THE EFFECT OF *PSIDIUM GUAVA* LEAF EXTRACT ON BLOOD PRESSURE IN GUINEA PIGS, AND WEIGHT, LIPID PROFILES AND SERUM LIVER ENZYMES IN FRUCTOSE FED SWISS ALBINO MICE

By

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Abstract

Background: Hypertension is an elevation of arterial blood pressure greater than normal. HTN affects 1 billion people globally and accounts for approximately 7.1 million deaths annually. Dyslipidemia is abnormal lipid accumulation in the body due to either defect in lipid metabolism or clearance. It is highly prevalent worldwide including in Ethiopia, where it affects especially urban populations. Hypertension and dyslipidemia are highly correlated and they are significant risk factors for cardiovascular disorders. Some medicinal plants are used in traditional medicine for the treatments hypertension and dyslipidemia. *Psidium guava* (*P. guava*) is one medicinal plant which is widely cultivated and popularly used in traditional medicine treatment of diseases such as diabetes, vomiting, diarrhea and dyslipidemia.

Objective: The study was designed to identify the effect of *P. guava* leaf extract on hypertension in guinea pigs. And also to investigate lipid profiles, liver function tests and weight of mice fed a high fructose diet.

Materials and Method: The study was conducted on twelve guinea pigs to measure the effect of *P. guava* leaf extract by using invasive and organ bath methods. Other experiment was done on 30 fructose fed Swiss albino mice to investigate the effect of *P .guava* leaf extracts on weight, lipid profiles and liver serum enzymes . Fructose fed mice for four weeks were treated with different doses of extracts for additional three weeks while maintaining high fructose diet. Then weight of mice were measured three times during the experiment. And also the end of seventh week, animals were sacrificed and lipid profile (TC, TG, HDL-C and LDL-C) and serum enzymes (AST, ALT and ALP) were analyzed by using chemical analyzer. The result was statically analyzed by using paired t-test independent student t test, one –way ANOVA and mean comparison, Microsoft excel.

Result: The ethanol extract of *P. guava* leaf showed a significant ability to relax isolated aortas of guinea pigs in *ex vivo* experiments, after induction ofaortic muscle contraction by high potassium concentrations. *P. guava* leaf extracts also lowered the systolic and diastolic blood pressure of live guinea pigs in *in vivo* experiments. *P .guava* leaf extracts also significantly reduced the weight , improved lipid profiles and reduces liver serum enzymes in fructose-fed mice.

Conclusion: The effect of *P .guava* leaf extract in reduction of hypertension, improvement of lipid profiles and possible protection against liver damage may be due to numerous benefits of phytochemicals.

Key terms: *Psidium guava*, hypertension, blood pressure, lipid profile, guinea pigs, mice, dyslipidemia, serum enzymes, hepatoprotective, effect.

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Acronyms and abbreviations

ACC1 acetyl-CoA carboxylase 1

ACE angiotensin-converting enzymes

ALP alkaline phosphatase

ALT alanine aminotransferase

AST aspartate aminotransferase

BP blood pressure

CCBs Calcium channel blockers

CHE cholesterol esterase

CHO cholesterol oxidase

ChReBP carbohydrate response element binding protein

CPT-1 carnitine *O*-palmitoyl transferase 1

CRP C reactive protein;

CVDs cardiovascular diseases

DBP diastolic blood pressure

DHAP dihydroxy- acetonphosphate

ET-1 endothelin-1

HDL-C high density lipoprotein cholestreols

HFCS high fructose corn syrup

HTN hypertension

ICAM-1 inter-cellular adhesion molecule-1

LDL-C low density lipoprotein cholesterol

LFTs Liver Function Tests

MABP mean arterial blood pressure

MC3R melanocortin 3 receptor

NCDs non-communicable diseases

NPC1L1 niemann-Pick C1-Like 1

PPRA peroxisome proliferators activated receptor

POD peroxidase

PP pulse pressure

SBP systolic blood pressure

TC total cholesterol

TG triglycerides

T2DM type 2 diabetes mellitus

1 INTRODUCTION

Non-communicable diseases (NCDs), also known as chronic diseases, are not passed from person to person. They have long duration and generally slow progression. The four main types of NCDs are cardiovascular diseases (including heart attacks and stroke), cancers, chronic respiratory diseases (such as chronic obstructive pulmonary disease and asthma) and diabetes (World Health Organization, 2010). They are a severe threat to the health of people as well as global health care systems and economic development, due to long-term costs of treatment and the negative effects on productivity of countries (Khan *et al.,* 2013). Of the 57 million deaths that occurred globally in 2008, 36 million (almost two thirds) were due to NCDs. Cardiovascular diseases (CVD) are the number one cause of death in the world (World Health Organization, 2010). In particular, low and middle-income countries (LMIC) bear a large burden of CVDs, accounting for 80% of the global CVD-related deaths and 87% of CVD-related disability (Hendriks *et al.,* 2012).

Metabolic syndrome (MS) is major cause of CVDs and is associated with the clustering of metabolic and pathophysiological cardiovascular risk factors: impaired glucose tolerance (IGT), dyslipidemia, obesity and hypertension (Otani, 2011). The central (visceral, or ―apple" shape) type of obesity represents the most dangerous kind of obesity, which can lead to a cascade of metabolic abnormalities, including insulin resistance, pre-diabetes, type 2 diabetes mellitus (T2DM), dyslipidemia, elevated arterial blood pressure, atherosclerosis, increased coagulation and inflammation that in consequence cause serious risks for CVD (Rygiel, 2009; Otani, 2011; Bloomgarden, 2007).Figure 1.1 illustrates the relative proportions of global deaths caused by NCDs in 2008.

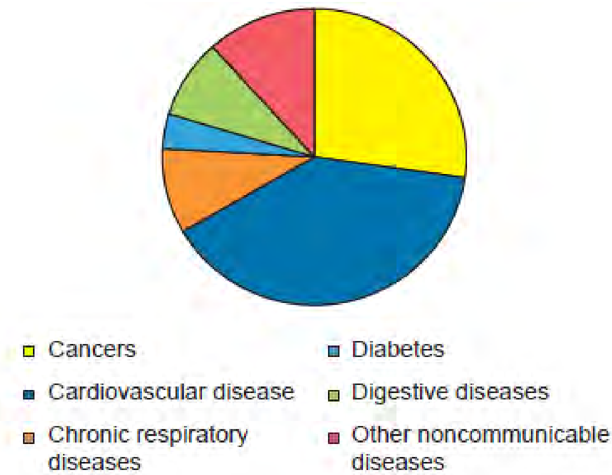


Figure 1.1: Non-communicable disease (NCD) deaths in people under the age of 70 in 2008 (World Health Organization, 2010).

1.1 Hypertension

Hypertension is defined as an elevation of systolic blood pressure (SBP) greater than or equal to 140 mm Hg and/or diastolic blood pressure (DBP) greater than or equal to 90 mm Hg (Matthew *et al.,* 2012). Any patients being treated with anti-hypertensive medications, whatever their measured blood pressure is, are also defined as having hypertension. Hypertension is a major cardiovascular risk factor and its prevalence is increasing throughout the world, including Ethiopia and the rest of sub-Saharan Africa (Khan *et al.,* 2013; Mekoya, 2007, Twagirumukiza *et al.*, 2011). It predisposes a person to myocardial infarction, congestive heart failure, renal failure, stroke, peripheral vascular insufficiency and premature mortality. Risk factors for hypertension include age, obesity, male sex, ethnicity, smoking, diabetes, excess alcohol intake and hypercholesterolemia (Nichols and Eliot, 1996; Lawes *et al.*, 2008; Gaurav *et al.*, 2012; Crews *et al.*, 2010).

1.1.1 Prevalence of hypertension

Hypertension is the one of the most common health problems, affecting approximately one billion people globally, and accounts for approximately 7.1 million deaths annually (Robitaille *et al.,* 2012). It also affects approximately 72 million Americans (Carson*et al.,* 2011). Prevalence of hypertension

increases with age and is more prevalent in some ethnic groups; for example the prevalence of hypertension is higher among African-Americans when compared with white Americans and Hispanics across all age groups (Carson *et al.,* 2011; Ferdinand and Welch, 2007). An increase in its prevalence leads to dramatic rises in the incidence of CVDs and their consequences, which overwhelm health care systems; it also has financial implications for national and local medical treatment plans (Nichols and Eliot, 1996; Lawes *et al.,* 2008; Gaurav *et al.,* 2012). The majority of patients with hypertension require two or more drugs to achieve blood pressure control, according to the report of the African Union Conference of Ministers of Health (CAMH, 2013). Hypertension is also widely prevalent in Ethiopia, especially in Addis Ababa, when compared to other regions of the country; this is may be due to lifestyle differences, including overweight, obesity and physical inactivity, which are important determinants of high blood pressure (Fikru *et al.,* 2009).

1.1.2 **Causes of hypertension**

There are different factors that predispose to hypertension, as illustrated in Figure1.2. There are two major groups of risk factors: these are modifiable and non-modifiable factors (Huma *et al.*, 2012)

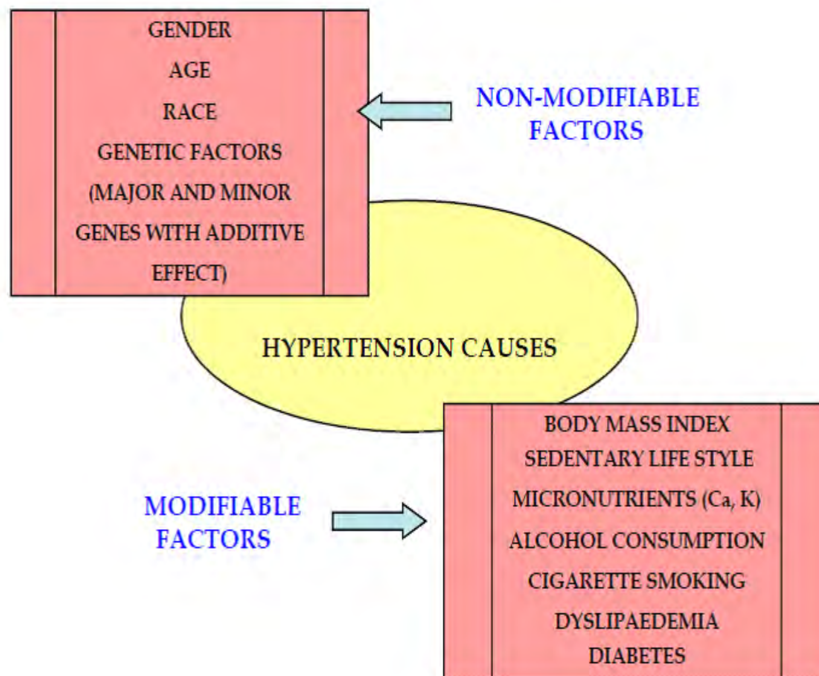


Figure1.2: The major causes of HTN (Huma *et al.*, 2012)

1.1.3 **Types of hypertension**

Hypertension can be classified into primary (essential) hypertension, which accounts for 90-95 % of cases, and secondary hypertension, which accounts for 5-10% of cases. Although no single cause has been identified for primary hypertension, there are many possible causes, such as sedentary lifestyle, diet (high sodium, for example), stress, and increased sympathetic nervous system activity (Landsberg *et al.,* 2013). Genetic predisposition, hypokalemia and others also contribute for primary hypertension (Lakshmi *et al.,* 2011). Hypertension is mostly observed in adults after the age of 40 (Wamala *et al.,* 2009). Secondary hypertension accounts for 5-10 % of hypertension and its causes include chronic kidney disease (including renal artery stenosis), Cushing's syndrome (adrenal cortical hypersecretion of cortisol), phaeochromocytoma (adrenal medulla tumor), drugs such as non-steroidal anti-inflammatory drugs (NSAIDs) and oral contraceptives (Lakshmi *et al.,* 2011).

1.1.4 **Classification of Hypertension**

There are a number of classifications of hypertension based on its severity levels. The Joint National Committee (JNC) on prevention, detection, evaluation, and treatment of high blood pressure (JNC) classification system is shown in Table 1.1 (Martin, 2008).

Table 1.1: Classification of hypertension according to JNC guidelines (Martin, 2008)

SBP/DBP	*JNC7 Category*
<120/80	*Normal*
120-129/80-84	
130-139/85-89	*Pre-hypertension*
≥ 140/90	*Hypertension*
140-159/90-99	*Stage 1*
160-179/100-110	
≥ 180/110	*Stage2*

1.1.5 Biochemistry of hypertension

There are three basic general pathways that, when perturbed, can lead to hypertension, but the mechanisms of primary hypertension are overall very complex and involve overlapping pathways. One pathway involves dysfunction of arterial sub-endothelial cells; another involves increased activity of the renin-angiotensin-aldosterone system; the third pathway involves central nervous system dysfunction, leading to elevated sympathetic activity (Hall *et al*., 2010).

1.1.6 Pathophysiology of hypertension

Blood pressure (BP) is the related to cardiac output and peripheral vascular resistance. Therefore, maintenance of a normal BP is dependent on the balance between the cardiac output and peripheral vascular resistance (Kalish *et al.,* 2003). Cardiac output is determined by stroke volume and heart rate; stroke volume is related to myocardial contractility and to the size of the vascular compartment, whereas peripheral resistance is determined by functional and anatomic changes in small arteries and arterioles (Lakshmi *et al.,* 2011)

The major complications of untreated hypertension include: stroke, myocardial infarction, congestive heart failure, left ventricular hypertrophy and renal failure (Kalish *et al.,* 2003). See Figure 1.3, which shows the pathophysiology of hypertension.

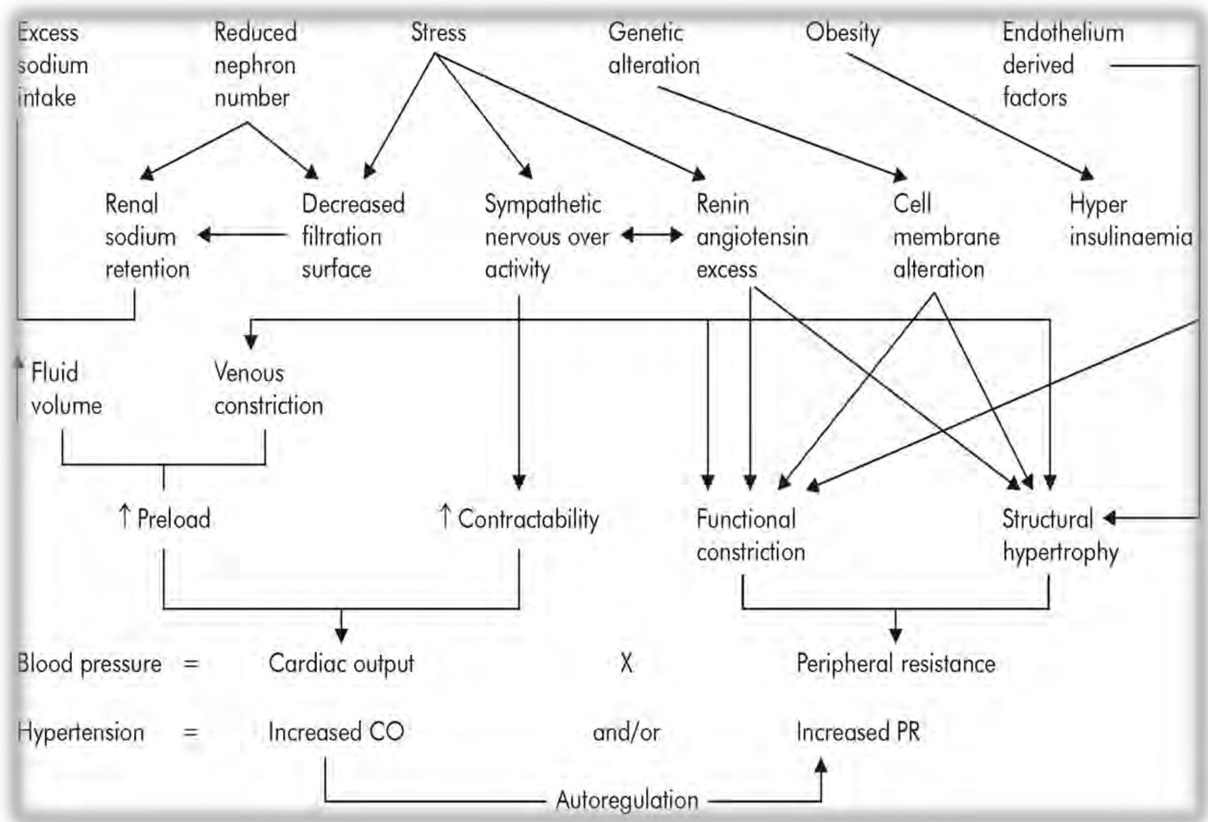


Figure1.3: Pathophysiology of hypertension (Vikrant and Tiwari, 2001).

CO=cardiac output, PR=peripheral resistance, ⟷ the one is cause and effect for other.

1.1.7 Prevention and treatment of hypertension

Because of the global increase in prevalence of hypertension and its complications, there is an urgent need for improved public health and medical strategies and programmes to prevent and treat hypertension, and to promote healthy lifestyles, particularly among urban populations of Ethiopia (Fikru *et al.,* 2009).Lifestyle modification, using appropriate diet (in particular, low sodium diet) and moderate physical activity, is a primary means of prevention and early treatment of hypertension (Sharma *et al.,* 2010).

There are a number of drugs that are used in hypertension treatment:

Diuretics (thiazides, loop diuretics, potassium-sparing diuretics) are the known diuretics that used in treatment of hypertension. Thiazides such as hydrochlorothiazide inhibit Na^+ and Cl^- reabsorption in

distal tubules of the kidney, whereas loop diuretics (e.g. furosemide) inhibitthe $Na^+/K^+/Cl^-$co-transporter in the ascending loop of henle, and potassium sparing diuretics such as amiloride correct potassium loss during the treatment with thiazide and loop diuretics. There a number of side effects of diuretics, including hyperglycemia, hypercholesterolemia, increased urine frequency, gout and hypokalemia (Schrier, 1999).

Angiotensin converting enzyme (ACE) inhibitors such as captopril, lisinopril,rampril and others are another group of antihypertensive drugs, which work by blocking the conversion of angiotensin I to angiotensin II by the angiotensin converting enzyme (ACE). Hyperkalemia, edema, changes to sense of taste and persistent dry cough are potential side effects of ACE inhibitors (Martin, 2008).

Angiotensin receptor blockers (ARBs) such as losartan and irbesartan, treat hypertension by direct blocking of angiotensin II subtype 1 receptor, hence reducing the action of angiotensin II. Hyperkalemia, headache, or cold or flu symptoms, edema and acute renal failure are possible side effects of ARBs (Wallace, 2003).

Calcium channel blockers (CCBs) can be classified in two as dihydropyridines (amlodipine and nifedipine, for example), which produce excellent blood pressure control by directly relaxing the smooth muscles surrounding muscular arteries, and non-dihydropyridines (verapamil and diltiazem, for example), which reduce blood pressure by both inducing vasodilation and by decreasing myocardial contractility(Gaspar and Hajagos-Toth, 2013). Peripheral edema, reflex tachycardia, headaches, dizziness and others side effects are seen in patients used these drugs (Martin, 2008).

Alpha blockers, for example doxazosin and prazosin, are drugs that reduce hypertension by inhibiting the α-receptors of smooth muscles (Vikrant and Tiwari, 2001*)*. They lower blood pressure but are rarely used for treatment of hypertension because several studies suggest that they may have detrimental effects on cardiovascular outcomes.

Beta blockers, for example propranonol, metoprolol, atenolol and pindolol, improve hypertension by blocking beta-receptors in the brainstem and in the peripheral tissues, including the heart, and decrease sympathetic nervous system activity. Main side effects of this group are alteration of the level of lipids and glucose, asthma and bradycardia (Wallace, 2003).

1.2 Dyslipidemia

Fats, oils, waxes, and sterols are collectively known as lipids, which play an important role virtually in all aspects of biological life. They are sources of hormones or hormone precursors, help in digestion, providing energy, storage function and metabolic precursors for other biochemical products; they act as functional and structural compounds in biological membranes, and form insulation to allow nerve conduction or to prevent heat loss (Devaranavadgi *et al.,* 2012).

Lipoproteins are complex aggregates of lipids and proteins that render the lipids compatible with the plasma and other aqueous environments of body fluids and enable their transport throughout the body of all vertebrates and insects to tissues where they are required.

Table 1.2: The classification of lipoproteins and their functions according to the National Cholesterol Education Program National (NCEP, 2014).

Apoprotein	Lipoprotein Classes	Function
A-I	Chylomicrons, HDL	Activates lecithin cholesterol acyltransferase (LCAT) d(LCAT)
A-II	Chylomicrons, HDL	Inhibits LCAT, enhances hepatic lipase activity.
A-IV	Chylomicron	Unknown function
B-100	VLDL, IDL, LDL	Necessary for binding to cell receptors, LPLs.
B-48	Chylomicron	Necessary for binding to cell receptors, LPLs.
C-I	Chylomicron, VLDL, HDL	Cofactor for LCAT
C-II	Chylomicron, VLDL, HDL	Activates LPL
C-III	Chylomicron, VLDL, HDL	Regulates LPL
D	HDL	Essential for LCAT activity and Cholesteryl ester transfer.
E	All	Binds to specific cell receptors.

On average, 60 to 70% of total serum cholesterol in humans consists of low density lipoprotein cholesterol (LDL-C); 20 to 30% of total serum cholesterol is high density lipoprotein (HDL-C), and 10 to 15% is very low density lipoprotein (VLDL-C). Low serum levels of high density lipoprotein cholesterol (HDL-C) and elevated triglycerides (TG), elevated total cholesterol (TC), and elevated LDL-C and elevated very low density lipoproteins (VLDLs) are causes of dyslipidemia according to National Cholesterol Education Program National (NCEP, 2014)

Dyslipidemia is one of the most important risk factors for many chronic non-communicable diseases resulting in serious morbidity; and cause high mortality and medical costs worldwide (Khader *et* al., 2010; Ascaso *et al.*, 2007; Alberta, 2006). Generally, it is defined as the serum TC, LDL-C, TG and apo B levels above the 90th percentile, or HDL-C and apo A levels below the 10th percentile of the general population (Khader *et al.,* 2010). However, what constitutes a normal or abnormal cholesterol profile is determined by person's cardiovascular risk factors. For example, a serum LDL-C above 70 mg/dL is considered abnormal in any person who has known coronary artery disease, whereas an LDL-C level above 100 mg/dL is considered abnormal in any diabetic; in a person with no major cardiovascular risk factors, an LDL of 130 mg/dL or less is considered normal. Dyslipidemia is a common disorder and results from abnormalities in lipid transport or a disorder in the synthesis and degradation of plasma lipoproteins (Nor and Yatim, 2011).Even though fats like cholesterol and triglycerides play important roles in normal body functions, they are risk factor for our health when their levels are abnormal, resulting in increased risk of atherosclerosis, coronary heart disease (CHDs) and stroke (Vuyyuru *et al.,* 2012).

1.2.1 **Prevalence of dyslipidemia**

The prevalence of dyslipidemia can vary based on nationality, ethnicity, genetics, and socio-cultural and economic factors. Dyslipidemia has clearly been shown to increase in individuals between the ages of 40 and 60 worldwide. A 2002 World Health Report indicated that high plasma cholesterol levels are responsible for 56% of CHD and 18% of nonfatal cerebrovascular diseases worldwide (Cetin *et al.,* 2010). Dyslipidemia affects one individual of every two American adults. It is a major risk factor for CVD and CVD-related death (Jellinger *et al.,* 2012). According to one study on risk factors for CVD among diabetic patients in southwest Ethiopia, 63% of them were dyslipidemia (Tamiru and Alemseged, 2010). The introduction of highly active antiretroviral therapy (HAART) has led to a marked reduction in AIDS related morbidity and mortality, but its introduction to patients

may cause co-morbid problems such as dyslipidemia, insulin resistance, and diabetes (Tadewos *et al.,* 2012).

1.2.2 **Causes of dyslipidemia**

There are two main causes, primary and secondary, of dyslipidemia. Primary causes are the most severe forms of dyslipidemia and are caused mainly by genetic disorders of lipoprotein metabolism (Hassan, 2013). Secondary causes are more common and are more complex conditions that usually combine genetic predisposition with environmental cause (Mesquita *et al.,* 2010).

1.2.2.1 Familial hypercholesterolemia (FH)

Familial hypercholesterolemia (FH) is a group of genetic diseases that involve defective clearance of LDL from the circulation and can be due to mutations in genes encoding the LDL receptor or its processing, or ApoB mutations that decrease binding of LDL to its receptor. It is characterized by elevated LDL-cholesterol (LDL-C) level, which deposits in tissues, causing external manifestations of the disease, namely tendinous xanthomas, palpebral xanthelasmas, and corneal arcus. More importantly, LDL-C deposits in blood vessels, leading to premature CVD (Fahed and Nemer, 2011). Each child of a parent with heterozygous FH has a 50/50 chance of inheriting the condition, and it affects boys and girls equally (Jarvik *et al.,* 2008; Soutar *et al.,* 2007).

1.2.2.1.1 **Heterozygous Familial hypercholesterolemia (FH)**

Heterozygous FH is not uncommon, occurring in 1 in every 500 people, and is a single gene defect in which affected individuals inherited one abnormal allele from one parent and a normal allele from the other parent. Different genes can be affected, and are generally involved in LDL receptor function, LDL metabolism or LDL transport. Most forms are autosomal dominant, so a child has a 50% chance of inheriting heterozygous FH from his or her parents. (Jarvik *et al.,* 2008). In heterozygous FH, plasma LDL levels are usually between 250 and 500 mg/dL and the LDL responds well to statins.

Males may have significant coronary artery disease in their 30s and 40s, whereas females lag behind males by about 10 years in their progression to overt coronary artery disease.

1.2.2.1.2 Homozygous Familial hypercholesterolemia (FH)

Homozygous FH, in which affected individuals inherit two abnormal alleles of a gene involved in LDL metabolism, is rare, occurring in 1 in every million people (Fahed and Nemer, 2011). In homozygous FH, plasma LDL levels are extremely high, often between 600 and 1000 mg/dL, or even higher, and patients can have severe coronary artery disease and myocardial infarctions in their teens and early 20s. They do not respond well to statins or other currently used lipid-lowering drugs, though experimental drugs have recently been used, so they are treated periodically with plasmapheresis methods, where their blood is circulated through a device that absorbs out LDL cholesterol from their blood.

Secondary causes of dyslipidemia result from other disorders that indirectly affect the metabolism of lipoproteins such as T2DM, autoimmune diseases, different classes of drugs like thiazides, β-blockers, sex hormones, liver disease, obesity, chronic renal insufficiency (CRI), hypothyroidism , excess alcohol intake and pregnancy (Mesquita *et al.,* 2010).

1.2.3 Hepatic fructose metabolism and adverse effects of fructose diets

1.2.3.1 The Liver

The liver is the largest organ in the body and is located in the upper right quadrant of the abdomen. It is divided into four lobes; these are in turn composed of multiple lobules, which contain the hepatocytes (Castro *et al.,* 2008).

1.2.3.2 Functions of the Liver

The liver is responsible for about 500 body functions that are necessary for survival. Liver breaks nutrients down and builds up body tissue. It has an extensive blood supply: about three-fourth of blood flow through it every minute. It receives oxygen-rich blood from the hepatic artery. The portal vein delivers blood containing nutrients, toxins, and other substances absorbed from the intestines to the liver. The liver processes this blood, and then sends it to the heart via the hepatic vein (Fabbrini *et*

al., 2010).It also plays a role in digestion, sugar and fat metabolism, blood clotting, growth regulation, and the body's immune defense (Castro *et al.,* 2008; Tennent *et al.,* 2007). About 90% of the body's nutrients pass through the liver from the intestines and some of them are stored in the liver (Asahina *et al.,* 2009).Liver also acts to remove harmful substances from the blood (Percival, 1997). It is crucial in producing blood cells in the developing fetus (Fabbrini *et al.,* 2010)

1.2.3.3 Liver Damage

Damage of liver can be due to various causes. These include chronic hepatitis B or C, heavy alcohol use, environmental and industrial toxins, some drugs, autoimmune hepatitis, hemochromatosis, celiac disease, alpha-1-antitrypsin deficiency, non-alcoholic steatohepatitis, and primary biliary cirrhosis. As it is known that the liver performs many vital functions; it is not surprising that liver injury can have an effect on almost all body systems (Kaur and Patil, 2012). Liver damage progresses through chronic inflammation (hepatitis) and this can lead to mild scarring (focal fibrosis), then to extensive fibrosis (cirrhosis), where normal liver tissue is replaced almost entirely by scar tissue. These are commonly due to fatty liver and alcohol intake (Chalasani, 2012). If the liver becomes too heavily damaged, with extensive replacement of healthy tissue by fibrosed tissue, it is no longer able to carry out its normal functions (Bruha *et al.,* 2012). Unless liver detoxifies toxins and metabolic by-products such as ammonia, these chemicals may build up in the blood, leading to impaired brain and mental functioning, encephalopathy, coma and death as well as jaundice, severe edema and internal bleeding (Kauri and Patil, 2012).

The liver may also be damaged by alterations in cellular fatty acid transport that facilitates fat accumulation in liver and skeletal muscle instead of in adipose tissue. The fatty acid translocase, CD36, regulates tissue FFA uptake from plasma. CD36 expression is decreased in adipose tissue, but increased in the liver and skeletal muscle, of insulin-resistant animals and insulin-resistant human subjects who have increased intrahepatic and intra-myocellular TG content. This suggests that alterations in tissue fatty acid transport could be involved in the pathogenesis of ectopic TG accumulation by diversion of plasma fatty acid uptake from adipose tissue toward other tissues such as liver and muscle (Fabbrini *et al.,* 2010). Figure 1.4shows how fat transport may be diverted to other tissues rather than adipose tissue.

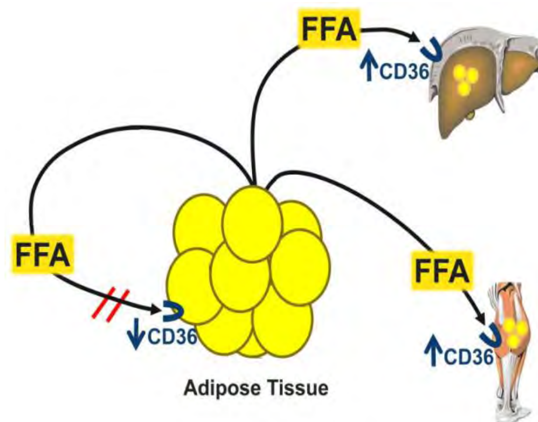


Figure 1.4: Fat transport may be diverted to other tissues rather than adipose tissue

CD36 translocator protein, FFA free fatty acids, // blocking translocation, ↓ inhibition of translocation of fatty, ↑ increased fatty translocation

1.2.4 Fructose and its metabolic syndromes

Fructose is a simple sugar present in fruits and honey that is responsible for their sweet taste. However, the major source of fructose worldwide is sucrose or table sugar, which is derived from sugar cane and sugar beets (Khitan and Kiml, 2013).

Sucrose is a disaccharide comprised of fructose and glucose. After ingestion, sucrose is degraded in the gut by sucrase, releasing fructose and glucose, which are absorbed. In addition to sucrose, the other major source of fructose is high fructose corn syrup (HFCS), which was introduced in the early 1970s as an additional sweetener. HFCS consists of fructose and glucose mixed in a variety of concentrations, but most commonly as 55% fructose and 45% glucose. In the United States, HFCS and sucrose are the major sources of fructose in the diet, and HFCS is a major ingredient in soft drinks, desserts, and various processed foods (Lustig, 2010).

1.2.4.1 Fructose metabolism in the liver

The liver is the principal site of fructose metabolism, as it possesses the fructose-specific Glut5 transporter. Although adipose tissue possesses Glut5 mRNA and protein, the level of the transporter protein itself in adipose tissue is quite low (Miller and Adeli, 2008). The kidney and small intestine also possess Glut5 transporters, but their function is to transport fructose molecules across their

lumen, either for urinary excretion (to eliminate any systemic fructose that escapes hepatic clearance) or for release into the portal circulation, which passes directly to the liver (Lim *et al.,* 2010).

Despite the similarity in their chemical structures, fructose and glucose are metabolized in very different ways; they utilize different transporters; fructose metabolism is insulin-independent; and from an ingested sucrose load, 20% of the glucose and 100% of the fructose is metabolized in liver. Fructose also bypasses the two highly regulated steps of glycolysis, catalyzed by hexokinase and phosphofructokinase. Instead, fructose enters the glycolytic pathway at a level that is not regulated: it is metabolized to fructose-1-phosphate primarily by fructokinase, and has no negative feedback system; ATP is used for the phosphorylation process. As a result, continued fructose metabolism results in intracellular phosphate depletion, activation of AMP deaminase, and uric acid generation, which is harmful at the cellular level (Khitanand and Kiml, 2013).

Many investigators have implicated fructose in the pathogenesis of the metabolic syndrome and nonalcoholic fatty liver disease (NAFLD). Metabolic syndrome is a constellation of dyslipidemia, HTN, impaired glucose tolerance, and central obesity (Lim *et al.,* 2010). Fructose causes obesity and related cardio-metabolic diseases which are rising in prevalence, creating global public health problems. As a result this requires strategies that can be efficiently applied to the population at risk for CVD. The central (visceral, or ―apple" shape) type obesity, associated with production of adipokines by visceral fat, represents the most dangerous form of obesity and can lead to a cascade of metabolic abnormalities, including insulin resistance, prediabetes, T2DM, dyslipidemia, hypertension, atherosclerosis, increased coagulation and inflammation that in consequence cause serious risks for CVDs (Rygiel,2009).

1.2.4.2 Dyslipidemia and muscle insulin resistance

Free fatty acids formed from fructose, packaged as triglycerides into heavily fat-laden VLDLs, and cleared with low efficiency, cause dyslipidemia and other risk of CVDs. An excess accumulation of fats such as cholesterol and TG in the blood from fructose metabolism causes coronary heart disease (CHD) and stroke (Vuyyuru et *al.,* 2012; Dekker *et al*, 2010). Excess circulating lipidis also taken up by skeletal muscle to form intra-myocellular lipid accumulation, which leads to muscle insulin resistance (Miller and Adeli, 2008; Basciano *et al*, 2005).

1.2.4.3 Hypertension

Fructose is phosphorylated by fructokinase, which uses ATP as the phosphate donor, depleting the hepatocyte of intracellular ATP and increasing generation of ADP and AMP. The scavenger enzyme, AMP deaminase1, generates the waste product, uric acid, from AMP. Uric acid acts within vascular smooth muscle to inhibit endothelial nitric oxide synthase and results in reduction of nitric oxide production, which promotes hypertension. Uric acid has been implicated in increased risk of coronary artery disease (Lustig, 2010).

1.2.4.4 Hepatic steatosis

Getting an excess fructose load results in excess mitochondrial acetyl-CoA formation which exceeds the ability of the tricarboxylic acid (TCA) cycle to metabolize the excess acetyl-CoA (Singh *et al.,*2011).The excess acetyl-CoA is converted to citrate, exits into the cytosol via the citrate shuttle, and serves as the substrate for *de novo* lipogenesis. Acetyl-CoA dimerizes and it is decarboxylated to form malonyl-CoA, which inhibits mitochondrial β-oxidation (Klop *et al.,* 2013). TGs, newly formed by *de novo lipogenesis*, can overwhelm the lipid export machinery and accumulate in the liver, forming intra-hepatic lipids and lead to hepatic steatosis (Landsberg *et al.,* 2013).

1.2.4.5 Hepatic insulin resistance

Fructose-1-phosphate activates dual specificity mitogen-activated protein kinase kinase 7 (MKK7), which stimulates the hepatic enzyme, mitogen activated protein kinase 8 (MAPK8). This kinase is thought to be the bridge between hepatic metabolism and inflammation (Lim*et al.,* 2010). Furthermore, the intermediate, diacylglycerol (DAG), which accumulates during *de novo* lipogenesis, activates hepatic protein kinase C, ε type (PKCε) (Kolovou *et al.,* 2005). Both MAPK8 and PKCε trigger serine phosphorylation and subsequent inactivation of IRS1, which leads to hepatic insulin resistance (Schultz *et al.,* 2013).

1.2.4.6 Leptin Resistance

Leptin is a multiple-function adipocytokine involved in the regulation of food intake, energy storage and carbohydrate and lipid metabolism (Das *et al.,* 2013). Its production is higher when adipose tissue becomes more abundant, and it is stimulated by insulin and inhibited by TNF-α, estrogens, FFAs and growth hormone (Myers *et al.,* 2008). Insulin resistance leads to leptin resistance and hence continuous intake of food that exacerbates dyslipidemia and metabolic syndrome (Das *et al.,* 2013).

1.2.4.7 Hyperglycemia and T2DM

Fructose, a gluconeogenic precursor, increases synthesis of the fork head box protein o1 (FoXo1). Hepatic insulin resistance, made worse by elevated fructose concentrations, prevents the phosphorylation of FoXo1, which allows this protein to enter the nucleus and induce the transcription of enzymes that promote gluconeogenesis. Increased hepatic glucose results in hyperglycemia, and is likely to contribute to the development and progression of T2DM (Lim *et al.,* 2010).

1.2.4.8 Obesity

Fructose also contributes to increased food consumption that leads to obesity. Direct effects of fructose on the central nervous system (CNS) include stimulation of production of brain hormones that stimulate appetite. Reduction of hypothalamic malonyl-CoA levels results in increased AMP kinase concentrations, driving further food intake (Zoulikha *et al.,* 2012). Indirect effects of fructose on the CNS include hypertriglyceridemia, which reduces leptin transport across the blood–brain barrier and hyperinsulinemia, which blocks the leptin signal transduction pathway, resulting in reduced satiety and increased appetite, again driving further food intake (Chang and Li, 2011).Generally, excess fructose intake induces causes the following:

- Increased synthesis of FOXO1 due to excess fructose load which promotes gluconeogenesis and hyperglycemia that leads to diabetes.

- Substrate-dependent phosphate depletion, which increases uric acid formation that contributes to HTN. This is by reducing nitric oxide (NO) production through inhibiting endothelial nitric oxide synthase.
- Activation of MAPK8 and PKCε, which contributes to serine phosphorylation of IRS-1 and hepatic insulin resistance, which in turn promotes hyperinsulinemia and influences substrate deposition into fat. This antagonizes central leptin signaling results leptin resistance. This promotes continued fructose intake.
- Excess formation of citrate, which serves as the substrate for *de novo* lipogenesis that results in formation of excess malonyl-CoA, which inhibits β-oxidation. As a result there is an accumulation of acyl-CoA, which is an intermediate substrate for the formation of free fatty acids, VLDL, DGA and hepatic lipid droplet. This results in muscle insulin resistances, dyslipidemia and obesity as a result of the accumulation of TGs in adiposities.

Figure 1.5: Metabolic disorders of hepatic fructose metabolism (Lim *et al.,* 2010).

Abbreviations: ACL, ATP-citrate lyase; ACC1, acetyl-CoA carboxylase 1; apo B, apolipoprotein B-100; ChReBP,carbohydrate response element binding protein; CPT-1, carnitine O-palmitoyl transferase 1; FAS, fatty acid synthase; DAG, diacylglycerol; FOXO1, forkhead box protein O1; GLUT-5, solute carrier family 2, facilitated glucose transporter member 5; IRS-1, insulin receptor substrate 1; LPL, lipoprotein lipase; MAPK8, mitogen-activated protein kinase 8; MKK7, mitogen-activated protein kinase kinase 7; MTTP, microsomal triglyceride transfer protein; NO, nitric oxide; PFK, 6-phosphofructokinase; PGC-1β, peroxisome proliferator-activated receptor γcoactivator 1β; Pi, inorganic phosphate; PKCε, protein kinase C εtype; PP2A, protein phosphatase 2a; pSer-IRS-1, serine phosphorylated IRS-1; SReBP-1c, sterol regulatory element binding protein-1c; TCA, tricarboxylic acid.

1.2.5 **Consequences of dyslipidemia**

There are a number of consequences of dyslipidemia:

1.2.5.1 Hypertension (HTN)

Free fatty acids (FFAs) can contribute to HTN through activating the calcium-independent isoenzyme of protein kinase C, which is a vital element in mediating signal transduction and cell regulation. FFAs act as potent activators of phosphorylation of protein kinase C (Kotsis *et al.,* 2010). This may be due to their activity to release calcium ions (Ca^{2+}) from the sarcoplasmic reticulum and increase Ca^{2+} influx from extracellular space through plasma membrane Ca^{2+} channels. Four Ca^{2+} ions bind to calmodulin (CAM) to form a Ca^{2+}-CAM complex, which activates myosin light chain (MLC) kinase, and in turn causes the phosphorylation of the 20 kDa MLC, stimulates actin-myosin interaction and promotes vascular smooth muscle (VSM) contraction, which may induce elevations of blood pressure (Khalil, 2013).

Normal endothelium senses hemodynamic forces and biochemical signals from the blood, and in turn, responds by synthesizing and releasing vasoactive substances (Collins and Tzima, 2012). Endothelium releases nitric oxide (NO), which causes a decrease of BP by initiating smooth muscle relaxation. NO activates soluble guanylyl cyclase, which initiates guanosine triphosphate (GTP) transformation to cyclic GMP. Activation of cyclic guanosine monophosphate (cGMP) dependent proteinkinase G is followed by cytosolic Ca^{2+} removal from the cell and inhibition of contractile apparatus (Dobutovi *et al.,* 2011).Action of proteinkinase G has direct influence on phosphorylation of gap junctions, and activity of potassium and calcium channels. Phosphorylation of potassium channels causes K^+ outflow from cell, when phosphorylated calcium channels decrease Ca^{2+} influx. If cytoplasmicCa^{2+} concentration decreases below 500 nM, the contraction is stopped. This happens because of Ca^{2+}becoming unbound from CAM, followed by detachment from myosin light chain kinase, causing its inactivation. Dephosphorylated myosin light chain prevents the myosin head frombinding to actin, causing relaxation of smooth muscle (Stankevicius *et al.,* 2003).

Endothelial dysfunction may also be caused by accumulation of lipids, which is considered to be an early process in the development of HTN and atherosclerosis (Khan *et al.,* 2013).Especially,

oxidation of these fatty acids and modification of the apoB protein make LDL highly susceptible to phagocytosis by tissue macrophages and monocytes. These phagocytic cells adhere to the endothelium and then penetrate into sub-endothelial spaces, perhaps in response to chemotactic factors including oxidized LDLs. The presence of large amounts of lipid within macrophages and monocytes yields foam cells, which appear to be necessary to generate fatty streaks in the vessel wall (Oparil, 2003). These fatty streaks recruit additional macrophages that can damage endothelial cells, releasing further cytokines and other pro-inflammatory factors. The net result of these reactions eventually causes an atherosclerotic plaque (Yamada, 2001).

Physiologically elevated FFA levels also produce insulin resistance in endothelial cells by inhibiting insulin induced increase in nitric oxide and blood flow. FFAs were found to inhibit insulin action at the level of insulin stimulated glucose transport and/or phosphorylation by inhibiting insulin signaling(Boden, 2012*)*.Elevated cellular levels of FFA may result in insulin resistance in skeletal muscle and liver, as well as reduce beta-cell function, and this has been referred to as lipotoxicity (Sikaris, 2004).

1.2.5.2 Renal disorders

Dyslipidemia can potentially accelerate progression of renal disease by several mechanisms. First, reabsorption of fatty acids and cholesterol contained in the filtered proteins (albumin and lipoproteins) by tubular epithelial cells can stimulate tubule-interstitial inflammation, foam cell formation, and tissue injury (Vaziri, 2006). Second, accumulation of lipoproteins in glomerular mesangium can promote matrix production and glomerulosclerosis (Tsimihodimos *et al.,* 2011). In this context, native and oxidized lipoproteins, particularly LDL, stimulate production of matrix proteins by cultured mesangial cells and promote generation of proinflammatory cytokines, which can lead to recruitment and activation of circulating and resident macrophages (Vaziri, 2006). In addition, impaired HDL-mediated reverse cholesterol transport can further contribute to tissue injury by limiting the unloading of the excess cellular cholesterol and phospholipid burden (Tsimihodimos *et al.,* 2011). Moreover, hereditary lecithin cholesterol acyltransferase (LCAT) deficiency, which is associated with a marked reduction in HDL cholesterol and impaired HDL-mediated reverse cholesterol transport, results in progressive renal disease (Vaziri, 2006). Both chronic renal

insufficiency and nephritic syndrome lead to acquired LCAT deficiency and impaired HDL metabolism (Tsimihodimos *et al.,* 2011).

1.2.5.3 Cardiovascular diseases

Among numerous factors that contribute to the high risk of CVDs, dyslipidemia is the most common factor that contributes to high morbidity and mortality. Oxidized LDL is highly susceptible to phagocytosis by tissue macrophages and monocytes. As already described, these phagocytic cells adhere to the endothelium and then penetrate into sub-endothelial spaces, producing foam cells which appear to be necessary to generate fatty streaks and atherosclerotic plaques in vessel walls (Oparil, 2003). This may also worsen hypertension (Yamada, 2001).

1.2.5.4 Impact of dyslipidemia on energy metabolism

Very low density lipoprotein cholesterol and chylomicrons are the principal vehicles for the delivery of fatty acids to the skeletal muscles and myocardium for energy production and to the adipose tissue for energy storage. Fatty acids and glucose are the principal sources of fuel for mechanical, biochemical, and biophysical functions of the body (Gallis *et al.,* 2011). Thus impaired LPL-mediated lipolysis of VLDL and chylomicrons, as well as diminished VLDL receptor-mediated uptake of VLDL by skeletal muscle and myocardium, can necessarily limit the availability of fatty acid fuel in these tissues (Bonanni*et al.,* 2011). Similarly, insulin resistance, which is partly a consequence of dyslipidemia, can increase the problem by limiting the availability of glucose for energy production in the muscle tissue (Vaziri, 2006).

1.2.6 **Prevention and treatment of liver damage and dyslipidemia**

Dyslipidemia causes liver damage in many ways especially in the case of excess fructose metabolism since 100% of fructose metabolism occurs in liver.

1.2.6.1 Liver damage prevention and treatment

Dyslipidemia and diabetes can cause liver damage through accumulation of fats in hepatocytes, and drugs that improve lipid profiles and diabetes can therefore reduce liver damage that is caused by this abnormal lipid accumulation. But almost all of these drugs have their own side effects on the liver (Chalasani *et al.,* 2012).

1.2.6.2 Prevention and treatment of dyslipidemia

Despite there being a number of ways of treating dyslipidemia, it is not controlled in as many people as it should be. This is due to poor lifestyle and lack of awareness of diagnosis (Sharma *et al.,* 2010). Practices of healthy lifestyle such as physical activity, reduction of food items which have high cholesterol and saturated fatty acids can improve lipid profiles (Blanco *et al.,* 2008). The Mediterranean diet, using olive oil as the primary source of fat, fruits and vegetables,nuts, fish, milk and dairy product consumption, reduces thedevelopment of dyslipidemia (Houston *et al.,* 2009). The phytosterols are plants sterols which are structural similar to cholesterol, and they lower plasma cholesterol levels primarily through competitive inhibition of cholesterol absorption at the intestinal level. Phytosterols added to margarine, yoghurt, cereal and dairy products have been proposed for hypercholesterolemia therapy due to their decreasing effect on blood lipids (Blanco *et al.,* 2008).

Medical therapy can be initiated if lifestyle changes are insufficient. Multiple medications exist, and differ not only in their mechanism of action but also in type of lipid that they may affect and the magnitude of their benefits (Reiner *et al.,* 2011).

Statins

Statins such lovastatin, simvastatin, pravastatin, atorvastatin and others are drugs that decrease serum LDL-C levels (Amara *et al.,* 2011). They do this by competitively inhibiting HMG-CoA reductase, the rate-limiting enzyme that converts HMG-CoA into mevalonic acid during cholesterol synthesis. The mechanism is by changing the conformation of the enzyme during binding to its active site. Hence statins prevent HMG-CoA reductase from attaining a functional structure (Tokoro *et al.*, 2004*)*. Statins also raise the level of LDL receptors, in the liver in particular, which remove LDL

from the bloodstream (Dobritoiu and Forsea, 2011). Liver cells sense the reduced levels of liver cholesterol and seek to compensate by synthesizing moreLDL- receptors to draw cholesterol out of the circulation. This is accomplished via protease enzymes that cleave a protein called "membrane-bound sterol regulatory element binding protein", which migrates to the nucleus and cause, through increased transcription, increased production of various other proteins and enzymes, including the LDL receptor (De Loecker and Preiser, 2012). The LDL receptor then relocates to the liver cell membrane and binds to plasma LDL and VLDL particles. LDL and VLDL are drawn out of circulation into the liver and are metabolized in the liver (Reiner *et al.,* 2011). Statins also reduce hepatic synthesis of apolipoprotein B-100, which in turn cause a reduction of the synthesis and secretion of lipoproteins rich in triglycerides and increase of receptors for apolipoproteins B or E, and also raises the level of HDL-C by an uncertain mechanism (Amara *et al.,* 2011).

Statins may cause adverse effects on health such as liver toxicity, muscle toxicity, renal insufficiency, skin toxicity and hypothyroidism (Dobritoiu and Forsea, 2011).

Fibrates

Fibrates are 2-phenoxy-2-methyl propanoic acid derivatives. Fibrates include bezafibrate, ciprofibrate, clofibrate, fenofibrate, and gemfibrozil. These are another group of anti-dyslipidemic agents, widely used in the treatment of different forms of dyslipidemia (Staels *et al.,* 1998). In contrast to statins, fibrates do not stop cholesterol biosynthesis but these drugs decreases plasma levels of fatty acid and triacylglycerol by stimulating beta-oxidation of fatty acids mostly in peroxisomes and partially in mitochondria (Klop *et al.,* 2013). Fibrates are able to activate gene transcription because they act as synthetic ligands for peroxisome proliferator-activated receptor (PPARα) (Reiner *et al.,* 2011). PPARα is a ligand-activated transcription factor and member of the nuclear hormone receptor superfamily (Staels *et al.,* 1998). PPARα transmits signals from lipid soluble factors, such as fatty acids, eicosanoids, hormones and vitamins, to genes in the nucleus by binding to DNA within specific response elements (PPRE). Activation of PPARs by these drugs is not directly by binding with PPARs. However, in response to fibrate drugs, PPAR-*a* heterodimerizes with retinoid X receptor-*a* (RXR-*a*), and the resulting heterodimer modulates the transcription of genes containing peroxisome proliferator responsive elements (PPREs) in their promoter sequence (Marrs, 2010). This causes the product of beta-oxidation, acetyl-CoA, to enter the Krebs cycle, where

it is further oxidized to carbon dioxide, resulting in more reduced energy carriers like NADH and FADH2 production, and reducing dyslipidemia (Klop *et al.,* 2013).

Fibrates are believed to cause oxidative stress, which ultimately increases hepatocyte proliferation, oxidative DNA damage, and cause nausea and diarrhea, headaches, and liver dysfunction. These drugs should not be used by nursing mothers or during pregnancy (Goldenberg *et al.,* 2008).Figure 1.10 shows the action mechanism of fibrates in reduction of serum cholesterol level (Goldenberg *et al.,* 2008).

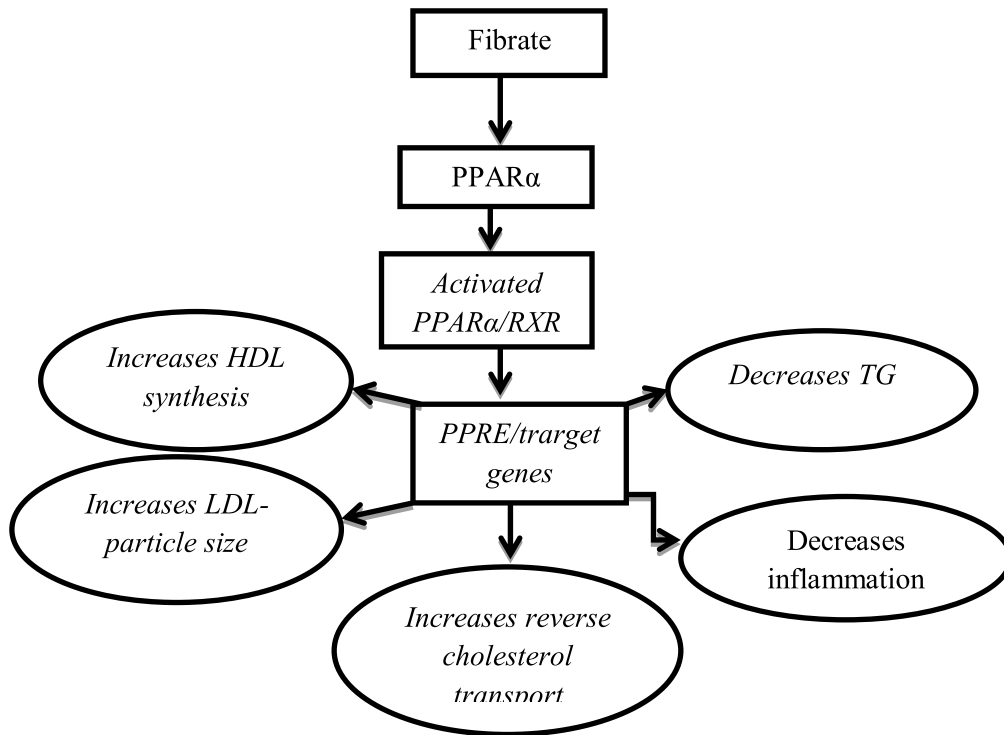


Figure 1.6:Action mechanism of fibrates (Goldenberg *et al.,* 2008).

LDL-low density lipoprotein, HDL-high density lipoprotein, PPARα- peroxisome proliferator-activated receptor α, RXR -retinoid X receptor, PPREs- peroxisome proliferator responsive elements, ↓ - decrease, ⊤ -increase.

Cholesterol absorption inhibitors

Cholesterol absorption inhibitors, for example ezetemibe, treat dyslipidemia by decreasing the dietary absorption of cholesterol in the small intestine. Ezetemibe causes a decrease in the cholesterol delivery to the liver which in turn clears more cholesterol from the blood (Tenenbaum and Fisman, 2012). Ezetemibe interferes with the dietary and biliary absorption of cholesterolin binding to the Niemann-Pick C1-Like 1 (NPC1L1), an important mediator of cholesterol absorption found on epithelial cells in the gastrointestinal tract (GI) tract and on hepatocytes. NPC1L1 protein recycles between the plasma cell membrane and endocytic recycling compartment. When the extracellular cholesterol concentration is high, cholesterol is incorporated into the cell membrane and is sensed by cell surface–localized NPC1L1 (Phan *et al.,* 2012). NPC1L1 and cholesterol are then internalized together through clathrin/ AP2-mediated endocytosis and transported along microfilaments to the endoplasmic reticulum (ERC) in vesicles. The ERC is where cholesterol and NPC1L1 are stored. When the intracellular cholesterol level is low, ERC-localized NPC1L1 moves back to the plasma membrane (PM) along microfilaments in order to absorb cholesterol. Ezetimibe hinders the interaction of the NPC1L1/ cholesterol complex with the AP2-clathrin complex (Domagala *et al.,* 2003).

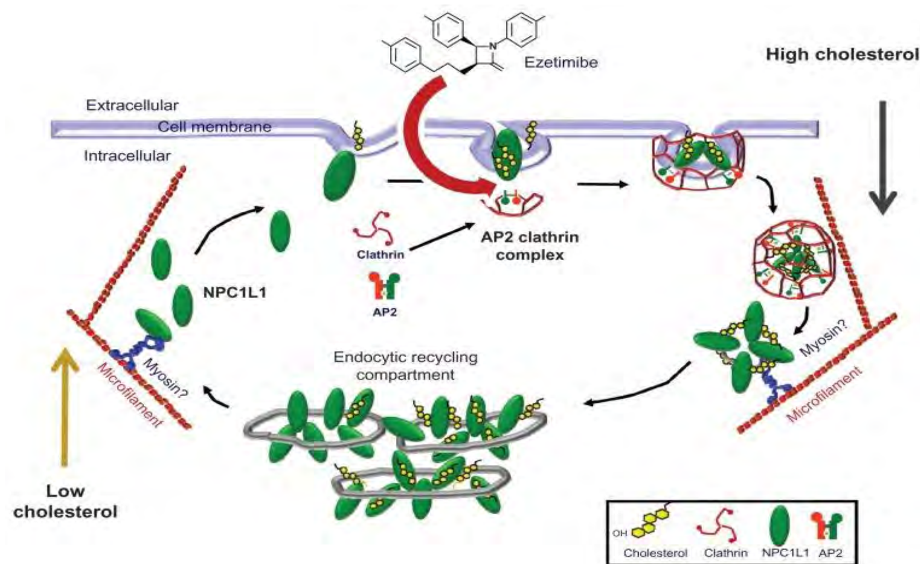


Fig 1.7: Action mechanism of ezetimibe (Phan *et al.,* 2012).

NPC1L1-Niemann-Pick C1-Like 1, AP2-clathrin

The most commonly reported adverse effects are upper respiratory tract infection, diarrhea, sinusitis and pain in extremity (Domagala *et al.,* 2003).

Bile Acid Sequestrants: Cholestyramine, colesevelam and colestipol

These are polymers that have the capability to bind negatively charged bile acids and salts in the small intestine, reduce enterohepatic bile acid circulation and increases the conversion of cholesterol to bile acids and therefore the excretion of cholesterol in feces. The resulting decreased hepatocyte cholesterol content promotes the increased synthesis of LDL-C-receptors that removes LDL-C. Thus they are commonly used to reduce the LDL-cholesterol level. Bile acid sequestrants also can be used safely in combination with statins, and they have recently been approved also for the treatment of type 2 diabetes *(Hou and Goldberg, 2009).*

1.3 **Medicinal plants**

The term, medicinal plants, includes various types of plants have medicinal activities. These medicinal plants are considered as a rich resource of ingredients which can be used in drug development and synthesis (Hassan, 2012).Over three-quarters of the world population relies mainly on plants and plant extracts for health care. More than 30% of the all plant species, at one or other time were used for medicinal purposes (Joy *et al.,* 1998).Plants may contain multiple medically useful products that act synergistically, and they may also act synergistically with synthetic pharmaceuticals. Medically beneficial plants that are eaten regularly may provide preventive medicine against various diseases (Hassan, 2012).

Ethiopian plants have shown remarkably effective medicinal value for many ailments that affect people and livestock. About 80% of the Ethiopian population is said to depend on traditional medicine for their healthcare delivery and most of this comes from plants (Belayneh *et al.,* 2012). Medicinal plants found in the Ethiopian flora were at one time estimated to consist of over 700 species, and perhaps even 1000 plant species. About 300 of the traditional medicinal plant species of Ethiopia are frequently mentioned in many sources as being of medicinal value. Many other medicinal plants of Ethiopia are being used medicinally but are less well known or studied (Belayneh *et al.,* 2012).

1.3.1 Medical plants used in treatment of dyslipidemia

Many plants play a role in reducing LDL and increasing HDL. Garlic has a reducing effect on plasma LDL-C and TG without significant change in HDL-C. This is due to its having of organo-sulphur compounds that significantly lower the hepatic activity of HMG-CoA reductase, which is the rate limiting enzyme in cholesterol biosynthesis (Mathew *et al.,* 2003). Studies of anti-dyslipidemic and antioxidant potential of *Kalanchoe crenata* whole plant extracts in streptozotocin-induced diabetic nephropathy in rats, showed that methanol extracts of *K.crenata*, through their lowering of SODand CAT activities, enhanced MDA levels, and thus improved dyslipidemia (Fondjo *et al,* 2012). A fructo-oligosaccharide fraction from *Psacalium decompositum* produced an anti-inflammatory effect, lowered weight gain, and improved triglyceride and total cholesterol, in fructose-induced obese Wistar rats (Merino-Aguilar *et al.,* 2014).

1.3.2 Medicinal plants used in treatment of hypertension

According to a WHO report, about 70 to 80% of the world‘s population relies on non-conventional medicine mainly from herbal sources in their primary health care (Kumar *et al.,* 2011). This is true especially in developing countries where the cost of consulting a western style doctor and the price of medication are beyond the capacity of most people (Kumar *et al,* 2011). Vascular smooth muscle relaxation that is important in reduction of HTN can be achieved by various mechanisms such as potassium channel opening, calcium channel blocking and receptor antagonism (Ghayur and Gilani, 2005; Gilani *et al*., 2010; Suresh *et al.,* 2006*). Rauwolfia serpentine* is a plant that has ability to deplete catecholamines from peripheral sympathetic nerve endings, decrease in sympathetic tone and is used in treatment of HTN, especially in hypertensive emergencies (Joshi*et al.,* 2012). *Annona* leaf extract in normotensive Sprague Dawley rats was reported to lower an elevated BP by decreasing the peripheral vascular resistance because some essential oils present in the plant exhibit vasodilator activities (Nwokocha*et al.,* 2012). Garlic is another herb that is thought to reduce BP by stimulating nitric oxide production, resulting in smooth muscle relaxation and vasodilatation (Nahida and Feroz, 2011). *Lupinus albus* seed extract treatment of renovascular hypertensive guinea pigs showed a dose-dependent reduction of blood pressure (Cherinet *et al.,* 2002).

1.3.3 **Medicinal plants used in protection of liver**

Methanolic extracts of *Apium graeolens Linn* (Apiaceae) had significant hepatoprotective effects on carbon tetrachloride induced hepatotoxicity in albino rats, comparable with standard hepato-protective drug, silymarin(Singh *et al.,* 2012). An oral administration of n-heptane extract of leaves of *Cassia fistula (Amaltas)* showed significant protection against paracetamol induced hepatotoxicity in rats when compared to standard hepato-protective agent (Singh *et al.,* 2012). Another study of the effects of pink guava puree supplementation on antioxidant enzyme activities and organ function of spontaneous hypertensive rats showed that liver function tests, ALT, AST, LDH and GGT,were significantly lower in the treated groups when compared to control group. This may be due to ability of *guava* to inactivate free radicals that causes liver damage according to the National Health Survey (NHS, 2010).The aqueous extract of *guava* leaves on paracetamol (PCM) induced acute liver injury in rats, showed the dose dependent hepato-protective activity (Roy and Das, 2011). Another study showed that an aqueous extract of *guava* leaf had hepato-protective effects on erythromycin-induced liver damaged rats at low extract doses, but was hepatotoxic at high extract doses (Salgado*et al*, 2006).

1.3.4 ***Psidium guava* (*P.guava*)**

*Psidium guavaL.*is a small medicinal tree that is native to Mexico, Central America, and northern South America. Brazil is among the world's top producers and most of the country's production is destined for the food industry. It is popularly known as guava (family Myrtaceae) and has been used traditionally as a medicinal plant throughout the world for a number of ailments. There are two common varieties of guava: the red (*P. guajava* var. *pomifera*) and the white (*P. guajava* var. *pyrifera*) (Barbalho*et al.,* 2012).

1.3.5 **Distribution of the plant**

Psidium guava is a perennial plant. It grows to a height of 3-10m and its leaves are opposite with short petioles (Sengupta *et al.,* 2011). It is often cultivated throughout tropical and subtropical countries and it can grow in almost all types of soils and tolerates a soil pH of 4.5 to 8.2. However, to obtain a good yield, the tree needs deep, fertile soils with sufficient moisture (Samba, 2009). The common names of *P. guava* are guava in English, kuawa in Hawaian, guayaba in Spanish speaking

countries, goiaba in Brazil and zeitun or zeituna in Amharic (Samba, 2009). Even though there are no studies or books on geographical distribution of guavain Ethiopia, it is assumed to be distributed throughout the country. It can grow in all types of the soil and withstand different temperatures. Mostly the known areas of its distribution are Hawassa, Meki, Wonji, Dire Dawa, Mekelleand Addis Ababa and it grows sparsely in other parts of Ethiopia.

1.3.6 Phytochemistry of *Psidium guava* plant

Different parts of this plant have different chemical compositions (Table 1.3).

Table 1.3: Chemical compositions of different parts of P.guava plant (Mittal et al., 2010)

Parts	Constituents
Fruit	Vitamin C, vitamin A, iron, calcium, manganese, phosphoric, oxalic and malic acids, saponin combined with oleanolic acid. Morin-3-O-α-L-lyxopyranoside and morin-3-O-α-L-arabopyranoside, flavonoids, guaijavarin, Quercetin, Myricetin and apigenin. Essential oil contains hexanal ,-2-hexenal , 2,4-hexadienal, 3-hexenal, 2-hexenal, 3-hexenyl acetate 6-methyl-5-hepten-2-one, limonene, octanol, ethyloctanoate(pink guava fruit),fiber,fatty acid.
Leaves	α-pinene, β-pinene, limonene, menthol, terpenyl acetate, isopropyl alcohol, longicyclene, caryophyllene,β-bisabolene, caryophyllene oxide, β copanene,farnesene, humulene, selinene, and guayavolicacids, cineol,quercetin, 3-L-4-4-arabinofuranoside (avicularin) and its 3-L-4-pyranoside (Essential oil), resin, tannin eugenol andetc
Bark	Polyphenols resin and crystals of calcium oxalate.
Root	Tannin, leukocyanidins, sterols, Gallic acid, carbohydrates, salts, tannic acid.
Seed	Proteins, starch, oils, phenolic, flavonoid compounds,flavonol glycoside, quercetin-3-O-β-D-(2''-Ogalloyglucoside)-4'-O-vinylpropionate.
Wings	Calcium, magnesium, phosphorous, potassium, sodium, fluoride, copper, iron, zinc, manganese, and lead. Flavonoids, sesquiterpene alcohols and Acid triterpenoids.

1.3.7 **Caloric and nutritional value of *Psidium guava***

Psidium guava is one of the most popular, nutritionally rich fruits, as indicated in Table 1.6, having unique flavor, taste, and health promoting qualities that make it common in the new functional foods category often called ―super fruits". Guava is low in calories and fats but contains numerous vitamins, minerals antioxidants, polyphenolic and flavonoid compounds that may play important role in prevention of cancers, aging, cell differentiation, apoptosis, and may have immune-enhancing properties (Kamath *et al.,* 2008; Sandhar *et al*, 2011).

Table1.4: Nutritional value of guava fruit per 100 gram (Kamath *et al., 2*008)

Kcalories	77-86
Moisture	2.8-5.5g
Crude fiber	0.9-1.0g
Protein	0.1-0.5g
Fat	0.43-0.7g
Ash	9.5-10g
Carbohydrate	9.1-17mg
Calcium	17.8-30mg
Phosphorous	0.30-0.70mg
Iron	200-400 I.U
Carotene (Vitamin A)	0.046mg
Thiamin(vitamin B1)	0.03-0.04mg
Riboflavin(vitamin B2)	0.6-1.068mg
Niacin	40 I.U.
Vitamin B3	35 I.U
Ascorbic acid (vitamin C)	228mg

1.3.8 **Medical uses of *P .guava***

Psidium guava is medically important in treatment of different diseases throughout the world. Medicinal properties of guava fruit, leaf and other parts of the plant are well known in traditional systems of medicine. Phytochemical compounds such as carotenoids and polyphenols, the major classes of antioxidant pigments give guava a relatively high potential antioxidant value among plant foods (Joseph and Priya, 2010, 2011). See Table 1.5 for some medical uses of *P. guava.*

Multiple potential medicinal effects of *P. guava* fruit, roots leaves or branches have been described in animal and human studies but there are no published studies of the medicinal effects of *P.guava* in humans in Ethiopia (Kumar, 2012; Sanda *et al.*, 2011). However, in some areas of Ethiopia, guava is recommended by traditional medicine practitioners for various ailments, including nausea and vomiting, diarrhea, and as an antimicrobial. There is experimental data from animal and human studies suggesting that *P. guava*leaf, root, fruit or branches may be beneficial for diabetes and dyslipidemia (Deguchi *et al.*, 2010; Zhao*et al.,* 2012; Hassan *et al.*, 2012; Uboh *et al.,* 2013; Nor and Yatim, 2010; Owen *et al.*, 2008; Rai *et al.*, 2009; Rawi *et al.*, 2011; Bahrani *et al.,*2012; Shinde *et al.,* 2013; Nahida and Feroz, 2011; Khan, 2014; Shakeera *et al.,* 2013). Ant-inflammatory effects of *P. guava* have also been described (Dutta and Das, 2010; Weni, 2011; Fanta *et al.,* 2010). Psidium guava also used in treatment of hypertension in animal studies (Larson *et al.,* 2010; Shivashankara and Acharya, 2010; Chiwororo and Ojewole, 2008). Animal studies also suggest that *P. guava* may have strong anti-oxidant properties (Chen and Yen, 2006; Tachkittiruagrod *et al.*, 2006; Vanitha *et al.*, 2012). Hepatoprotective benefits (Rai *et al.*, 2009; Osman *et al*, 2010; Deguchi and Miyazaki, 2010;) and cytotoxic or anti-cancer properties have also been suggested in non-human studies (Jun *et al.*, 2011; Mohd *et al.*, 2012; Lee and Park, 2010) are also potential medical applications of *P. guava*. Several studies show anti-diarrheal effects of *P. guava* (Chanu *et al.*, 2011; Ezekwesili *et al.*, 2013) and antimicrobial activity in experimental systems (Henie *et al.*, 2009; Beatriz *et al.*, 2012). However, several detrimental effects of *P. guava* have been described; for example, (Ekaluo *et al.*, 2013) found that leaf extracts of *P.guava* caused impaired spermatogenesis in rats.

1.3.9 **Pharmacokinetic properties of *P. guava***

The *Psidium guava* plant has so many advantageous pharmacokinetic properties. This is due to the presence of many different phytochemical compounds such as vitamin C, vitamin A, iron, calcium, manganese, phosphoric, oxalic and malic acids, saponin combined with oleanolic and others in its fruit(Joseph and Priya, 2011). Quercetin, α-pinene, β-pinene, limonene, menthol are compounds from its leaves; polyphenols and resin exist in its bark; gallic acid andtannin are found in its roots; and phenolics and flavonoids are found in its seed and wings (Shruthi *et al.,* 2013).

The antioxidant activity *P.guava* is due to flavonoids which are considered to be dependent on the presence of ortho-phenolic functions (Tachakittirungrod *et al.,* 2007*)*. Lycopene is another very crucial chemical compound that exists in *guava* leaf and is important in phytochemical treatment of different types of cancers andCVDs (Nunez Rueda, 2005).
Some structures of pharmacological active compounds of guava plant leaf are illustrated as follow:

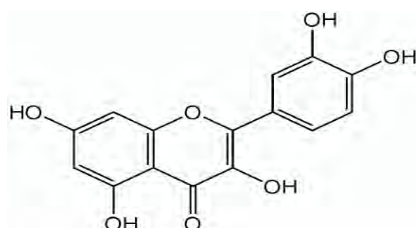


Figure 1.8: Chemical structure of quercetin (Shruthi et al., 2013)

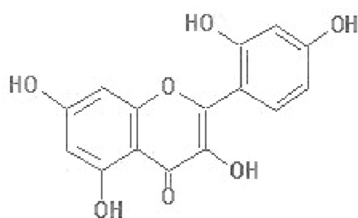


Figure 1.9: Chemical structure of motin (Tachakittirungrod et al., 2007*)*

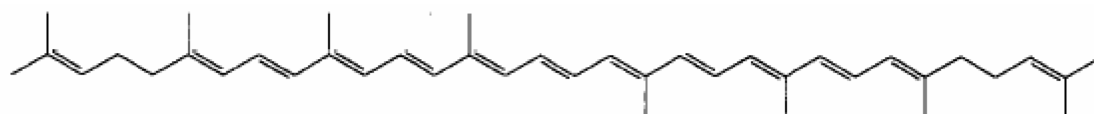


Figure 1.10: chemical structure of lycopene (Nunez Rueda, 2005)

1.4 Significance of the study

Hypertension is silent killer and it does not show considerable symptoms for many years, until complications occur. It is dangerous because it makes the heart work too hard and contributes to atherosclerosis (―hardening of arteries"). Besides increasing the risk of heart disease and stroke, it can also lead to other conditions such as congestive heart failure, kidney disease and blindness. Dyslipidemia is another serious health problem which is much correlated to hypertension. Dyslipidemia may contribute hypertension by narrowing the blood vessels. Hypertension and dyslipidemia are often associated with one another, and may exacerbate each other to some degree. As a result, there is an urgent need for strategies and programmes to prevent and control hypertension, dyslipidemia and liver disease in order to promote healthy life styles, particularly among the urban populations of Ethiopia. Since most anti-hypertensive and anti-dyslipidemic drugs are sometimes associated with unpleasant side-effects, there is a need of effective treatments for hypertension and dyslipidemia.

This study investigated the effect of guava leaf extract on blood pressure in guinea pigs as well as in an *ex vivo* model of hypertension in guinea pig aortas; and hepato-protective effects, as well as effects on dyslipidemia, of guava leaf extract in fructose-fed mice. This study may provide information that is relevant to treatment and prevention of hypertension, dyslipidemia and liver damage in humans.

1.5 Hypothesis of the study

The null hypothesis (Ho) is that the crude extract of *P. guava* leaf has no effect on blood pressure in guinea pigs or on dyslipidemia in fructose fed mice.

The main hypothesis here is that, based on prior knowledge of *P. guava*, guava leaf extracts will lower blood pressure in guinea pigs, will improve lipid profiles in dyslipidemic mice, and will have a hepato-protective effect in mice.

2 OBJECTIVES

The present study was designed to attain the following general and specific objectives.

2.1 General objective

- To investigate the effect of *P. guava* leaf extracts on blood pressure guinea pigs and on relaxation of isolated aortas of guinea pigs
- To investigate the effect of *P. guava* leaf extracts on weight , serum lipid profiles and serum liver enzymes of fructose fed mice

2.2 Specific Objectives

- To examine the effect of different doses of leaf extract of *P.guava* on blood pressure of in vivo guinea pigs.
- To study the ability of *P. guava* leaf extract to relax smooth muscle of aortas taken from guinea pigs.
- To study the effect of different doses of *P. guava* leaf extract on the lipid profiles (TG, TC, HDL-C, VLDL and LDL-C) of fructose-fed Swiss albino mice.
- To evaluate the effect of different concentrations of *P. guava* leaf extract on serum liver function tests in high fructose fed Swiss albino mice .
- To investigate the effect of *P. guava* extracts on weight in fructose-fed Swiss albino mice.

3 MATERIALS AND METHODS

3.1 Study area

The Study was conducted at Addis Ababa University, School of Medicine, laboratories of Biochemistry (dose preparation and measurement), Physiology (experiments on guinea pigs), Pharmacology (experiments on mice) , and Ethiopian Public Health Institute (extraction of the leaf).

3.2 Study design

The study design was randomized experimental animal study.

3.3 Plant leaf collection and authentication

Sufficient amount of fresh leaf of *P. guava* was collected from Hawassa which is 273 km from the capital city of Ethiopia. The leaves with seeds were identified and authenticated by the National Herbarium of Addis Ababa University, and voucher number 085679/ *P.guava L./*2013. was given and deposited at the same institute for further reference

3.4 Preparation of *P .guava* leaf extract

3.5 kg of fresh *P.guava* leaf was washed and dried under shade. Dried leaves were powered with an electrical grinder then weighed. Powdered leaves weighing 732gram were extracted with 1.5 liters of a ethanol/water mixture (70 % ethanol by using Rota evaporator. The extraction was done at room temperature for 72 hours, and then filtered by using Whatman No 1 filter paper. The filtrate was evaporated in a 37 degree water bath till dry, to remove water and ethanol. The resulting ethanol and water-free extract was dissolved in freshly prepared normal saline.

Figure 3.1: Fresh *P. guava,* which was collected from Hawassa by me

3.5 Dosage selection

Dose selection of *P. guava* leaf extract in mice was based on the average amount of *P. guava* leaf extract used in previous published studies (Basha and Kumara, 2012; Abreu *et al.,* 2006). In our case the graded dose 100mg/kg, 200mg/kg and 300mg/k was used oral gavage for mice experiments. For experiments with guinea pigs, *P. guava* leaf extracts were used at 12, 26, 42, 60 and 80 mg/kg intravenously. These doses used in guinea pigs were determined by pilot experiments using different doses of leaf extract, because no studies were available in the medical literature. For *ex vivo* experiments using isolated guinea pig aortas, doses of extract used were from zero to 10 mg/mL, which were chosen based on pilot studies, again because no pre-existing data was available in the medical literature.

3.6 Animal preparation

Twelve male guinea pigs weighing between 300 and 600 g, and thirty male Swiss-albino mice weighing between 25 and 32 g were purchased from Ethiopian Public Health Institute (EPHI). The guinea pigs and mice were given one week acclimatization period in the pharmacology animal house, School of Medicine, Addis Ababa University. They were allowed free access to water and pellet food at controlled ambient temperature of 22 ± 2°C and 50 ± 10% relative humidity, with 12 hour light/12 hour dark cycles.

3.7 Ethical Considerations

Any activity of the study and its results were assumed to do not unnecessary harm the study animals. The protocol was approved by research and ethical committee of Biochemistry department with protocol number M.Sc. thesis 01/13. Animals were taken care of throughout the experimental period. They were anesthetized with pentobarbital prior to removing blood to prevent pain. At the end of experiment, they were sacrificed by neck dislocation and their blood was collected and examined.

3.8 Procedure for measuring guinea pig blood pressure:

3.8.1 *In vivo* experiment using invasive method

The *in vivo* experiment was carried out according to methods described previously (Ghayur and Gilani, 2005; Gilani *et al.,* 2010).Six male guinea pigs (300 – 500g) were anaesthetized with pentobarbital (60 mg/kg, i.p.). The trachea was exposed using a scalpel blade and cannulated, and the animals were artificially ventilated (Bioscience, 815-51190-1, Sheemess, Kent, UK). The right carotid artery was cannulated with a heparinized saline-filled catheter attached to a pressure transducer (BBC,Goez,Metrawatt, Model SE 120) for continuous recording of blood pressure. The ethanol leaf extract of *P.*guava was injected in the form of bolus injection via a cannula inserted into the external jugular vein followed by a saline flush (0.2 mL). Pulse pressure was obtained by subtracting diastolic blood pressure (DBP) from systolic blood pressure (SBP), and mean arterial blood pressure (MABP) was also determined from the sum of DBP plus one-third of pulse pressure. All readings were expressed as the mean ± standard error of the means. A typical experimental tracing of guinea pig blood pressure measurement is shown in Figures 3.1

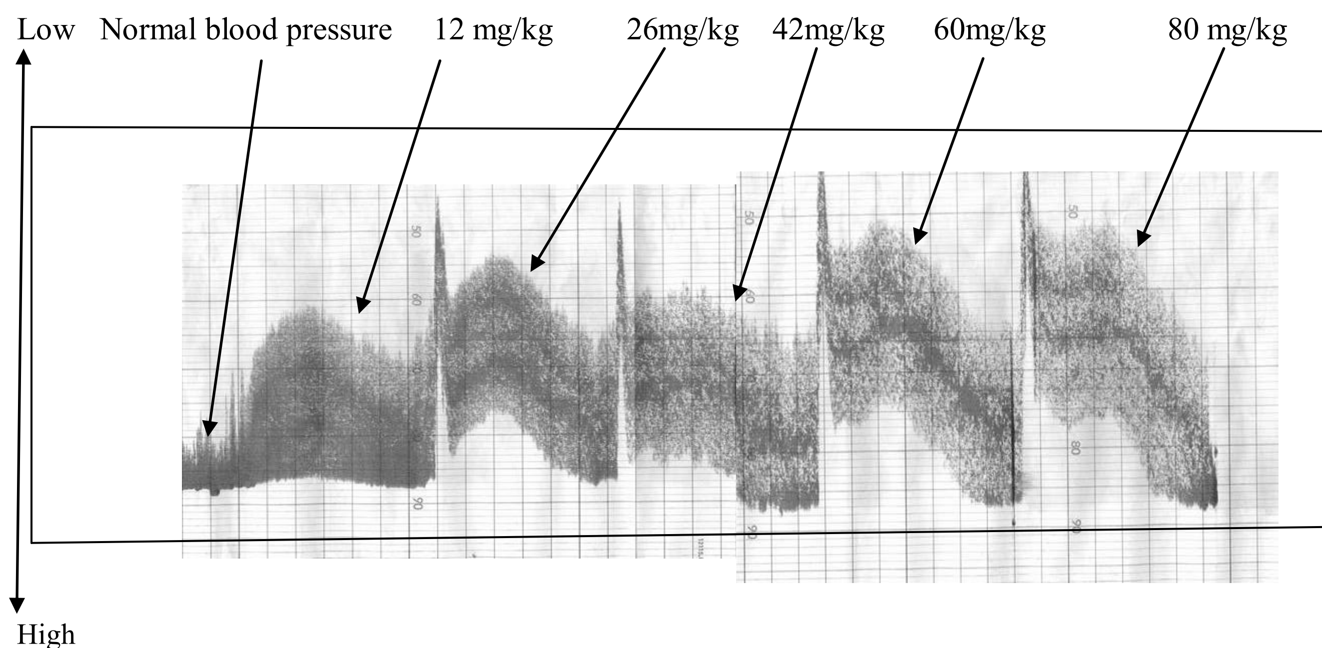


Figure 3.2: A typical experimental tracing of guinea pig blood pressure measurement

3.8.2 *Ex vivo* experiment using organ bath incubation of isolated guinea pig aortas

Six male guinea-pigs (400- 600g) was sacrificed according to the method described by (Ghayur and Gilani, 2005). The descending thoracic aorta was quickly removed and placed in Krebs-Henseleit solution. Excess fat and connective tissues were trimmed off and the whole length of aorta was then cut spirally resulting in long strip. From this strip a short strip (2 to 3 cm) was prepared to be used for the experiment. The tissue was kept moist with Krebs-Henseleit solution during the whole procedure and the strip preparation was mounted in a 2.5 ml tissue bath containing Krebs–Henseleit solution, maintained at 37 ^{0}C and continuously bubbled with a mixture of 95% $O_2$and 5% CO_2.

The composition of the physiological salt solution used was: NaCl 6.9 g/L, $NaHCO_3$ 2.1 g/L, $CaCl_2$0.36 g/L, KCl0.373 g/L, KH2$PO_4$0.16 g/L, $MgSO_4$0.141 g/L and glucose 2g/L, pH 7.4. A resting tension of 1g was applied to the tissue and an equilibrium period of 1hour and 10minutes was allowed to equilibrate before addition of any drug or the test leaf extract. During this period the bath fluid was changed every 15minutes. High K^+ (80 mM KCl) was added to the bath solution to induce sustained contraction of the aortic smooth muscle tissue. The *P. guava* leaf extract was later tested for

its ability to inhibit the aortic contraction induced with high K^+ (80 mM). A change in isometric tension of the strip was measured via a force displacement transducer (FT- 03) using a grass model 7E polygraphs.

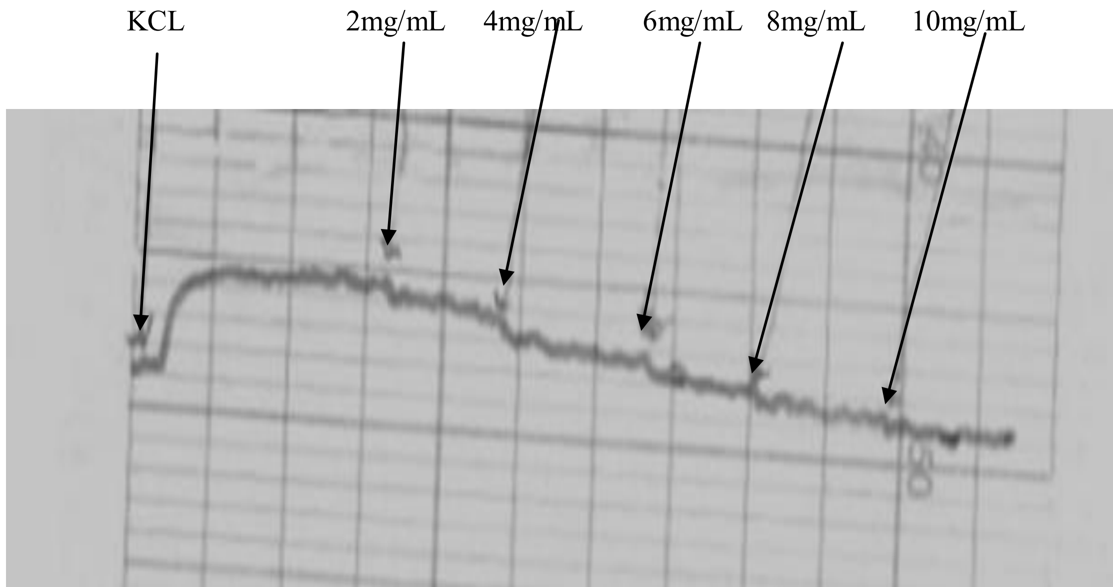


Figure 3.3: A typical tracing from the ex vivo experiment on isolated guinea pig aorta

3.9 **Grouping and administration of leaf extract to mice**

Male mice were randomly grouped into five groups, each of with six mice per cage. One group of mice was fed standard pellet diet throughout the 7-week experimental period. The other groups of mice were fed a continuous high fructose diet in their drinking water (42% fructose) as it was described by (Bray *et al.,* 2004) plus standard pellets, then treated with different doses of *P .guava* leaf extract (zero, 100, 200 and 300 mg/kg)once daily by oral gavage. The weight of all mice was measured at zero days, and at the end of the 4th and 7th week.

3.10 **Variables**

3.10.1 **Dependent variables**

The systolic and diastolic blood pressure, serum total cholesterols, triglycerides, HDL-C, LDL-C and liver enzymes such as (AST, ALT, and ALP) and weight of the animals are dependent variables.

3.10.2 **Independent variable**

P. guava leaf extract

3.11 **Statistical tests**

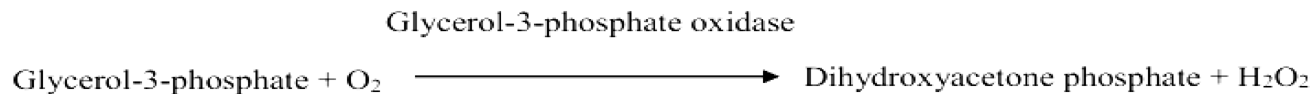
The data for the systolic, diastolic pressure, pulse pressure and mean arterial blood pressure of guinea pigs, data from isolated aorta experiments, serum lipid profiles, liver enzymes and weights of the mice were analyzed by using SPSS Version 16. The data statistically were compared by repeated measures using ANOVAs, two tailed student‘s t-test and independent t-test, paired t-test, excel. The data were expressed as means plus or standard error of the means.

3.12 **Blood sample collection and biochemical analysis**

Guinea pigs were sacrificed, by neck dislocation, at the end of experiments. Mice were sacrificed by pentobarbital anesthesia and blood was collected by heart puncture. Blood was left at room temperature to allow coagulation, then centrifuged at 4500 rpm for 5 minutes. Serum (the supernatant) was removed and stored at -20 degrees Centigrade until analysis.

3.12.1 **Serum Triglyceride (TG) Measurement**

Principle: TGs were measured enzymatically in serum or plasma using a series of coupled reactions in which TGs are first hydrolyzed to produce glycerol by lipase, and further converted to Glycerol-3-phosphate in the presence of glycerol-kinase (GK). Glycerol-3-phosphate is then oxidized using glycerol phosphate oxidase (GPO). Hydrogen peroxide, H_2O_2, one of the reaction products, reacts with 4-aminophenazone + 4-chlorophenol in the presence of the enzyme peroxidase (POD) to produce a colored product in a chromogenic recation. Absorbance is measured at 500 nm and the intensity of color is proportional to the concentration of quinoneimine dye, which is directly related to the triglyceride in the original serum sample.



Reaction Reagent= pipet buffer 50mmol/L, pH=6.8, lipase> 12000 U/L glycerol kinase>1000U/L, glycerol phosphate oxidase>1000U/L, 4-amino phenazone phenol=3mmol/L, non-ionic tension-active 2g/L.

Procedure:10 microliters of serum sample and one mL of Reaction Reagent were mixed at room temperature in a cuvette and incubated for 10 minutes. Then the absorbance was read at 500nm against a blank sample containing buffer alone instead of serum. Standard concentrations of triglyceride were used to determine absolute triglyceride concentrations of serum TG.

3.12.2 **Serum total cholesterol (TC) determination**

Principle: This method for the measurement of total cholesterol in serum involves the use of three enzymes: cholesterol esterase (CE), cholesterol oxidase (CO) and peroxidase (POD). In the final reaction the mixture of 4-aminoantipyrine and phenol are condensed in a chromogenic reaction to form ared quinoneimine dye, which is measured at 500 nm in a spectrophotometer against a blank tube containing buffer only instead of serum. The absorbance at 500 nm is proportional to the concentration of cholesterol in the original serum sample. Standard concentrations of cholesterol were used to determine the absolute cholesterol concentrations in serum samples.

Reactions

Reaction Reagent: pipes200mmol/L, pH=7.0, sodium cholate 1mmol/L, cholesterol esterase>250U/L, cholesterol oxidase > 250U/L, perioxidase>1000U/L,4-aminoantipyrine 0.33mmol/L and non-ionic tension-active 2g/L.

Procedure: 10 microliters of serum sample and one mL of Reaction Reagent were mixed in a cuvette and incubated at room temperature for ten minutes, and then absorbance was read at 500nm.

3.12.3 **Serum HDL cholesterol (HDL-C) determination**

Principle: In this method a magnesium/dextran sulfate solution is first added to the specimen to form water-soluble complexes with non-HDL cholesterol fractions. These complexes are not reactive with the measuring reagents added in the second step. With addition of a second reagent, HDL-cholesterol esters are converted to HDL-cholesterol by PEG-cholesterol esterase. The HDL-cholesterol is acted upon by PEG-cholesterol oxidase, and the hydrogen peroxide produced from this reaction combines with 4-amino-antipyrine and HSDA under the action of peroxidase to form a purple/blue pigment that is measured spectrophotmetrically at 600 nm.

1. ApoB containing lipoproteins +α-cyclodextrin +Mg^{2+}+dextran SO_4 ⟶ soluble non-reactive complexes with apoB-containing lipoproteins

2. HDL-cholesterol esters —(PEG-cholesterol esterase)→ HDL-unsterified cholesterol + fatty acid

3. Unsterified cholesterol + O_2 —(PEG-cholesterol oxidase)→ Cholesterone +H_2O_2

4. H_2O_2+5-aminophenazone+N-ethyl-N-(3-methylphenyl)-N'- succinyl ethylene diamine —peroxidase)→ Qunoneimine dye + H_2O

Reaction reagent: Goods buffer (pH 6.0), cholesterol esterase 375 U/L, cholesterol oxidase 750 U/L, Peroxidase 975 U/L, ascorbate oxidase 2250 U/L,DSBmT 0.75mmol/L,4-aminoantipyrine 0.25mmol/L, detergent 0.375%, preservative 0.05%

Procedure: Ten microliters of serum sample were mixed with 1mL of Reaction reagent in a cuvette and incubated at 37°C for 5 minutes for minutes. Absorbance was determined at 600 nm against a blank cuvette containing 10 microliters of buffer alone (no serum). Standard solutions of known concentrations of HDL were used to determine the absolute HDL concentrations in serum samples.

3.12.4 Serum low density lipoprotein cholesterol (LDL-C) determination

Low density lipoprotein (LDL) cholesterol (in mg/dL) concentration in serum samples was calculated from measured values of total cholesterol, triglycerides and HDL-cholesterol by using the Friedewald equation:

[Total cholesterol] = [VLDL-cholesterol] + [LDL-cholesterol] + [HDL-cholesterol]

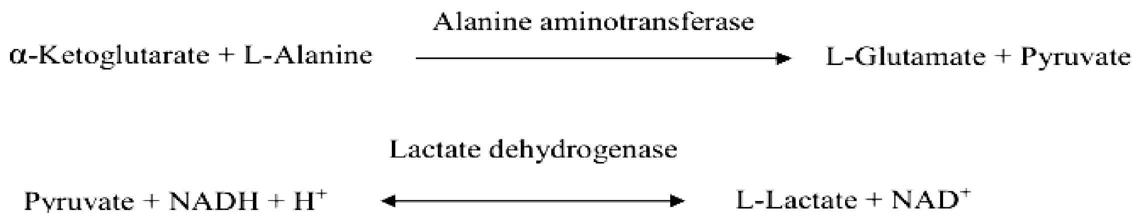
[LDL] = [Total cholesterol] – [HDL] – [Triglyceride]/5

3.13 Liver function tests

Liver Function Tests (LFTs) are the most commonly requested screening blood tests use in investigation of suspected liver disease, monitoring of disease activity, or simply as ‗routine' serum analysis(Hall and Cash, 2012).

3.13.1 **Serum alanine aminotransferase (ALT) assay**

This ALT assay procedure is based on principles outlined by Wroblewski and LaDue and International Federation of Clinical Chemistry (IFCC, 2002).ALT transfers the amino group from alanine to α-oxoglutarate to form pyruvate and glutamate. The pyruvate formed is then reduced to lactate and NAD+ is generated in the presence of lactate dehydrogenase and NADH. The activity of ALT is proportional to the rate of oxidation of NADH at 340nm. The following equation shows the reaction catalyzed by action of ALT (alanine aminotransferase):

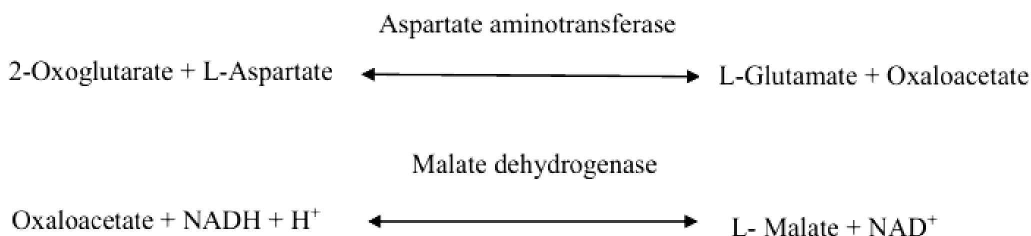


Reagent = tris buffer 150mmol/L, Ph=7.3, l-alanine 750mmol/L, lactate dehydrogenase >1350U/L, NADH 1.3mmol/L, 2-oglutarate 75mmol/L.

Procedure: Reagent (1 mL) was pre-incubated at 37^oC for 10 minutes, then serum sample (100 microlitres) was added and the cuvette contents mixed. The cuvettes were inserted into the spectrophotometer cuvette holder and the absorbance was recorded at 340 nm exactly after 1, 2 and 3 minutes against an air blank (empty cuvette). An absolute activity of ALT was determined by comparison with ALT of known activity that was available with the assay kit used.

3.13.2 **Serum aspartate aminotransferase (AST) assay**

Principle: In the assay reaction, the AST catalyzes the reversible transamination of L-aspartate and α-ketoglutarate to oxaloacetate and L-glutamate. The oxaloacetate is then reduced to malate in the presence of malate dehydrogenase (MDH) and NADH with the concurrent oxidation of the NADH toNAD+.The amount of NADH generated in unit time is proportional to the activity of AST in the serum sample.

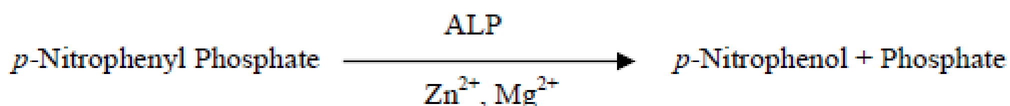


Reaction Reagent: Tris buffer, pH 7.65 (37ºC) 80 mmol/L, LDH ≥ 900 U/L, L- Aspartate 240 mmol/L, α-Oxoglutarate 12 mmol/L, NADH 0.20 mmol/L, MDH ≥ 6 00 U/L

Procedure: Reaction Reagent (1000 microlitre) was incubated in a cuvette at 37 °C. Serum (100 microlitres) was added to the cuvette, the solution mixed and the cuvette placed in the cuvette holder of the spectrophotometer.Absorbance was adjusted to zero with air. The absorbance was recorded at 340 nm exactly after 1, 2 and 3 minutes. Absolute AST activity in serum samples was determined by comparison with AST enzyme of known activity that came with the assay kit used.

3.13.3 **Alkaline phosphatase (ALP)**

Principle: Alkaline Phosphatase Assay Kit is designed to measure ALP activity directly in biological samples .The improved method utilizes p-nitrophenyl phosphate that is hydrolyzed by ALP into a yellow colored (P-nitrophenol) (maximal absorbance at 405nm). The rate of the reaction is directly proportional to the enzyme activity.



Reaction Reagent = 2-Amino-2-methyl-1-propanol 0.35 mol/L, pH 10.4; p-nitrophenyl phosphate 16.0 mmol/L; HEDTA 2.0mmol/L; zinc sulfate 1.0 mmol/L; magnesium acetate 2.0 mmol/L

Procedure: Reaction Reagent (1 mL) was pre incubated at 37°C. The spectrophotometer was adjusted to zero absorbance with air (empty cuvette). Serum sample (20 µL) was added and the cuvette contents mixed by inversion. The cuvette was inserted into the spectrophotometer cuvette holder and absorbance was recorded at 405 nm exactly after 1, 2 and 3 minutes. Absolute ALP activity in serum samples was determined by comparison with ALP enzyme of known activity that came with the assay kit used.

4 RESULTS

4.1 Result of blood pressure studies in guinea pigs

4.1.1 Effect of leaf extracts of *P. guava in vivo* on guinea pig blood pressure

The effect of ethanol extract of *P. guava* leaf on the systolic blood pressure in guinea pigs before and after treatment with various doses (12, 26, 42, 60 and 80 mg/kg) of extract injected into the right jugular vein was examined. The mean systolic blood pressure (SBP) of guinea pigs treated with each dose of leaf extract tested was significantly ($p<0.05$, $P<0.001$) lower than control (non-treated) guinea pigs (Figure 4.1). At a leaf extract dose of 12 mg/kg, systolic blood pressure was reduced from 84.52 ± 0.94 mmHg to 77.50± 1.84 mmHg and, higher doses of *P. guava* leaf extract cause increasingly higher reductions in systolic blood pressure(Figure 4.1). Thus, 80 mg/kg leaf extract reduced systolic blood pressure from 84.52 ± 0.94 mmHg to 69 ± 2.24mmHg.

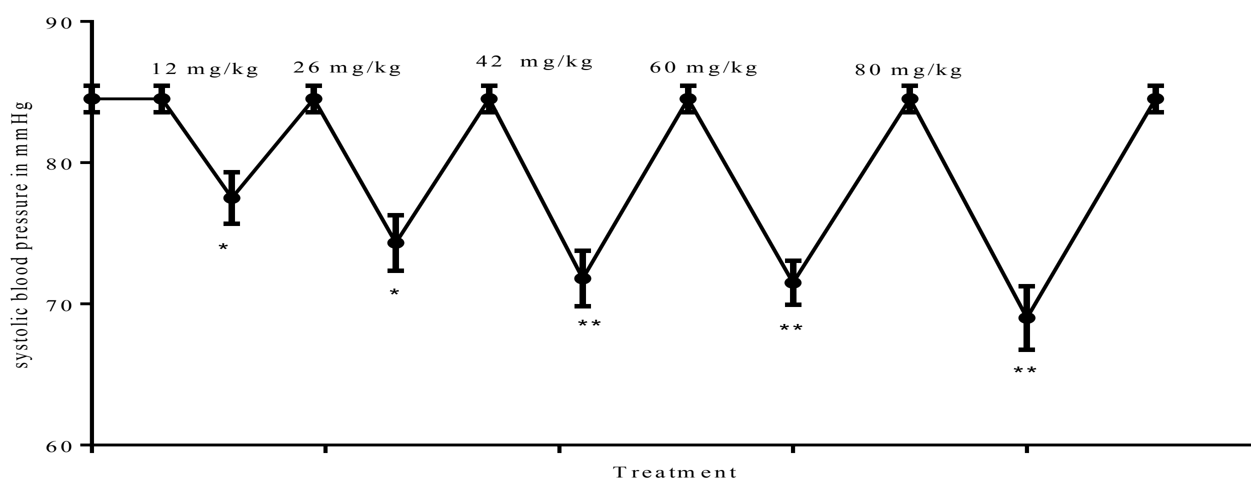


Figure 4.1: Effect of *P. guava* leaf extract on systolic blood pressure of guinea pigs.

A group of six guinea pigs was treated intravenously (via right external jugular vein) with increasing doses (12, 26, 42, 60, 80 mg/kg) of *P .guava* leaf extract. Blood pressure was continuously monitored via a transducer connected to the left carotid artery. An average systolic blood pressure before treatment was 84.52 ± 0.94 mmHg . After each treatment with leaf extract, blood pressure was allowed to return to baseline prior to treatment with the next dose of extract.
Comparison was done with paired t-test and $p <0.05$ was considered significant,*=$p<0.05$,
**=$p<0.001$.

The effect of *P. guava* leaf extract on the diastolic blood pressure of guinea pigs before and after treatment with various doses (12, 26, 42, 60 and 80) mg/kg of leaf extract given through the external jugular vein was examined. The mean diastolic blood pressure (DBP) of guinea pigs treated with each dose of extract significantly ($p<0.05$, $P<0.001$) was lower than the diastolic blood pressure of the guinea pigs before treatment with extract (Figure 4.2). Mean DBP of non-treated guinea pigs was 62.67 ± 1.20 mm Hg, whereas treatment with 12 mg/kg extract lowered DBP to a mean of 56.17 ± 1.78 mmHg, and treatment with increasing doses of extract caused progressively higher drops in DBP, showing a dose-dependent effect (Figure 4.2), as seen with the effect of extract on systolic blood pressure. Thus, 80 mg/kg leaf extract caused a drop in DBP from 62.67 ± 1.20 mm Hg to 45.33 ± 1.48 mmHg.

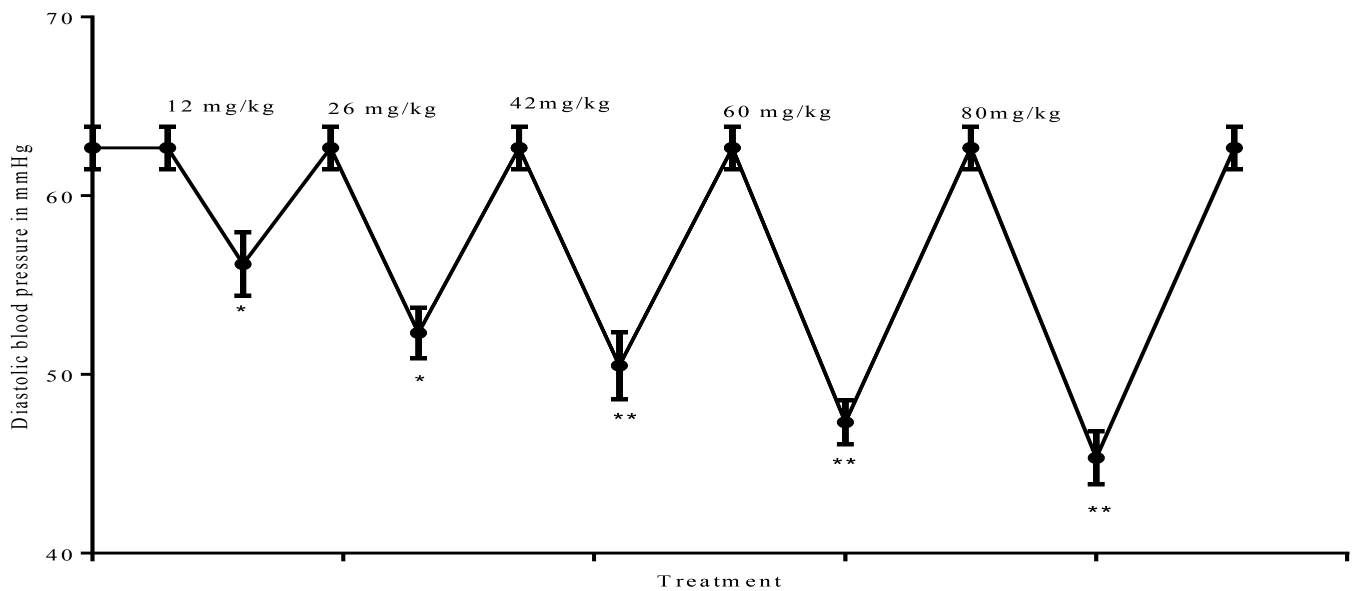


Figure 4.2: Effect of ethanol extract of *P. guava* leaf on diastolic blood pressure of guinea pigs.

A group of 6 guinea pigs was treated intravenously (via right external jugular vein) with increasing doses (12, 26, 42, 60 and 80 mg/kg) of *P. guava* leaf extract. Blood pressure was continuously monitored via a transducer connected to the left carotid artery. An average diastolic blood pressure before treatment was 62.67 ± 1.20 mm Hg. After each treatment with leaf extract, blood pressure was allowed to return to baseline prior to treatment with the next dose of extract.

Comparison was done with paired t-test and $p <0.05$ was considered significant,*=$p<0.05$, **=$p<0.001$.

The calculated pulse pressure (SBP-DBP) on guinea pigs before and after treatment with each dose of leaf extract (12, 26, 42, 60 and 80 mg/kg) was found to be unchanged at all doses. Based on paired t-test analysis there were no significant differences (p=0.526) between pulse pressure before and after treatment of guinea pigs with ethanol extract of *P .guava* leaf, suggesting that the leaf extract lowered systolic and diastolic blood pressure equally (Figure 4.3). However, at higher doses of extract (greater than or equal to 42 mg/kg), there was a slight trend towards an increase in pulse pressure, though this was not statistically significant (Figure 4.3).

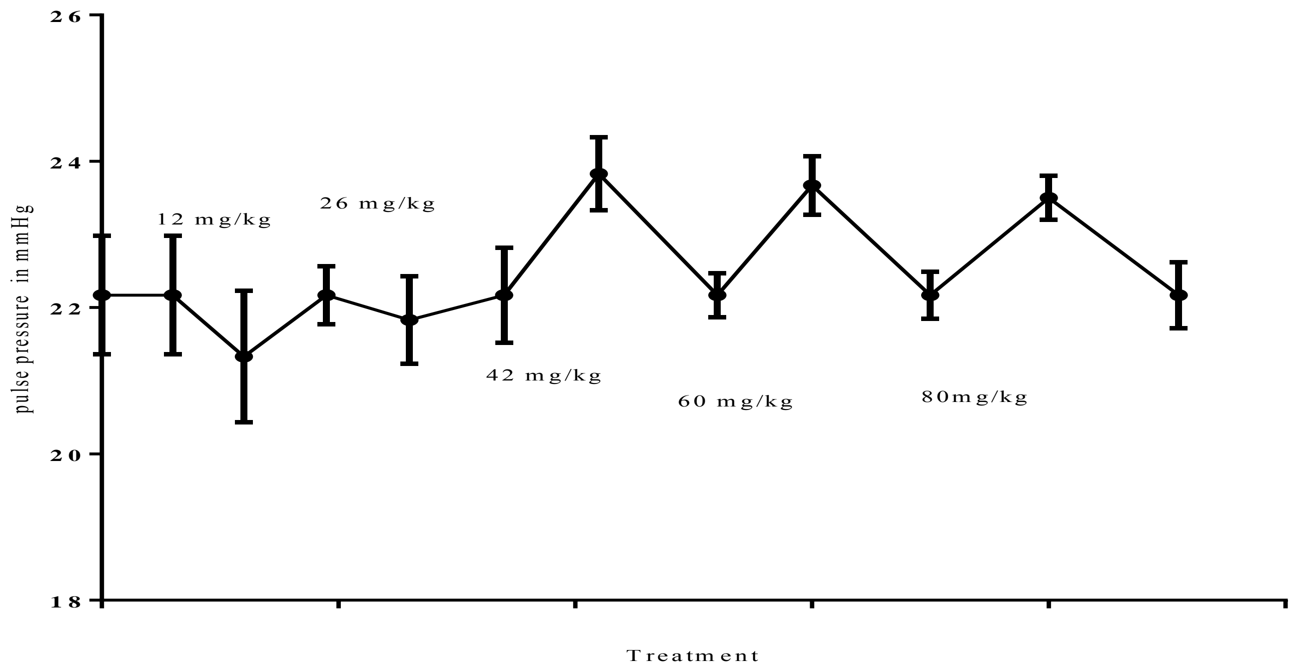


Figure 4.3: Effect of ethanol extract of *P. guava* leaf on pulse pressure (SBP-DBP) in guinea pigs Comparison was done with paired t-test and p <0.05 was considered significant.

The calculated Mean Arterial Blood Pressure in guinea pigs is:

$$\text{DBP} + \frac{1}{3}(SBP - DBP)$$

$$or DBP + \frac{1}{3}(pulse pressure)$$

The mean arterial blood pressure of guinea pigs before and after treatment with *P. guava* leaf extract with doses of (12, 26, 42, 60 and 80) mg/kg was measured. Mean arterial blood pressure of the guinea pigs treated with *P.guava* leaf extract with each dose of extract was significantly reduced (p<0.05, P<0.001) compared with non-treated guinea pigs, again in a dose dependent manner, with higher doses of extract causing an increasing lowering of mean arterial blood pressure (Figure 4.4).

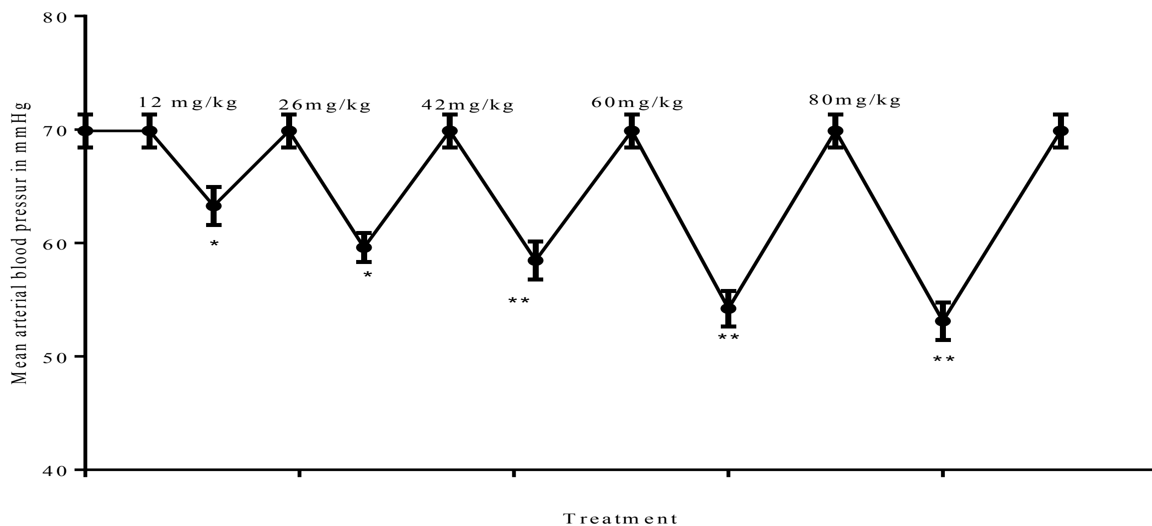


Figure 4.4: Effect of ethanol extract of *P. guava* leaf on mean arterial blood pressure in guinea pigs Comparison was done with paired t-test and p <0.05 was considered significant, *=p<0.05, **=p<0.001.

4.1.2 **Result of *ex vivo* experiments on isolated guinea pig aortas**

For *ex vivo* experiments, to achieve a baseline contraction (100%) of guinea pig aortas, the tissues were treated with high potassium chloride concentrations (K^+ =80mM). Mean percentage relaxation of guinea pigs aortas treated with various doses of *P. guava* ethanol leaf extracts (2, 4, 6, 8 and 10 mg/mL) is shown in (Figure 4.5). The mean percentage relaxation of guinea pig aorta treated with 2mg/mL of extract was found to be 5.72± 3.00% and it was not significantly relaxed (p=0.536 as compared with the baseline contraction). However, treatment of isolated aortas with increasing doses of leaf extract caused significant relaxation of aortas *ex vivo*. Percentage relaxation of aortas at higher doses of extract were 22.95±2.50% for 4 mg/mL extract; 49.55± 3.50 % for 6 mg/mL extract; 82.35± 4.10% for 8 mg/mL extract; and 99.37± 2.90% for 10 mg /mL extract), ($p<0.05$ for extract doses between 4mg/mL and 6 mg/mL extract and $p<0.001$ for 8mg/mL and 10 mg/mL extract). This was dose-dependent, with higher doses of extract causing progressively greater relaxation of aortic muscle than lower doses (Figure 4.5).

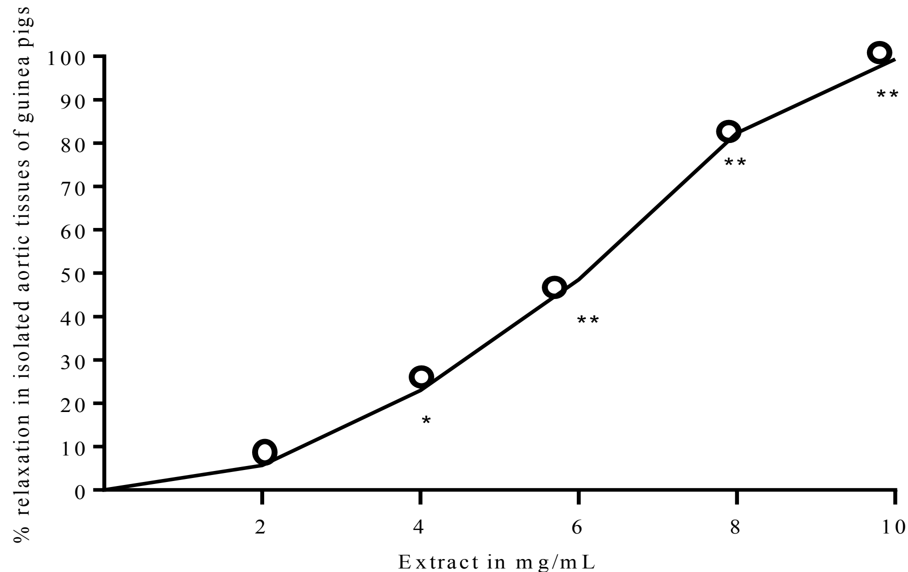


Figure 4.5: Effect of *P. guava* leaf extracts on contraction of aortic tissue of guinea pigs after inducing aortic smooth muscle contraction with 80 mM potassium chloride.

Thoracic aortas were removed from each of six guinea pigs and placed *ex vivo* into an organ bath containing 80mM potassium chloride, to induce maximal aortic muscle contraction (see Materials and Methods).Comparison was done with one way ANOVA, and p <0.05 was considered significant, *=p<0.05, **=p<0.001, O=point of treatment.

4.2 Effect of ethanol leaf extract of *P .guava* on fructose fed obese mice

4.2.1 Effect of ethanol leaf extract of *P. guava* on weight of mice

There were five randomly selected groups of mice for the experiment, in which one group of mice were fed standard pellet diet while the other four groups were fed a high fructose diet (42% fructose). After 4 weeks, mice in the high-fructose diet groups were treated by oral gavage with (100, 200 and 300 mg/kg of *P. guava* leaf extract once daily for another additional 3 weeks, while maintaining them on the high-fructose diet. The mean weight of fructose-fed mice was 28.17g± 2.13 at day zero, 36.67g ±1.51 after 4 weeks, and 40.33g ± 1.21 after 7 weeks. The mean weight for mice fed standard diet alone was 27.17g ±2.14 at day zero, 29.17g ± 2.14 after 4 weeks and 31g ± 1.26 after 7 weeks. There was significant ($p<0.05$, $p<0.001$) gain of weight in only fructose fed mice as compared to mice fed standard diet of mice (Figure 4.6).

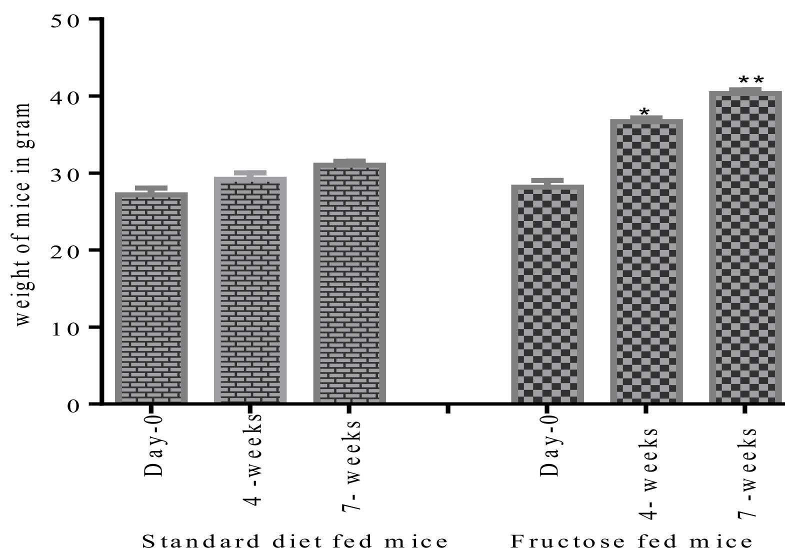


Figure 4.6: Effect of fructose feeding on body weight of mice.

Mice fed standard pellet diet and high-fructose diets were weighed on day zero, and at the end of 4 and 7 weeks.

*=$p<0.05$, .The comparison was done by one-way ANOVA, independent t-test and $p<0.05$ was considered as statically significant.

Mice fed with a high-fructose diet and treated with 100mg/kg of *P. guava* leaf extract showed significant reduction(p<0.05) in mean body weight during the three week period, (35.67 ±1.51 g) as compared with the fructose fed control mice (mean weight = 40.33±1.21 g). Similarly, mice fed with a high fructose diet and treated with 200 and 300 mg/kg of ethanol extract of *P. guava* leaf had a significantly reduced (p<0.001) in their mean body weight (33.67 ±2.25 g and 33 ±1.41 g at 3 weeks) as compared with the fructose fed control (40.33±1.21 g), respectively (Figure 4.7).

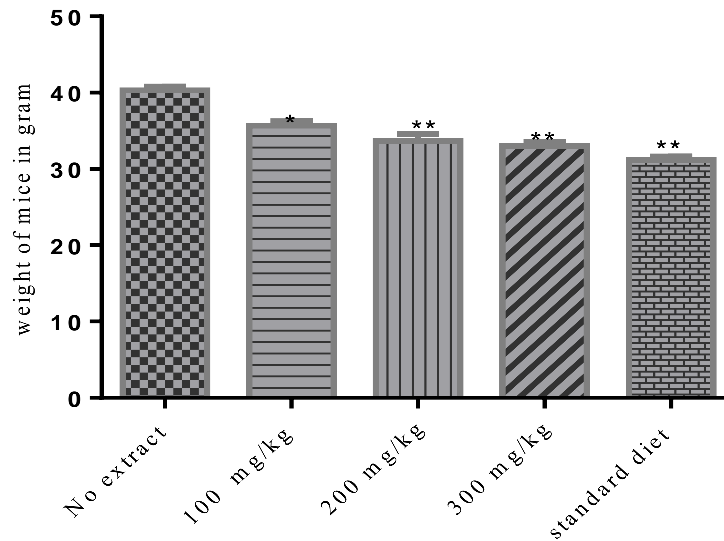


Figure 4.7:Effect ethanol extract of *P.guava* leaf on weight of fructose fed mice

Groups of six mice were fed a high-fructose diet for 4 weeks, then treated once daily by oral gavage with different doses of *P .guava* leaf extract for a further 3 weeks, while being maintained on the high-fructose diet. At the end of the three –week treatment period, mice were weighed. Mice fed a standard pellet diet for a total of 7 weeks are shown for comparison.

*=P<0.05, **= p<0.001, the comparison was done with independent t-test and one-way ANOVA and p<0.05 was considered as statically significant.

4.2.2 Effect of *P. guava* leaf extracts on serum lipid profiles of mice

The serum lipoprotein, high density lipoproteins (HDL) cholesterol, total serum cholesterol (TC) and serum triglycerides (TG) were measured by using a chemistry analyzer. Low density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation, to evaluate the effect of fructose and *P .guava* leaf extract on lipid profiles of fructose fed mice.

The total serum cholesterol (TC) of fructose fed mice was found to be 192.67 ±15.40 mg/dL. Fructose-fed mice treated with graded doses of *P. guava* leaf extract (100, 200 and 300 mg/kg) showed significant reductions ($p<0.001$) in their total serum cholesterol level (144.83 ± 13.19, 120.67 ± 14.80 and 119.17 ± 2.51mg/dL, respectively) after three weeks of treatment as compared with the fructose fed mice (Figure 4.8).

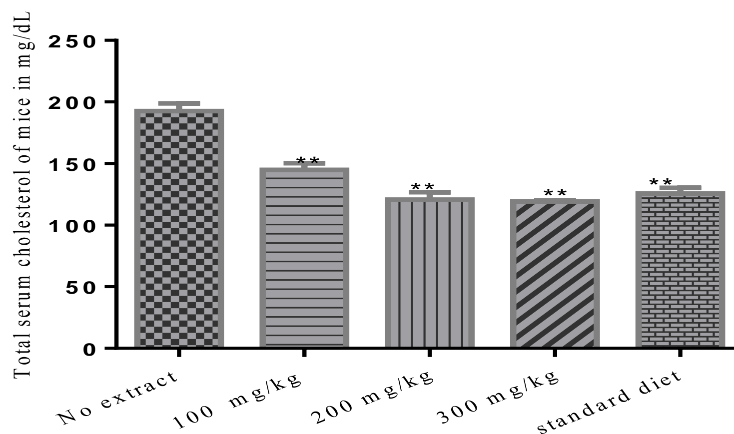


Figure 4.8 :Effect of *P.guava* leaf extract on total serum cholesterol level in fructose fed mice

Mice fed a high fructose diet for 4 weeks were treated with increasing doses of *P. guava* leaf extract for a further 3 weeks, and then serum total cholesterol levels were measured. During the three-week extract-treatment period, mice were maintained on the high-fructose diet. Cholesterol levels of mice fed a standard pellet diet for 7 weeks are shown for comparison.

**= $p<0.001$, the comparison was done with independent t-test and one-way ANOVA and $p<0.05$ was considered as statically significant.

The mean serum triglycerides (TG) of high-fructose fed mice was found to be 173.84± 6.96 mg/dL. High-fructose fed mice treated with increasing doses of *P. guava* leaf extract at 100, 200 and 300 mg/kg showed significant reductions ($p<0.001$) in their serum triglycerides, (91.67 ± 7.71, 67.67 ± 16. 31, 54.67 ± 6.97mg/dL, respectively) after three weeks of treatment as compared with the fructose fed mice (Figure 4.9).

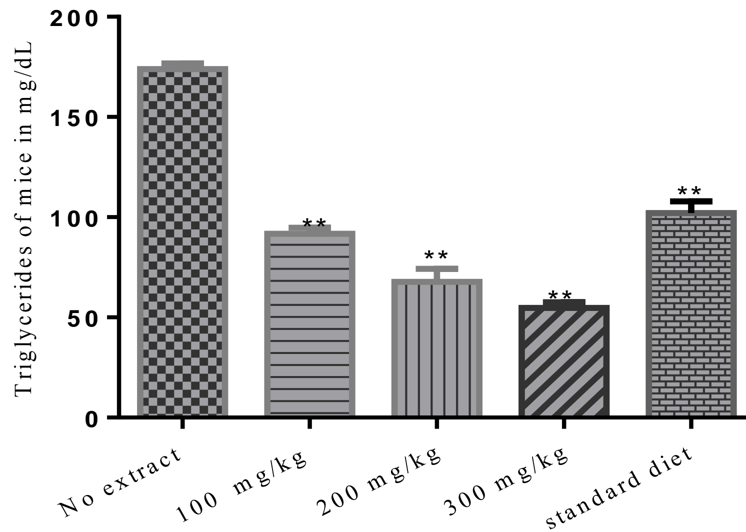


Figure 4.9: Effect of *P.guava* leaf extract on serum triglyceride in fructose fed mice

Mice fed a high fructose diet for 4 weeks were treated with increasing doses of *P. guava* leaf extract for a further 3 weeks, and then serum triglyceride levels were measured. During the three-week extract-treatment period, mice were maintained on the high-fructose diet. Triglyceride levels of mice fed a standard pellet diet for 7 weeks are shown for comparison.

**=$p<0.001$, the comparison was done with independent t-test and one-way ANOVA and $p<0.05$ was considered as statically significant.

The serum High Density Lipoprotein cholesterol (HDL-C) level of fructose fed mice was found to be (44.17 ± 8.01 mg/dL. Fructose-fed mice treated with 100mg/kg of *P. guava* did not show a significant difference in their serum high density lipoproteins cholesterol levels (48.17 ± 16.06 mg/dL) as compared with fructose fed mice (44.17 ± 8.01 mg/dL). However, fructose-fed mice treated with 200 and 300mg/kg of *P .guava* ethanol extract showed significant increases (p<0.001) in high density lipoproteins cholesterol level (73.50 ± 8.21 and 75.67 ±1.83mg/dL, respectively) as compared with fructose fed mice (44.17 ± 8.01mg/dL) (Figure 4.10).

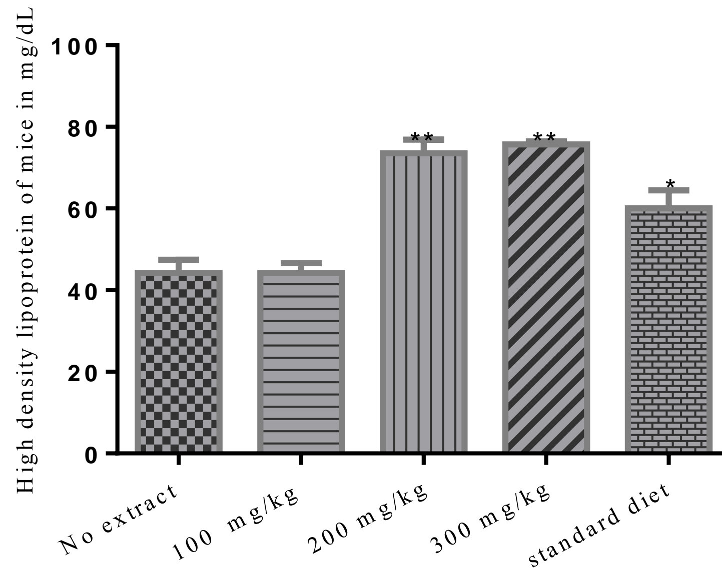


Figure 4.10: Effect o of *P.guava* on serum high density lipoprotein cholesterol in fructose fed mice

Mice fed a high fructose diet for 4 weeks were treated with increasing doses of *P. guava* leaf extract for a further 3 weeks, then serum high density lipoprotein (HDL) cholesterol levels were measured. During the three-week extract-treatment period, mice were maintained on the high-fructose diet. Serum HDL levels of mice fed a standard pellet diet for 7 weeks are shown for comparison.

*=P<0.05, **= p<0.001, the comparison was done with independent t-test and one-way ANOVA and p<0.05 was considered as statically significant.

The mean Low Density Lipoprotein cholesterol (LDL-C) of fructose fed mice was found to be 97.06± 9.43 mg/dL. Fructose fed mice treated with graded doses of *P. guava*leaf extract (100, 200 and 300 mg/kg) showed significant reduction ($p<0.001$) in their serum low density lipoproteins cholesterol levels (51.20 ± 15.98 mg/dL, 58.80 ± 10.49 mg/dL and 33.01 ± 3.96 mg/dL, respectively) after three weeks of treatment as compared with the fructose fed mice(Figure 4.11).

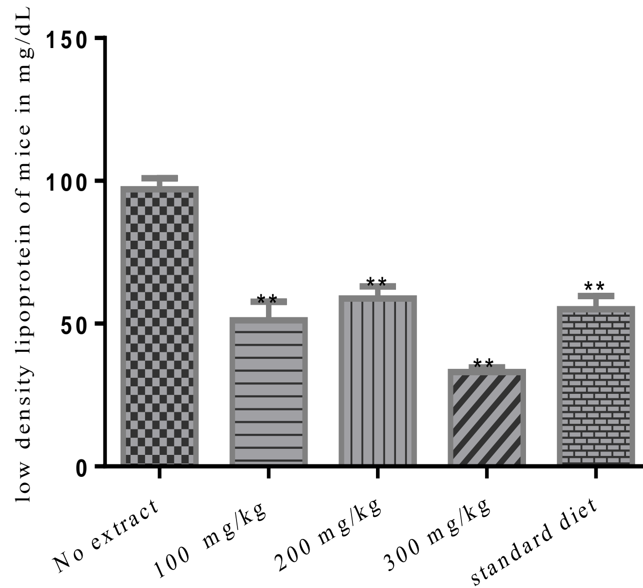


Figure 4.11: Effect of *P.guava* leaf extract on serum low density lipoprotein cholesterol in fructose fed mice

Mice fed a high fructose diet for 4 weeks were treated with increasing doses of *P. guava* leaf extract for a further 3 weeks, then serum low density lipoprotein (LDL) cholesterol levels were determined using the Friedewald equation. During the three-week extract-treatment period, mice were maintained on the high-fructose diet. Serum LDL levels of mice fed a standard pellet diet for 7 weeks are shown for comparison.

**=$p<0.001$, the comparison was done with independent t-test and one-way ANOVA and $p<0.05$ was considered as statically significant.

4.2.3 **Effect of *P. guava* extract on serum liver enzymes**

The major serum liver enzymes (aspartate aminotransferase,alanine aminotransferase andalkaline phosphatase) were measured to assess the effect of fructose and *P.guava* leaf extract on the liver function of mice fed a high fructose diet.

The mean serum aspartate aminotransferase level of control fructose fed mice was found to be 566.33 ± 22.60 U/L. Fructose-fed mice treated with 100 and 200mg/kg of *P. guava* did not show a significant difference in serum aspartate aminotransferase level (522.17 ± 65.28 U/L, 471.17 ± 20.32 U/L, respectively) as compared with control fructose fed mice (566.33 ± 22.60 U/L). However, fructose-fed mice treated with 300mg/kg of *P. guava* leaf extract showed significant reduction (p<0.001) in mean serum aspartate aminotransferase level (310.67 ± 15.10 U/L) as compared with control fructose fed mice (566.33 ± 22.60 U/L) (Figure 4.12).

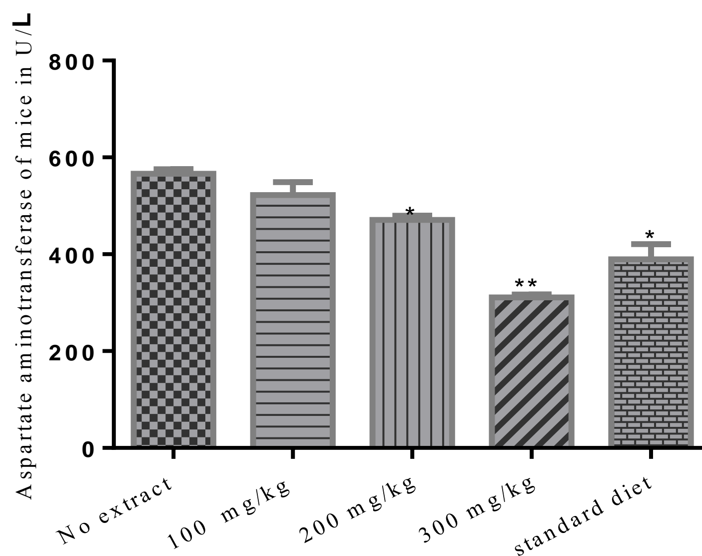


Figure 4.12 Effect of *P.guava* leaf extract on serum aspartate aminotransferase in fructose fed mice

Mice fed a high fructose diet for 4 weeks were treated with increasing doses of *P.guava* leaf extract for a further 3 weeks, and then serum aspartate aminotransferase (AST) levels were measured. During the three-week extract-treatment period, mice were maintained on the high-fructose diet. Serum AST levels of mice fed a standard pellet diet for 7 weeks are shown for comparison.

*=p< 0.05, ** =p<0.001, the comparison was done with independent t-test and one-way ANOVA and p<0.05 was considered as statistically significant.

The mean serum alanine aminotransferase (ALT)level of fructose fed mice not receiving leaf extract was found to be 184 ± 12.97 U/L. Fructose-fed mice treated with 100, 200 and 300 mg/kg of *P. guava* extract showed significant reductions ($p<0.05$, $p<0.001$) in mean serum alanine aminotransferase level (111.83 ± 14.73 U/L, 70.33 ± 10.70 U/L and 75.17 ± 1.91 U/L, respectively) as compared with control fructose fed mice(184 ± 12.97 U/L) (Figure 4.13)

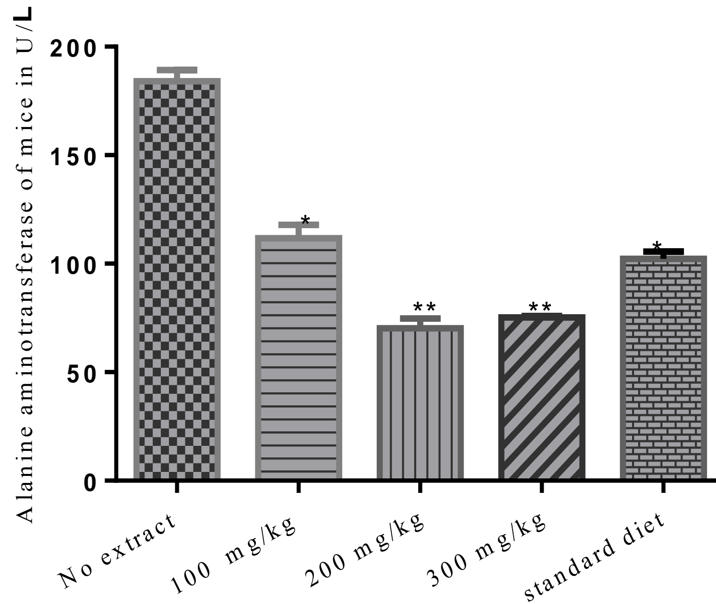


Figure 4.13: Effect of *P.guava* leaf extract on serum alanine aminotransferase in fructose fed mice

Mice fed a high fructose diet for 4 weeks were treated with increasing doses of *P .guava* leaf extract for a further 3 weeks, and then serum alanine aminotransferase (ALT) levels were measured. During the three-week extract-treatment period, mice were maintained on the high-fructose diet. Serum ALT levels of mice fed a standard pellet diet for 7 weeks are shown for comparison.

*=P<0.05, **=p<0.001, the comparison was done with independent t-test and one-way ANOVA and p<0.05 was considered as statically significant

The mean serum alkaline phosphatase level of fructose fed mice not treated with leaf extract was found to be 411.83 ± 31.39 U/L. Fructose-fed mice treated with 100, 200 and 300 mg/kg of *P.guava* leaf extract showed significant reductions (p<0.001) in mean serum alkaline phosphatase level (225.83 ± 18.18, 170.33 ± 49.96 U/L and 165.17 ± 53.71U/L, respectively) as compared with control fructose fed mice that were (411.83 ± 31.39 U/L)(Figure 4.14).

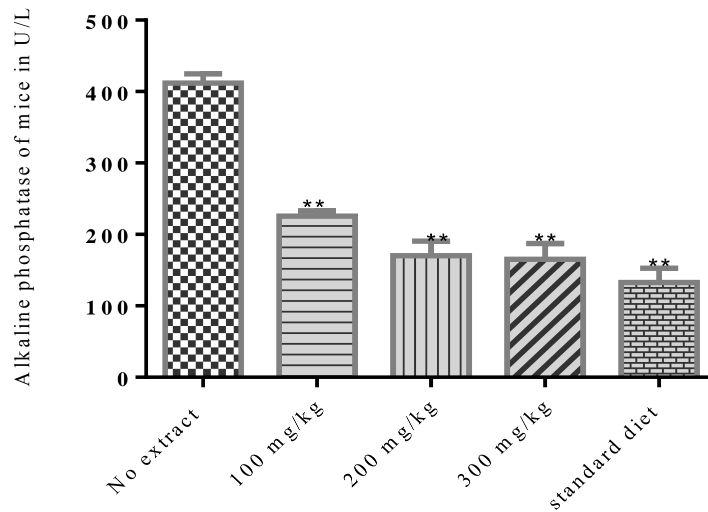


Figure 4.14: Effect of P .guava leaf extract on alkaline phosphatase in fructose fed mice

Mice fed a high fructose diet for 4 weeks were treated with increasing doses of P.guava leaf extract for a further 3 weeks, and then serum alkaline phosphatase (ALP) levels were measured. During the three-week extract-treatment period, mice were maintained on the high-fructose diet. Serum ALP levels of mice fed a standard pellet diet for 7 weeks are shown for comparison.

**= p<0.001, the comparison was done with independent t-test and one-way ANOVA and p<0.05 was considered as statically significant.

5 DISCUSSION

5.1 Effect of *P. guava* leaf extracts on blood pressure of guinea pigs

Hypertension a common health problem, affecting approximately one billion people globally, and accounts for 7.1 million deaths annually according to the report by Robitaille *et al.,* 2012. Study by Jellinger *et al.,* 2012 on dyslipidemia showed that, it affected one out of every two American adults. Hypertension and dyslipidemia are major cardiovascular risk factors and their prevalence is increasing throughout the world, including Ethiopia and the rest of sub-Saharan Africa, according Khan *et al.,* 2013; Mekoya, 2007, Twagirumukiza *et al.*, 2011.

According to Hassan, 2012, over three-quarters of the world population relies mainly on plants and plant extracts for health care this is because of ease of accessibility and low costs of plants. Plants may contain multiple medically useful products that act synergistically, and they may also act synergistically with synthetic pharmaceuticals. *P .guava* has many traditional medicinal uses in different countries, including Ethiopia. In Ethiopia, there are no major published studies of the effects of *P .guava* on except on anti-microbial and anti-inflammatory effect on rats, by Fanata *et al.,* 2010.

Both systolic and diastolic blood pressure of normal guinea pigs was reduced by intravenous treatment with *P. guava* leaf extract (Figures 4.1 and 4.2), and there was an equal effect on systolic and diastolic blood pressure, since the pulse pressure was unchanged by the leaf extract (Figure 4.3). However at leaf extract concentrations above 42 mg/kg, there seemed to be a possible trend towards a differential effect on systolic or diastolic blood pressure, though not statistically significant. To evaluate this further, higher concentrations of extract as well as greater numbers of guinea pigs could be used to evaluate a possible differential effect of *P. guava* leaf extract on systolic or diastolic blood pressure in guinea pigs.

The ethanol extract of *P. guava* leaf reduced systolic, diastolic and mean arterial blood in guinea pigs in a dose dependent manner (Figures 4.1, 4.2 and 4.4). This reduction may be due to the actions of lycopene, quercetin or other flavonoids present in the leaf of *P .guava*. Lycopene may reduce blood pressure due to its high antioxidant properties as a result of having different structural formulas used in attacking many free radicals and oxidized cholesterols. According to a report by Shivashankara and Acharya, 2010 on bioavailability of dietary polyphenols and the cardiovascular diseases, quercetin improved endothelial functions through inhibiting endothelial endothelin-1 (ET-1) and

promoting nitric oxide. Since endothelium-dependent vasorelaxation and proper endothelial function is largely dependent on the production and bioavailability of nitric oxide. Review by Larson *et al.,* 2010 on efficacy and mechanisms of quercetin in treatment of hypertension, shown vasorelaxation of isolated aortas of rats, by inhibiting the action of ET-1 at the gene transcription level, and promoted the production and action of nitric oxide on endothelium cells.

In the case of *ex vivo* experiments using isolated guinea pig aortas, high K^+ was applied to induced maximum contraction to the aortas. Ghayur and Gilani, 2005 described that K^+ at high doses (>30mM) induced smooth muscle contractions through opening of voltage-dependent slow Ca^{2+} channels, thus allowing influx of extracellular $Ca^{2+,}$ causing a contractile effect. The contracted of isolated aortic tissue of guinea pigs with high potassium chloride (K+=80mM) was not significantly relaxed (p=0.536) when the tissues were treated with 2 mg/mL of *P. guava* leaf extract as compared to baseline contraction, whereas significant ($p<0.05$, $p<0.001$) relaxation of aortas was seen with higher doses (4, 6, 8 and 10 mg/mL) of *P .guava* leaf extract.

Gilani *et al.,* 2010 described that smooth muscle relaxation can be achieved by various mechanisms such as potassium channel opening, calcium channel blocking and receptor antagonism. The present finding in (Figure 4.5) showed that *P. guava* leaf extract induces relaxation of contracted aortas with high K^+ (80 mM). The relaxation of aortas in this experiment may be due to components of *P. guava* leaf extract causing blockage of Ca^{2+} entry through voltage-dependent calcium channels. Relaxation of aortic tissue was due to blockage of Ca^{2+} entry as reported by Suresh *et al.,* 2006. A similar study was conducted by Chiwororo and Ojewole, 2008 on the effect of aqueous extract of *P .guava* leaf extract on rats‘ isolated aortas, showed significant relaxation in dose dependent manner.

5.2 Effect of *P. guava* leaf extracts on weight, lipid profiles and serum liver enzymes of fructose fed mice

The mean weight of the mice was significant increased ($p<0.001$) in 42% fructose fed mice as compared to mice fed standard diet at the end of fourth week and was even more pronounced at the end of the seventh week (Figure 4.6). This may be due to excess mitochondrial acetyl-CoA formation as a result of fructose metabolism, leading to *de novo* lipogenesis and formation of free fatty acids that may causes insulin and leptin resistance, which results further stimulation of intake of fructose. Study by Bocarsly *et al.,* 2010 on effect of high fructose feeding in weight of rats, showed similar

result. Similarly, review by Lim *et al.,* 2010on role of fructose in pathogenesis of NAFLD and the metabolic syndrome in , showed weight gain was obtained after feeding fructose.

The mean weight of the mice was significantly reduced ($p<0.05$, $p<0.001$) in fructose-fed mice treated with different doses of *P. guava* leaf extract, and this was more pronounced at higher extract doses (Figure 4.7). Weight reduction on treated mice may possibly be due to presence of gallic acid tannins, flavonoids, and lycopene that used in stimulation of glucose uptake and insulin sensitivity. A report by Shakeera *et al.,* 2013on the effect of *P .guava* leaf extract in alloxan-induced diabetic rats, showed weight reduction by inhibition of enzymes such as alpha-amylase, sucrase and maltase that are responsible for the formation of glucose from dietary products. Therefore *P. guava* leaf extract may possibly reduce weight in fructose fed mice through these mechanisms. However, further studies need to be done on fractionating biochemically *P. guava* leaf extracts to determine which particular biochemical components are responsible for the reduction in weight gain seen.

Total serum cholesterol, triglyceride and low density lipoprotein cholesterol were significantly increased ($p<0.001$) in fructose fed mice as compared to mice fed standard diet (Figures 4.8, 4.9 and 4.11), and the level of high density lipoprotein cholesterol was reduced fructose fed mice as compared to mice fed standard diet. This may be due to positive association of fructose in the production of total serum cholesterol, triglyceride and low density lipoprotein cholesterols. According to a report by Bocarsly *et al*., 2010, feeding high fructose significantly increase in body fat and triglyceride levels in rats. As a result feeding fructose may increase body fats, total serum cholesterol and triglycerides levels in mice.

Result in this part of the study, showed that there was significant reduction ($p<0.001$) in total serum cholesterol of fructose fed mice treated with extract of *P.guava* leaf as compared to that of control fructose fed mice (Figure 4.8). A review by Barbalho *et al.,*2012 on plant of multipurpose medicinal applications, described one possibility, that *P.guava* leaf extract reducing total serum cholesterol may be due to the presence of rutin and kaempferol, gallic acid, catechin, epicatechin or other components in leaf of *P .guava*, though again, further biochemical studies of fractionated components of leaf extract will need to be done to identify the particular components involved in this and other beneficial effects of dyslipidemia. As it was report by Zhao *et al.,* 2012, other possible explanation for the observed decrease in serum total serum cholesterol may be the ability of vitamin C to activate the enzyme, 7 α-hydroxylase, which enhance the conversion of plasma cholesterol to bile acids, thereby reducing the serum cholesterol levels. Similarly a study done by Shinde *et al.,* 2013 on effect

of *P.guava* leaf extracts in hyperlipidemic rats, showing significant decreases in level of total serum cholesterol. Thus ethanol extracts of *P.guava* leaf may reduce total serum cholesterol level by inhibiting the action of enzymes such as HMG-CoA reductase and pancreatic cholesterol esterase and activating bile acid formation.

Likewise serum triglyceride was significantly reduced ($p<0.001$) in extract-treated fructose fed mice as compared with non-treated fructose fed mice (Figure 4.9). Quercetin may suppress triglyceride formation by inhibiting diacylglycerol acyltransferase and acetyl-CoA carboxylase activity responsible for the synthesis of triglyceride. Shakeera *et al.,* 2013 found similar result with this result after conducted study on the effect of *P. guava* leaf extract in alloxan-induced diabetes rats.

High density lipoprotein cholesterol was significantly increased ($p<0.001$) in fructose-fed mice treated with 200 and 300 mg/kg daily of *P. guava* leaf extract, as compared to that of fructose fed mice not receiving leaf extract (Figure 4.10). The reduction in other cholesterols especially triglycerides and low density lipoproteins cholesterol may increase the level of high density lipoprotein cholesterol because high density lipoprotein cholesterol may exist free if there is less ―bad” cholesterol in our body . There was no significant raise in high density lipoprotein in mice treated with 100mg/kg of leaf extract. A study done by Nor and Yatim, 2010 on the beneficial lipid-lowering effects of pink guava puree in high fat diet-induced obese rats, showed significant increases in high density lipoproteins.

As it was described by Uboh *et al.,* 2013, low density lipoprotein cholesterol is another form of cholesterol that causes damage to body, especially when it is oxidized. Oxidation of low-density lipoproteins (LDL) is considered by many sources to be a very important early component in the development of atherosclerotic lesions. There was a statistically significant reduction ($p<0.001$) in low density lipoprotein cholesterol in extract-treated fructose-fed mice as compared to non-treated fructose fed mice (Figure 4.11). Bahraini *et al.,* 2012 described that antioxidants which interrupt oxidation of low density lipoprotein cholesterol, can be very helpful in the process of preventing and/or treating cardiovascular disease. As it was reported by Khan, 2014, the mechanism by which flavonoids reduce LDL is not totally known, but it is thought that they reduce free radical formation, protect LDL-α-tocopherol or regenerate oxidized LDL-α-tocopherol, and/or sequester metal ions which participate in oxidation reactions.

As shown in Figures (4.8, 4.9 and 4.11), there were significant reductions of total serum cholesterol, triglycerides and low density lipoproteins cholesterol even below the normal values, at higher leaf extract doses. The high density lipoprotein cholesterol was significantly increased in extract-treated mice even more than that of normal mice. Similarly Shined *et al.,* 2013 described that *P. guava* leaf extracts lowering total serum cholesterol, triglycerides and low density lipoproteins cholesterol and increases of high density lipoprotein cholesterol in hyperlipidemic rats below normal levels. This suggests that higher doses of *P. guava* leaf extract may alter serum cholesterol levels even in normal mice.

Serum liver enzymes tests are commonly used to screen for liver diseases monitor the progression of known disease and monitor the effects of potentially hepatotoxic toxins. In this study, the serum liver enzymes, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase, were significantly ($p<0.001$) increased in mice fed fructose as compared to mice fed normal standard pellet diet (Figures 4.12, 4.13 and 4.14). This change in serum liver enzymes may be due to damage of hepatocytes as a result of feeding fructose. Fructose may be involved in the pathogenesis of NAFLD and the metabolic syndrome; an excess fructose load leads to increased acetyl-CoA and malonyl-CoA formation, which inhibits mitochondrial beta-oxidation so promoting *novo* lipogenesis. A review by Lim *et al.,* 2010 on the effect of fructose in involved in the pathogenesis of NAFLD and the metabolic syndrome; showed formation of intra-hepatic lipid deposits, leading to liver disease.

The present study showed that there was significant reduction ($P<0.05$, $p<0.001$) in aspartate aminotransferase (AST) activity in fructose-fed mice treated with 200 and 300 mg/kg of *P. guava* leaf extract, as compared to fructose fed mice that were not treated with extract (Figure 4.12). *Psidium guava* leaf extract may possibly reduce liver toxicity by attacking free radicals, oxidized low density lipoprotein cholesterols and inhibiting diacylglycerol acyltransferase and acetyl-CoA carboxylase activity responsible for the synthesis of triglyceride as well. Similarly Roy and Das, 2011 reported that *P. guava* leaf extract protects liver damage in dose dependent manner on paracetamol (PCM) induced acute liver injury in rats.

As illustrated in Figure 4.13, the level of alanine aminotransferase (ALT) was also significantly reduced ($p<0.05$, $p<0.001$) fructose-fed mice treated with *P. guava* leaf extract, in dose dependent manner. This also may be possibly be due to antioxidant effects of some components in the leaf of *P.guava* such as ascorbic acid, lycopenes, rutin and extra compounds have ability to scavenge free radicals and oxidized cholesterols. These results agree with a previous report by NHS, 2010, on the

effects of pink guava puree supplementation, showing antioxidant activities in spontaneous hypertensive rats, showed significant reduction in ALT in the treated groups when compared to control group. As a result *P.guava* leaf extract may reduce alanine aminotransferase level in treated mice as compared to non-treated fructose fed mice due to ability of *P .guava* to attack free radicals and oxidized cholesterols that cause liver damage. The level of alanine aminotransferase was slightly lowered in mice treated with 200mg/kg as compared to mice treated with 300mg/kg per day. This is may be due to activeness of mice or other unknown factors.

The level of alkaline phosphatase also significantly reduced ($p<0.05$, $p<0.001$) in treated mice as compared to mice fed only fructose fed mice in dose dependent manner (Figure 4.14). These again may be due to different ant-oxidants in *P. guava* leaf extract that mage protect liver from damage with existence free radicals and oxidized cholesterols. This result is consistent with the result obtained by NHS, 2010, from effects of pink guava puree supplementation on antioxidant enzyme activities and organ function of spontaneous hypertensive rats.

As it was observed in Figures (4.12, 4.13 and 4.14), there is significant reductions ($p<0.05$) in all liver enzymes of extract-treated mice, especially with the dose of 300 mg/kg. This indicates that *P .guava* leaf extract may causes damage at higher doses by inactivating or inhibiting the action of enzymes below normal activity. Saguibo *et al.,* 2012 reported that *P. guava* leaf extract was hepato-protective at lower doses and hepato-toxic at higher dose on erythromycin-induced liver damaged rats.

6 CONCLUSION

From this study, it can be concluded as fructose fed mice increases body weight, levels of total serum cholesterol, triglycerides and low density lipoprotein cholesterols whereas reduces the level of high density lipoprotein cholesterol by increasing hepatic lipid droplet formation or hepatic insulin resistance, which in turn promotes substrate deposition into fat.

Psidium guava leaf extract reduced blood pressure in normal guinea pigs and induced relaxation to aortas contracted with the addition of high K^+ (80 mM) in dose dependent manner. The extract also has reducing effect on body weight, LDL-cholesterols, and serum enzymes and increasing high density lipoprotein as well, in fructose fed mice again dose dependent manner. High dose of *P. guava* leaf extract may have toxicity, since it reduced the levels of cholesterols and enzymes even more than normal levels.

The treatment quality of *P .guava* leaf extract is may be due to having many phenolic compounds within the leaf that used as antioxidant, calcium channel blockers, improver of endothelial functions, inhibiting some enzymes and other mechanism.

7 RECOMMENDATION

The prevention and treatment of hypertension and dysilipidemia is crucial in preventing the cardiovascular diseases, which are dangerous health problems worldwide. Thus from this study it is recommended that:-

- The extracting and identifying phytochemical compounds of *P. guava* leaf extract should be done to identify specific chemical compound which is responsible for treatment of hypertension, dyslipidemia and hepato-protectivity.
- The effect of *P.guava* leaf extract on weight has to be conducted to identify weather it prevent weight gain or induces weight loss.
- The exact chemicals, dose and toxicity of the chemicals that are important in treatment of hypertension, dyslipidemia and hepato-protectivity have to be checked before it used by man; therefore many studies should be conducted regarding these aspects of *P.guava* leaf.
- The other parts of this plant should be done comparatively to that of the leaf in order to identify best part of this plant in treatment of hypertension, dyslipidemia and hepato-protectivity.
- Action the exact action of mechanism of *P.guava* in treatment of hypertension, dyslipidemia should be evaluated in future.
- The effect of *P.guava* in treatment of both hypertension and dyslipidemia should be carried out in same animal model to evaluate exact correlation between hypertension and dyslipidemia.
- Other studies have to be conducted in more guinea pigs and extra doses of *P.guava* leaf to identify wither pulse pressure change could be significant.

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