4, which also serves as a scavenger and has the capacity to promote the proliferation of skeletal muscle cells, is inhibited by garlic extract, making it a prospective anti-diabetic medicine. A novel mechanism of action for the treatment and management of diabetes mellitus is provided by phytochemicals contained in garlic extract, which are most likely the source of DPP-4 inhibition (Kalhotra et al., 2020).

Garlic contains sulfur compounds that are connected to hypoglycemic activity by direct or indirect stimulation of insulin production, improved glucose utilization, and decreased glucose absorption (Maideen and Balasubramaniam, 2018). Only the organosulfur compounds diallylsulfide and allylmethylsulfide are capable of dramatically lowering hepatic CYP2E1 protein levels (Wargovich, 2006). One of the active ingredients in freshly crushed garlic extract, allicin, has several antibacterial and immunomodulatory effects. Due to its ability to change sulfhydryl groups, allicin has an inhibitory effect on sulfhydryl group enzymes within the cell (Dissanayake et al., 2021). Antioxidants, which scavenge free radicals in our systems, are said to

be responsible for these bioactivities (Alide et al., 2020). The present study was therefore carried out evaluate the effect of aqueous garlic extract treatment on fasting blood glucose, lipid profile and alkaline phosphatse hormone concentrationsinatazavir treated hyperglycemic *Rattus norvegicus albinus*

2.9 Effect of garlic on plasma alkaline phosphatase

The liver is the second largest organ and one of the most important organs in the body as it is involved in several metabolic activities of the body (Saleh et al 2020). Increase in alkaline phosphatase can be attributed tp hepatic damage resulting to increased rate of synthesis and release of functional enzymes from the biomembranes (Tsai et al 2019).

Studies have demonstrated that garlic can reduce the levels of alkaline phosphatase through its metabolites allicin and numerous sulfides, such as dialylsulfide, diallyl disulfide, diallyl trisulfide, methyl allyl disulfide, methyl allyl sulfide, ajoene, and vinyl dithiins (2-vinyl-1,3-dithiin, 3-vinyl-1,2-dithiin) that are produced (Ohaeri 2001, Sarghani et al 2022, Airhomwar et al 2023).However, a double blind randomized controlled clinical trial by Ali et al demonstrated no significant difference between the study subjects and the control in the levels of alkaline phosphatase following garlic extract interevntion (Ali et al 2020). The present study therefore evaluated the effect of aqueous garlic extract treatment on fasting blood glucose, lipid profile and alkaline phosphatse hormone concentrationsinatazavir treated hyperglycemic *Rattus norvegicus albinus*

CHAPTER THREE
METHODOLOGY

3.1 Study Area

This study was conducted within zoology department of University of Eldoret, Kenya. This laboratory is equippedand designed for animal studies

3.2 Study Design

This study was a randomized laboratory based experimental study in which thirty-nine (39) albino male rats aged three to four month and weighing 200 to 230 grams which were acquired from University of Nairobi Chiromo campus. The animals were allowed two weeks of acclimatization and getting accustomed to handling. The animals were kept in cages under 12/12 dark/light cycle and fed on rat pellets and allowed access to both water and rats' pellet *ad libitum*. After two weeks of acclimatization the experimental animals were divided randomly into three (3) groups, A, B and C with each group consisting of thirteen animals. Labeling with a permanent marker was done to the animals as A1 to A13, B1 to B13 and C1 to C13. For determination of baseline data of all the study variables before commencement of the treatment about 1ml of tail blood was obtained from each rat in the three groups fortnightly twice. Treatment 1 rats each received a daily treatment of atazanavir at 10mg/Kg only throughout the study period. Treatment 2 rats each received a daily atazanavir 10mg/Kg body weight as in treatment 1 plus garlic extract treatment of 250mg/kg body weight from the third week after initiation of atazanavir treatment. The control rats received 2mls of distilled water daily during the study period. Normal saline was used as the solvent. All treatments were administered through oral gavage route. The study period was 14 weeks.

3.3 Sample Size Determination

Based on blood glucose levels, a sample size estimate was made for this investigation. Fasting blood glucose (FBG) and postprandial blood glucose (PBG) values are 3.95 +/- 1.31 and 5.65 +/- 1.63 mmol/L, respectively (Zhu Wang et al, 2010).

Sample size is estimated using the Israel, 1992, statistical formula based on confidence intervals:

$$n = (Z^2_{1-\alpha/2}S^2)/d^2$$

Where; n=sample size

$Z_1-\alpha/2$= Z-value at α-level of significance where the value of $Z_{1-\alpha/2}$, with α=0.05 is equal to1.96 [from statistical tables]. The assumption here being that glucose concentration in blood is normally distributed in the rat population.

S= is the sample standard deviation of blood glucose levels which from the above figures is estimated at 1.6mmol/L.

d= the error we are willing to tolerate; in this case I chose d=1mmol/L level of precision in blood glucose concentration.

Therefore, n= $(1.96^2 \times 1.6^2)/1^2$ =9.83 = 10 animals per group or treatment.   3 animals were added per group to take care of atrocities making total no of animal 13 per treatment group.

3.4 Sampling Method

A systematic sampling criterion was used during data collection where the total study population was divided by the sample size to get the sampling co-efficient. Selection of study subjects by systematic random sampling involved selection of every 3rd animal until the sample size was achieved.

3.5 Research Instruments

A prelabeled and coded form was used to collect data on laboratory test results. A medical weighing scale was used for weight determination.23-gauge needle. 2 mls syringe was used for phlebotomy. Plain 4mls vacutainer tube was used to collect blood sample for obtaining plasma. Glucometer for measuring fasting blood sugar was On-call Plus TM by ACON Laboratories INC.1025 Mesa RM Road San Diego CA 92121USA,2016 model. Triglycerdies, cholesterol, HDL and LDL was done using Clinical chemistry analyzer (Name- FujiFilm, Model- FujiDry-Chem NX500i). Alkaline phosphatase level was determined using i-CHROMA TM Reader (BODITECH MED INC.) from Germany.

3.6 Materials and Methods

3.6.1 Research Animals

Male Winstar albino rats aged three to four months and weighing 200 to 230 grams were used in this study. The animals were acquired from Chiromo Campus of the University of Nairobi, Kenya and transported to the Department of Physiology and Zoology, university of Eldoret. Prior to the experiment, they acclimated for two weeks to the environment with an ambient temperature of $22\pm2'C$ and a 12-hour light/dark cycle. The rats had unrestricted access to food and water during the acclimatization and testing phases. Using a digital weighing balance, the test animals were weighed once each week. All animals in the study were handled and cared for in accordance with the internationally accepted standard guidelines for use of animals.

3.6.2 Garlic Collection and Preparation of Crude Extracts

The Garlic (Allium sativum) for this study was purchased from the local market. After collection the Garlic Bulbs were peeled and washed from the foreign particles under tap water aseptically and then cut into small pieces with a knife and then kept in the shade for 9 days at room temperature. The semi-dried pieces were then crushed using pestle and mortar and left to dry in the shade at room temperature finally dried plants was gridded into powder form by grinder machine and sieved by fine mesh the obtained powder of each plant was weighed. Active ingredients of garlic were extracted using technical ethanol as the solvent. The dry ground powder of garlic was mixed with ethanol at a volume ratio of 1:10 parts for 72 hours with mechanical shaking twice per day. The extract was filtered through Whatman filter number 1. The solution was evaporated by the evaporator and then concentrated in water bath at 40^0C. The resultant garlic pellets were placed in well labeled tubes and stored in a refrigerator and at 4^0C till was required for use. When required for use the pellets were weighed and reconstituted to give a concentration of 1500mg/ml solution. The garlic extract treatment dose was 250mg/kg body weight for each of the experimental animals and this was delivered by the gavage.

3.6.3 Atazanavir

2ml of atazanavir was drawn into a 2ml syringe and then gavage was mounted on to the syringe. The drug was delivered by sliding the gavage along the roof of the mouth and over the tongue

into the esophagus and the rat returned to the cage and observed for about 15 minutes. The gavage needle was wiped using gauze before using it on the next rats. At the end of the session the needle was cleaned using soapy water, rinsed well using distilled water and air- dried before storing.

3.6.4 Blood Sampling

1.0ml blood sample was taken from each rat's tail vein using a 2 ml syringe and 23-gauge needle into heparin anticoagulant vacutainer tubes. Part of the whole blood samples was used immediately to determine the blood glucose level using a glucometer. The remaining whole blood samples was then immediately centrifuged for 10minutes at 3000 r.p.m. to obtain plasma which was stored in a freezer at -20^0C till when it was required for assaying the variables of the study including Triglycerdies, cholesterol, HDL, LDL and alkaline phosphatase (ALP) levels. Blood sampling was done fortnightly until the end of the 14th week of the study.

3.6.5 Blood Glucose Determination

The blood sample drop of the was applied to a glucometer strip. For blood glucose testing, the hypo guard device was used with ON-CALL PLUS blood glucose test strips. For four hours, blood sugar levels were measured for each experimental group at 0 and 1 hours apart. The principle of blood glucose measurement is that when blood is placed in a strip, a chemical reaction occurs inide it, generating a small electrical current proportional to the glucose concentration

3.6.6 Determination of the Concentrations of Plasma Lipids and Alkaline phosphatase

Triglycerdies, cholesterol, HDL and LDL was done using clinical chemistry analyzer (name-fujifilm, model- fujidry-chem nx500i). Alkalne phosphatase levels were determined using i-chroma[tm] reader (boditech med inc.) from germany. The assay procedure was conducted according to the instructions of the manufacturers. The result of the test was entered into the form and laboratory results print-outs were pinned to each form.

3.7 Data Analysis

Data collected was checked for internal consistency and completeness. The differences between concentration of fasting blood glucose, total triglycerides, high density lipoproteins cholesterol and low-density lipoproteins cholesterol were analyzed using one- way ANOVA parametric test. Test of significance was set at $P < 0.05$.

3.8 Ethical Considerations

This study involved the use of animals. The permission to use the animals as experimental models from the ethical review board on the use of laboratory animals was granted from The University of East Africa, Baraton reference no B0606062022 (Appendix3) The perisson to conduct the study was also sought for and granted by school of graduate studies reference no. 57/351(Appendix 2) and national commission for science technology and innovation reference no 716859 (Appendix 4)

3.9 Study limitations and challenges

i.   Withrawal of blood for analysis was a challenge to the investigator owing to the small size of winstar rats

ii. Limited financial resources was a challenge. For this reason other liver enzymes (AST, ALT, GGT) and insulin were not done .

iii. Handling the small animals during sample collection without harming them was also a challenge.

CHAPTER FOUR

RESULTS

4.1 Mean plasma FBG concentration in atazanavir treated laboratory rats following aqueous garlic extract intervention

Mean plasma fasting blood glucose concentration level in the control group during the pretreatment period was 4.2mmol/l. At two weeks until the end of the study it remained steady at 4.0 mmol/l (Figure 1).
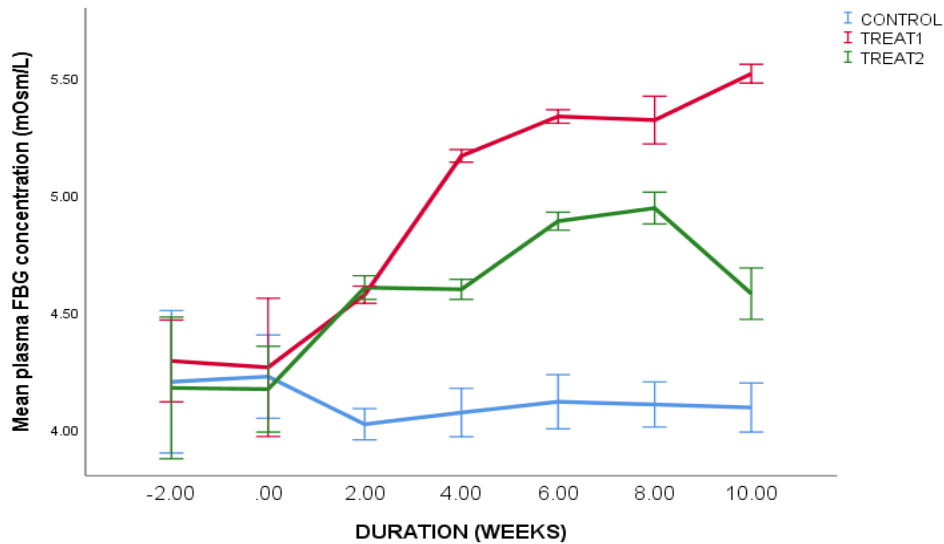

Fig 1. Mean plasma FBG concentration in atazanavir treated male laboratory rats following aqueous gralic extract intervention

Figure 4.1: Mean plasma fasting blood glucose concentration in atazanavir treated laboratory rats following acqueous garlic extract intervention

Treat 1 mean plasma fasting blood glucose concentration level during pretreatment period was 4.3mmol/l , two weeks after treatment with atazanavir 10mg/kg it was 4.5mmol/l in the fourth week it increased to 5mmol/l , in the sixth to eight week was 5.2mmol/l and in the tenth week was 5.5mmol/l. Treatment 2 mean fasting blood glucose concentration level during pretreatment period was 4.2mmol/l. Two weeks after initiation of treatment with atazanavir 10mg/kg was 4.6mmol/l. aqueous garlic extract 250mg /kg was given to treatment 2 on the third week. In the fourth week mean fasting blood glucose concentration level was 4.6mmol/l then at the sixth and eight week was 4.8mmol/l and at the end of the study it dropped to 4.4mmol/l (Figure 4.1).

Whereas the mean plasma fasting blood glucose concentration level of control was not significantly (P value 0.294) different across categories of experiment weeks, the mean plasma fasting blood glucose concentration level was significantly (P value 0.000) different in treatment 1and 2 across the experimental weeks

4.2 Mean plasma TTGLY concentration in atazanavir treated laboratory rats following aqueous garlic extract intervention

Mean plasma total triglyceride concentration in the control group during the pre-treatment period was 52mg/dl. At two weeks it was 47mg/dl on the sixth, eight and tenth week was 50mg/d (Figure 4.2).

Fig 2. Mean plasma TTGLD concentration in atazanavir treated male laboratory rats following aqueous garlic extract intervention

Figure 4.2: Mean plasma total tryglyceride concentration in atazanavir treated laboratory rats following acqueous garlic extract intervention

Treat 1 mean plasma total triglyceride concentration during pretreatment period was 50mg/dl, two weeks after treatment treatment with atazanavir was 55mg/dl on the fourth, sixth and eight

weeks was 65mg/dl then on the tenth week it was 63g/dl (Figure 2). Treat 2 mean plasma total triglyceride level pretreatment was 48g/dl two weeks after initiation of treatment with atazanavir it was 55g/dl at the fourth week it was 62g/dl then at the sixth and eight weeks it dropped to 58g/dl and at the end of the study it dropped further to 56g/dl (Figure 4.2). Whereas themean plasma total triglyceride concentration in control did not show significant (P value 0.148) difference across the experimental weeks, he mean plasma total triglyceride concentration was significantly (P value 0.000) different in treatment group 1and 2 animals across the experimental weeks.

4.3 Mean plasma HDLc concentration in atazanavir treated laboratory rats following aqueous garlic extract intervention

Mean plasma high density lipoprotein –cholesterol concentration in the control group during the pretreatment period was 54mg/dl. At two weeks it was 58mg/dl on the sixth, eight and tenth week was 59mg/dl (figure 4.3).
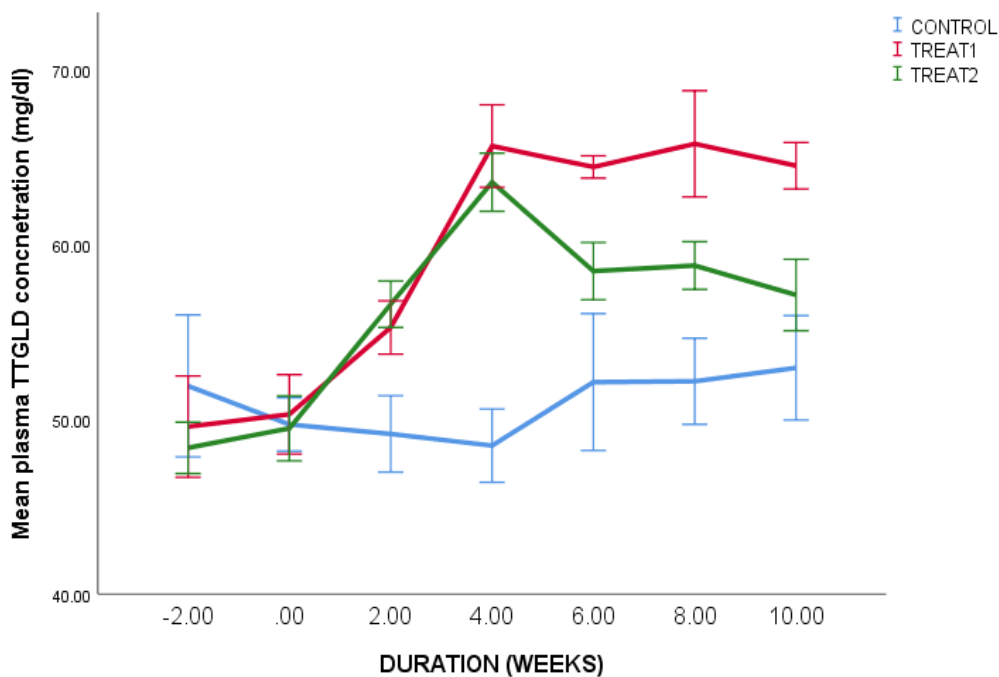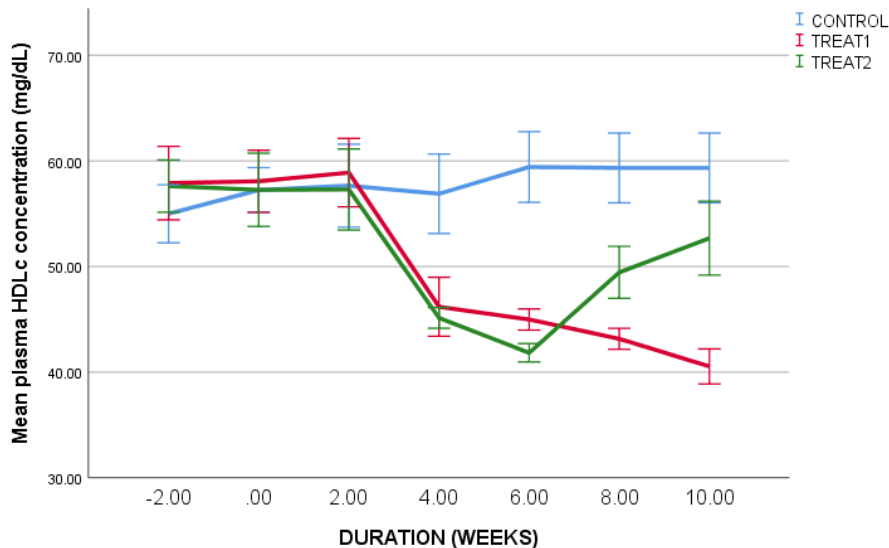

Fig 3. Plasma HDLc concentration in atazanavir treated laboratory rats following aqueous garlic extract intervention

Error Bars: 95% CI

Figure 4.3: Mean plasma high density lipoproteincholesterol concentration in atazanavir treated laboratory rats following acqueous garlic extract intervention

Mean plasma high density lipoprotein –cholesterol concentration in the Treat 1 was 58mg/dl in the pretreatment period, two weeks after treatment with atazanavir was 59mg/dl on the fourth week it was 46mg/dl, on the sixth week it was 45mg/, on the eight week was 42 mg/dl and on the tenth week it wa 40mg/dl (Figure 4.3). Mean plasma high density lipoprotein –cholesterol concentration in the Treat 2 was 58mg/dl in the pretreatment period, two weeks after treatment treatment with atazanavir was 58mg/dl on the fourth week it was 46mg/dl, on the sixth week it was 42mg/dl, on the eight week it rose to 50 mg/dl and on the tenth week it wa 52mg/dl (Figure 4.3). Whereas mean plasma high density cholesterol of control animals did not show significant (P value 0.98) difference across the experimental weeks the mean plasma high density cholesterol concentration was significantly (P value 0.000) different in treatment group 1 and 2 animals across the experimental weeks.

4.4 Mean plasma LDLc concentration in atazanavir treated laboratory rats following aqueous garlic extract intervention

Mean plasma low density lipoprotein –cholesterol concentration in the control group during the pretreatment period was 54mg/dl. At two weeks it was 56mg/dL on the fourth week it was 58md/dl on the sixth week 57g/dl, eight and tenth week was 56mg/dL (figure 4.4).
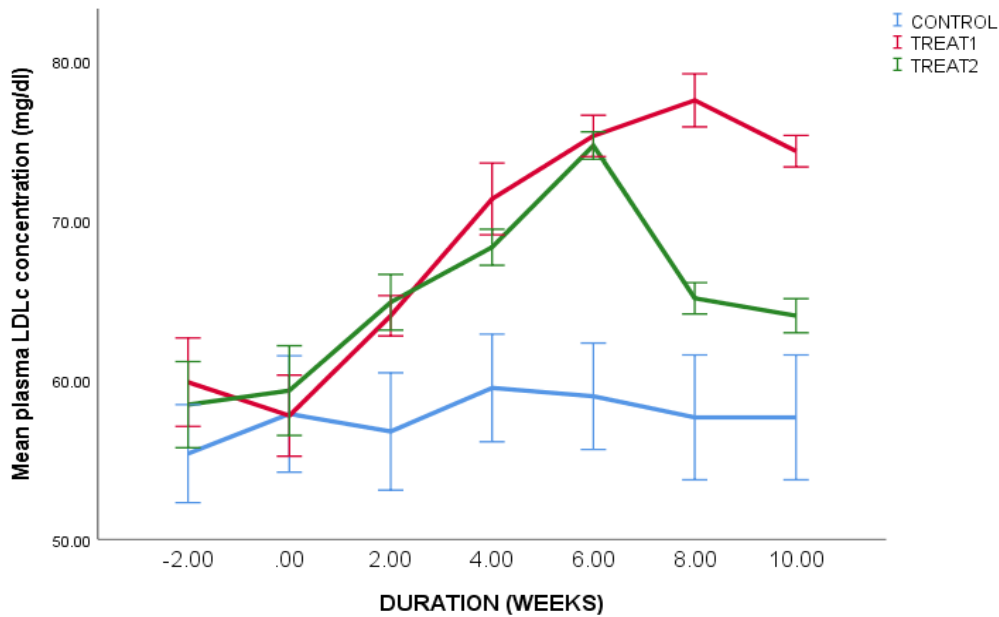
Fig 4. Mean plasma LDLc concentrations in atazanavir treatred male laboratory rats following aqueous garlic extract intervention

Figure 4.4: Mean plasma low density lipoprotein chiesterol concentration in atazanavir treated laboratory rats following acqueous garlic extract intervention

Mean plasma low density lipoprotein –cholesterol concentration   in the Treat 1was 60mg/dl in the pretreatment period, two weeks after treatment treatment with atazanavir was 64mg/dl on the fourth week it was 70mg/dl, on the sixth week it was 74mg/dl, on the eight week was 76 mg/dl and on the tenth week it was 74mg/dl (Figure 4.4).   Mean plasma high density lipoprotein – cholesterol concentration   in the Treat 2 was 58mg/dl in the pretreatment period, two weeks after treatment treatment with atazanavir was 64mg/dl on the fourth week it was 68mg/dl, on the sixth week it was 74mg/dl, on the eight week it rose to 64 mg/dl and on the tenth week it wa 62mg/dl (Figure 4.4).   The mean plasma low density cholesterol of control group was not significantly (P value 0.586) different across the experimental weeks.   On the other hand, the mean plasma of low density cholesterol concentration was significantly (P value 0.000) different in treatment group 1 and 2 animals across the experimental weeks.

4.5 Mean plasma ALP concentration in atazanavir treated laboratory rats following aqueous garlic extract intervention

Mean plasma alkaline phosphatase concentration level in the control group during the pretreatment period was 52u/l. At two weeks was 48u/l on the fourth week it was 48u/l on the sixth week 50u/l, eight and tenth week was 50u/l and tenth week 51u/l (figure 4.5).
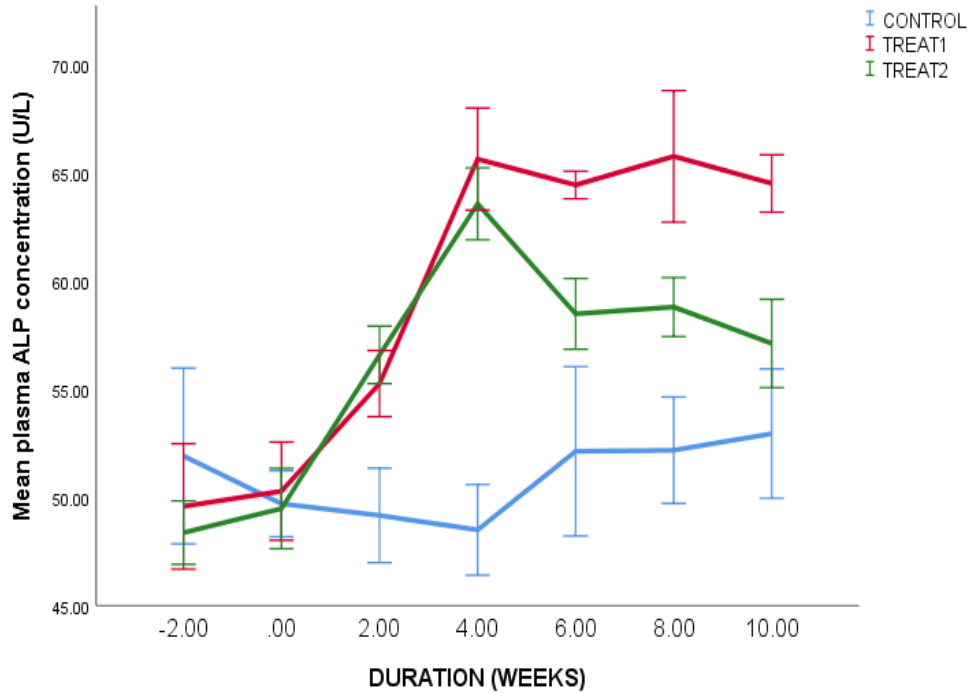
Fig 5. Mean plasma ALP concentration in atazanavir treated male laboratory rats following aqueous garlic extract intervention

Figure 5.5: Mean plasma alkaline phoaphatase concentration in atazanavir treated laboratory rats following acqueous garlic extract intervention

Mean plasma alkaline phosphatase concentration level in the Treat 1 was 50u/l in the pretreatment period, two weeks after treatment treatment with atazanavir was 54u/l on the fourth week it was 65u/l, on the sixth week it was 64u/l, on the eight week 65u/l and on the tenth week it was 64u/l (Figure 5.5). Mean plasma alkaline phosphatase concentration level in the Treat 2 was 48u/l in the pretreatment period, two weeks after treatment with atazanavir was 55u/l on the fourth week it was 62u/l, on the sixth week it was 58u/ll, on the eight week it rose to 58u/l and

on the tenth week it was 56u/l (Figure 4.5).   Whereas the mean alkaline phosphatase concentration was not significantly (P value 0.148) different in the control group, the mean plasma alkaline phosphatase concentration was significantly (P value 0.000) different in treatment group 1and 2 animals across the experimental weeks.

CHAPTER FIVE

DISCUSSION

5.1 Effect of garlic extract treatment on atazanavir-induced hyperglycemia

The findings of the present study show that the protease inhibitor atazanavir raised the mean fasting blood glucose level. These findings suggest that atazanavir treatment disrupted glucose regulation by either interfering with the insulin mechanisms or activation of hyperglycemic hormones actions**.** The endocrine changes that cause elevation of blood glucose may have included the secretion and release of hyperglycemic hormones such as cortisol, Thyroxine, adrenaline, ghrelin and growth hormone since these are usually associated with raised blood sugar levels. These hormones increase blood sugar levels through increased cellular protein synthesis and fatty acids breakdown, stimulation of glycogen release from the liver and gluconeogenesis. It can also be through inhibition of insulin secretion and activity. Hyperglycemic hormones also inhibit glucose uptake by the tissues and cause insulin resistance. On the other hand, hypoglycemic hormones that include insulin, somatostatin, amylin, asprosin, GIP and GLP-1 lower blood sugar levels by enhancing glucose transport into the cells of the body through GLUT-4 transporter molecules, inhibiting gluconeogenesis and mediating glucose storage in the liver and the skeletal muscles in the form of glycogen. In this context hypoglycemic hormones are released in response to hyperglycemic status of the body and therefore the sustained hyperglycemia could have also been due to inhibition of hypoglycemic hormones actions.

The B-islets cells of the pancreas perceive high blood glucose levels as part of normal homeostatic control of blood sugar levels and respond as follows. Through the glucose transporter GLUT2, glucose enters the beta cells and is subsequently transported through the glycolysis and respiratory cycle to make ATP through oxidation. Cell membrane depolarization

results from the closure of the ATP-controlled channels (K+). Ca+ inflow results from the Ca2+ channel opening up after that. The release of previously manufactured insulin from the secretory vesicles is triggered by an increase in Ca2+ in the cell. By means of the calcium responsive element binding protein (CREB), which is necessary for the synthesis, secretion, and release of new insulin molecules, the Ca2+ level also controls the expression of the insulin gene. Insulin synthesis stops when blood glucose levels drop below the typical physiologic values. The release of glucose from cellular reserves into the circulation is now compelled by the islets alpha cells' secretion of glucagon. To transport and convert glucose into glycogen for storage in the liver and skeletal muscle tissues, insulin is required for proper physiology. The insulin-dependent glucose transporter 4 (GLUT4) molecules help to do this (Reinhard et al,.2005). Treatment with atzanavir may have hampered the pancreatic -islets cells' ability to control insulin output. Additionally, there is a strong possibility that atazanavir may have increased the liver gluconeogenic enzyme's activity. The drug possibly also caused activation of the HPA-stress pathway, insulin resistance and secretion of hyperglycemic hormones such as growth hormone, adrenaline and thyroxine ((Mirella et al, 2011; Jenifer et al,2010; Sangiao-Alvardlos et al 2010; Victoria,2010; Amandio et al,2010; Maura et al,2009) in the experimental rats. On the other hand, because the GLUT4 transporter molecules that take glucose into the cells need insulin, the transfer of glucose from the ECF into the cell is insulin dependent. Therefore, the results of this investigation suggest that atazanavir-induced hyperglycemia may have been caused by decreased GLUT4 transporter molecule activity.

According to earlier research, protease inhibitor treatment for HIV causes metabolic syndrome (Lagathu et al., 2019; Barbaro, G.,2006; Hui et al., 2003;. Protease inhibitors, in particular, have been linked to type 2 diabetes mellitus and insulin resistance by reversibly inhibiting insulin

sensitivity and glucose transporters (Mustafa et al., 2006; Koster et al., 2003; Grundy et al., 2005). Atazanavir decreased glucose absorption in vitro, albeit at a much lower level than lopinavir and ritonavir at all concentrations, according to Mustafa et al.'s research (Mustafa et al., 2006). The PI combinations of atazanavir/ritonavir and liponavir/ritonavir have different effects on glucose metabolism. The results of the current investigation support Mustafa et al.'s finding that atazanavir decreased glucose absorption since they show that blood glucose levels rose in response to atazanavir therapy.

Protease inhibitor medication has been linked to anomalies in glucose metabolism in HIV-infected patients, including insulin resistance, hyperglycemia, and the onset of diabetes mellitus (Grinspoon S. 2003; Brown et al., 2005). Atazanavir cause the development of insulin resistance as a direct side effect of treatment. This lends support to the hypothesis that the atazanavir-induced hyperglycemia may have resulted from a disruption of the insulin-dependent glucose transport processes.

Hyperglycemia complicates the management of PLHIV by causing acute and longterm complications including cardiovascular disease that results in increased morbidity and mortality to these individuals who are already burdened with other comorbidities. Therefore, its important to intervene and control any possible metabolic disorders caused by both the HIV infection and protease inhibitors. In this study garlic extract was used to intervene the atazanavir effects on blood sugar levels among the experimental animals and the results demonstrated significant decrease in fasting blood sugar levels. In diabetic rats, garlic has been shown to act as an insulin secretagogue. Moreover, it protects insulin against the sulphydryl group, which is known to render insulin inactive. (Phil et al., 2011). Garlic may also lower blood sugar levels by inhibiting the therapeutic medication target dipeptidyl peptidase 4, a naturally occurring serine protease

that is found on cell surfaces and is well recognized to control glucose metabolism. Additionally, possessing scavenging properties and the ability to promote the development of skeletal muscle cells (Kalhotra et al., 2020). Significant steady and enhanced antioxidant activities can be found in garlic extract. Enhanced insulin production and release from pancreatic beta cells may have had a major role in the quick action of garlic (Wang et al 2017). The garlic extract may also have reversed the GLUT-4 molecules insensitivity to insulin so that the enhanced insulin secretion would achieve its physiologic phenomena. Garlic could have also mediated the suppression of the effects of any hyperglycemic hormones whose secretion was caused by atazanavir, activation of the hypoglycemic hormones and inactivation of hyperglycemic hormones mentioned above. The changes in fasting blood sugar observed in the present study are consistent with observation made by other studies (phil*et al.,* 2011, shoshi et al., 2017, Chhatwal *et al.,* 2012). This study observed that garlic extract has the capacity to reduce the atazanavir-induced increase in blood sugar levels in experimental animals.

5.2 Effect of garlic extract treatment on atazanavir-induced lipid changes

Treatment with atazanavir elevated the mean low density lipoprotein level significantly on the eight-week post-treatment. There was however, a significantly decline in all the experimental rats in response aqueous garlic extract intervention by the end of the study. Garlic extract intervention significantly reduced the mean plasma concentration levels of triglycerides and low density lipoproteins cholesterol. However, it elevated the level of high density lipoprotein cholesterol. The present study demonstrated that atazanavir significantly elevated the mean lipid triglyceride concentration level. On the other hand, atazanavir treatment reduced high density lipoprotein cholesterol level from 58mg/dl at the start of the study to 40mg/dl at the end of the study. It was observed that garlic extract intervention reduced high density lipoprotein

cholesterol level from 58mgdl to 42 at the 6th week however there was an elevation on the 8th week to 50g/dl to 52g/dl.

High levels of TG, LDL-C, small density lipoprotein particles, and low concentrations of HDL cholesterol, which are associated with CVD risk factors, are referred to as dyslipidemia. The antilipidemic action of garlic has been attributed to a number of different mechanisms. Garlic decreases the aggregation and secretions of chylomicrones from the intestines into the blood circulation as well as the expression of the intestinal microsomal tryglycerides transfer protein gene.By raising adipopectin levels, which then trigger fatty acid oxidation in the liver and muscle, garlic has a lipid-lowering effect.Adipopectin also favorably correlates with HDL-C and LDL-C sizes and negatively correlates with plasma total tryglycerides. Garlic regulates lipogenesis and aids in the relief of hepatic stasis by reducing the activity of enzymes involved in the production of hepatic fat, the NFkB pathway, and the gene expression of oxidative stress markers.Garlic has been shown to inhibit lipid synthesis enzymes, reduce platelet aggregation, prevent lipid peroxidation of oxidized erythrocytes and LDL, increase antioxidant status, and inhibit angiotension-converting enzyme by inhibiting human squalene monooxygenase and HMG-CoA reductase, enzymes involved in cholesterol biosynthesis. The more water-soluble sulfur compounds, including diallyl sulfide, have also been shown to be less cytotoxic and more efficient at preventing the formation of cholesterol. S-allylcysteine (SAC), which is present in old garlic extract, is one of these compounds. Garlic has been shown to reduce the in vitro oxidation of isolated human LDL by scavenging superoxide (ROS) and decreasing the production of lipid peroxides. The human enzymes required for cholesterol synthesis, such as squalene monooxygenase and HMG-COA (3-hydroxy- methylglutaryl-co enzyme A) reductase, may be inhibited by garlic and its various constituents. Garlic may, however, reduce LDL-c

levels by preventing the production of hepatic acid and lowering the activity of several enzymes, including hepatic cholesterol 7alpha hydrolase, HMG COA reductase, pentose phosphate pathway, bile acid excretion, microsomal tryglyceride activity, and hepatic acid synthase. Aslani et al. (2016), Sun et al. (2018), Poureza et al 2022) found that garlic substantially reduced tryglycerides, low density lipoprotein cholesterol, and total cholesterol.

According to previous studies (Yamakawa et al., 2014), dietary supplementation with garlic extract decreases the advancement of coronary calcification in high risk individuals with coronary artery disease and calcified plaques.

5.3 Effect of garlic extract treatment on ALP

Proteins called liver enzymes speed up bodily chemical processes connected to the liver's activities. These proteins are made by the liver cells, and a blood test may be used to determine their levels in the blood. Alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP), which is found in many tissues but is most prevalent in the liver, biliary system, and bone, are some of the major liver enzymes. To assess how effectively the liver is functioning and to check for any liver inflammation or injury, the levels of these enzymes are examined.

Atazanavir significantly elevated the level of alkaline phosphatase from 50u/l to 65u/l on the eight week compared to the control. In this study it was revealed that garlic extract intervention significantly reduced the mean plasma alkaline phosphatase level ie 62 u/l at the sixth week to 56ul at the end of the study. The asymptotic significance of the control was 0.148 compared to the treatment group 1 and 2 was 0.000. These findings show that atazanavir treatment caused increase in the concentrations of plasma ALP in the male laboratory rats and suggest that the atazanavir treatment may have caused injury and inflammation to the experimental rats' liver.

Elevated levels of all the three enzymes are indicative of liver injury, hepatic steatosis oxidative stressand inflammation.

Certain chemicals, including liver enzymes, have been shown to leak higher than usual amounts into the bloodstream from inflamed or damaged liver cells. Increased liver enzyme levels can result in cirrhosis, fluid retention, severe jaundice, or even liver failure. They can also cause fibrosis, which is a type of liver scarring (Pratt DS and Kaplan MM., 2000;Kim HC, Nam CM, Jee SH, et al, 2004;Tae Hoon Lee et al., 2012). The findings of the present study are in agreement with the previous findings regarding the effects of HAART on the liver enzymes. The use of antiretroviral drugs has been associated with systemic toxicity following its commencement (Llewellyn et al., 2016; Price JC and Thio CL., 2010; Segamwenge IL. and Bernard MK., 2018) or a change (Shiferaw et al., 2016; Tesfa et al., 2019; Sulkowski et al., 2004) HAART.

Because it is the organ in charge of the bulk of drug metabolism, the liver is a common location of drug-induced damage. This group includes antiretroviral drugs (Qinet al., 2019; Nez, M.2006; Overton et al., 2020; Fida et al., 2020). Non-nucleoside reverse transcriptase inhibitors have traditionally been associated with toxicity and liver damage, according to papers that are currently accessible (Dieterich et al., 2004). Although a number of mechanisms, such as direct cholestatic injury, hypersensitivity reaction, or mediation of immune reconstitution syndrome, have been proposed as the causes of hepatotoxicity linked to the use of NNRTIs, hypersensitivity seems to be the most frequently reported cause among NNRTIs in the literature (Rodrguez-Rosado, et al., 2001; Neff et al., 2006; Rivero et al., 2007). Protease inhibitors (PIs) are a crucial part of HIV therapy, particularly for those who have previously used the medication. Three frequently used PIs—atazanavir, darunavir, and lopinavir—are coupled with cobicistat or low-

dose ritonavir as pharmacologic enhancers (Anyanwu et al., 2020). The pharmacological class known as PIs is associated with adverse effects such dyslipidemia, hepatotoxicity, and lipodystrophy (Mayo Clinic2015). PIs come with warnings concerning raised ALT/AST values and acute hepatitis that may result in hepatic failure and mortality when combined with viral hepatitis or pre-existing liver disease. The increase in HAART-induced hepatotoxicity raises serious questions about the management and treatment of HIV/AIDS patients. Because of this, the present investigation examined the possible effects of a garlic extract intervention.

That liver injury and inflammation have been reversed through garlic extract intervention is a report by several studies By lowering hepatic steatosis, oxidative stress, and inflammation as well as restoring equilibrium to the liver's imbalanced metabolism, garlic supplements may have an effect. Oxidative stress, insulin resistance, and body composition indexes are all improved by garlic therapy. The NF-E2 related factor/heme oxygenase pathway may be activated by garlic's anti-oxidant capabilities. Additionally, NRF-2 activation increases the expression of genes associated with antioxidative defense while decreasing the expression of genes associated with lipogenesis. Garlic supplements may lessen dyslipidemia, lower intestinal tryglyceride absorption, and prevent the activation of lipogenic and hepatic adipogenic enzymes. The impacts of sterol SREP's and PPR alpha dependent pathways have been specifically identified for modifying lipogenesis by regulating the activity of lipogenic enzymes (Yu et al.2022). Other studies have shown that hepatic antioxidant function and lipid peroxidation markers, which are indicators of oxidative stress, may be improved by garlic compounds. (Zaidi 2020, Panjeshahin et al 2020, Ratsker et al 2022) It can prevent the liver from accumulating free fatty acids and going through oxidative stress. Results in this are in line with other studies that have shown that garlic significantly decreases hepatic triglyceride and

cholesterol, ALT AST and liver weight (Sarghani et al 2022). Extract of fermented black garlic demonstrated a hepatic protective effect by reduction in levels of alanine aminotransferase, alanine transaminase and alkaline phosphatase and hepatic malondialdehyde (Tsai et al 2019). Garlic therefore can be used as a supplement that can intervene the effects of atazanavir and other protease inhibitors.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

In conclusion the study found out that:

   i.   Garlic extract treatment lowered the atazanavir-induced hyperglycemia

   ii.   Garlic extract treatment lowered atazanavir-induced high levels of LDLc and TTG and elevated the atazanavir-induced low levels of HDLc

   iii.   Garlic extract treatment lowered the atazanavir-induced high levels of ALP

6.2 Recommendations

In view of the results of these study

   i.   Recommends that garlic extract to be used in clinical trial

   ii.   This study also recommends further studies to investigate dose dependent outcomes and more tests on pancreatic and liver injury (AST, ALT and insulin).

   iii.   We recommend larger animal models to be used for further investigations as they are phylogenically related closer to humans.

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APPENDICES
APPENDIX 1: MATERIALS AND REAGENTS

Thirty nine male Winstar rats with average weights of 200 to 230g.

Microfine syringe -100

Whatmans no 1 filter

Distilled water 400mls

Buckner funnel

2cc syringes – 200 pieces

Pestle and mortar

Glucometer machine

Glucometer strips -1000

Measuring cylinder

500ml beakers- 4

Rat housings/cages- 50

Room thermometer

Rat drinking water containers -50

Enough commercial rats feed or pellets

Rat labels-enough

Enough clean drinking water for the rats

Rat pellets containers-60 containers

APPENDIX 2: SGS PERMISSION TO CONDUCT RESEARCH.

MASENO UNIVERSITY
SCHOOL OF GRADUATE STUDIES

Office of the Dean

Our Ref: MSC/SM/00005/2016   Private Bag, MASENO, KENYA
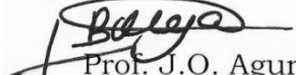57)351 22/351008/351011

FAX: 254-057-351153/351221
Email: sgs@maseno.ac.ke

Date: 10th May, 2022

To WHOM IT MAY CONCERN

RE: PROPOSAL APPROVAL FOR ARISI JEPHTER—MSC/SM/00005/2016

The above named is registered in the programme of Master of Science in Medical Physiology in the School of Medicine, Maseno University. This is to confirm that his research proposal titled "Effect of Garlic Extract Treatment on Lipid Profile, Plasma Glucose and Alkaline Phophatase Levels in Atazanavir Treated Rattus Norvegicus Albinus" has been approved for conduct of research subject to obtaining all other permissions/ clearances that may be required beforehand.

Prof. J.O. Agure
DEAN, SCHOOL

Maseno UniversityISO 9001:2008 Certified

APPENDIX 3: ETHICAL CLEARANCE

Annan,Beverlyne,Bo
sibori,Christine,Con

OFFICE OF THE CHAIRPERSON INSTITUTIONAL SCIENTIFIC ETHICS REVIEW COMMITTEE UNIVERSITY OF EASTERN AFRICA, BARATON P.O. BOX 2500-30100, ELDORET, KENYA, EAST AFRICA

B0606062022   June 6, 2022

TO: ArisiJephter (MSC/SM/0005/016)

    Maseno University

    School of medicine, Department of Medical Physiology Dear
Arisi,

RE: Effect of Garlic Extract Treatment on Lipid Profile, Plasma Glucose and Alkaline
    Phosphatase Levels of Atazanavir Treated Rattus Norvegicus Albinus.
This is to inform you that the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton has reviewed and approved your above research proposal. Your application approval number is UEAB/ISERC/06/06/2022. The approval period from is May 10th, 2022 -May 10th, 2023.

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used.

ii . All changes including (amendments, deviations, and violations) are submitted for review and approval by the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton.

iii. Death and life-threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton within 72 hours of notification.

iv. Any changes, anticipated or otherwise that may increase the risks or affected the safety or welfare of study participants and others, or affect the integrity of the
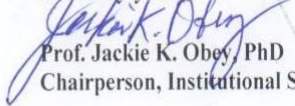
research must be reported to the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton within 72 hours.

v. Clearance for export of biological specimens must be obtained from relevant institutions.

vi. Submission of a request for renewal of approval at least 60 days prior to the expiry of the approval period. Attach a comprehensive progress report to support the renewal.

vii. Submission of an executive summary report within 90 days upon completion of the study to the Institutional Scientific Ethics Review Committee (ISERC) of the University of Eastern Africa Baraton.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology, and Innovation(NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

Sincerely yours,

Prof. Jackie K. Obey, PhD
Chairperson, Institutional Scientific Ethics Review Committee

A SEVENTH-DAY ADVENTIST INSTITUTION OF HIGHER LEARNING
CHARTERED 1991

University of Eastern Africa, Baraton
06 JUN 2022
P. O. Box 2500-30100, ELDORET
Research Ethics Committee

APPENDIX 4: NACOSTI LICENCE.

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **796859**

Date of Issue: **13/July/2022**

RESEARCH LICENSE

This is to Certify that Mr.. JEPHTER ARISI of Maseno University, has been licensed to conduct research in Kisumu on the topic: **EFFECT OF GARLIC EXTRACT TREATMENT ON LIPID PROFILE,PLASMA GLUCOSE AND ALKALINE PHOPHATASE LEVELS OF ATAZANAVIR TREATED RATTUS NORVEGICUS ALBINUS. for the period ending : 13/July/2023.**

License No: **NACOSTI/P/22/18770**

796859

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR
SCIENCE,TECHNOLOGY &
INNOVATION

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