ANTIOXIDANT AND ANTIHYPERLIPIDAEMIC POTENTIALS OF ETHANOLIC LEAF EXTRACT OF *Diodia sarmentosa* ON WISTAR RATS

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CERTIFICATION PAGE

This is to certify that this work: ***Antioxidant and Antihyperlipidaemic potentials of Ethanolic leaf extract of Diodia sarmentosa on Wistar rats*** was done by STEPHEN CHINEDUMIJE KORIE of registration number 20164994698 in the department of Biotechnology, School of Biological science, in partial fulfillment of the requirements for the award of Master of Science (MSc) degree in biotechnology.

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DEDICATION

I dedicate this work to the my family, starting with my parents Mr & Mrs. Stephen N.C Korie, and my siblings Chinwendum, Chisom, Chijiokem and Chimaobim for all their prayers, encouragements, and pray the good lord bless and keep us to reap the fruit of our labour.

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ABSTRACT

This study investigated the antioxidant and antihyperlipidaemic potentials of ethanolic leaf extract of *Diodia sarmentosa* on high fat diet-induced male wistar rats. The antioxidant potentials of the extract was ascertained by scavenging for hydroxyl and nitric oxide radicals. Thirty (30) male wistar rats (150g-200g) were divided into five (5) groups; Negative control (NC) group which was not induced nor treated, Positive control (PC) was induced but not treated, Low dose extract (LDE) was treated with 250mg/kg of the extract, High dose extract (HDE) was treated with 500mg/kg of the extract and Standard antihyperlipidaemic drug (SAD) group treated with 5mg/kg of Simvastatin. Hyperlipidaemia was induced by feeding the rats with high fat diet in form of Ghee and Coconut oil in the ratio of 3:1 for a period of six (6) weeks, and administration of the treatments started in the 4th week till the 6th week. Parameters like Superoxide dismutase (SOD), Catalase (CAT), Malondialdehyde (MDA), Glutathione peroxidase (GPx), Glutathione S-transferase (GST) and Lactate dehydrogenase (LDH) were assessed in the serum and heart tissue. In the serum, Lipid profiles like Total cholesterol (TC), High density lipoprotein cholesterol (HDL), Low density lipoprotein cholesterol (LDL), Very low density lipoprotein cholesterol (VLDL), Triglycerides (TG) and Atherogenic coefficient and indices were also assessed. Liver enzymes and other biomarkers like Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), Total Bilirubin (TB), Total Protein (TP), Albumin and Globulin were also assessed. Results obtained showed that extract inhibited nitric oxide and hydroxyl radicals in a concentration dependent manner, showing an inhibitory concentration at 50% (IC_{50}) of 907.17µg/ml ± 45.36 and 2173.44 µg/ml ± 100.11 respectively. Both doses of the extract increased SOD, GPx and CAT activities in the serum and heart tissue, but increase in CAT activity in the heart by the low dose was not significant compared to the PC. GST activities was decreased in both heart tissue and serum, but only the decrease in the heart tissue by the high dose was significant, and MDA levels was significantly decreased by both dose in the heart tissue and serum. LDH activity in the heart tissue was not significantly different in the LDE and HDE groups, while in the serum, LDH activity was significantly different in the LDE and HDE groups when compared to the PC group. The concentration ranges of the lipid profiles of the various study groups are; TC (80.53–177.25 mg/dl), TG (45.64–159.74 mg/dl), HDL (35.18–57.80 mg/dl), LDL (18.09–36.77 mg/dl) and VLDL (9.12–31.59 mg/dl). TC, TG, LDL, VLDL and Atherogenic indices and coefficient were significantly decreased ($p<0.05$) in the LDE and HDE groups, while HDL was significantly increased when compared to the PC group. Percentage Protection of the extract were 61.76% and 79.17% for the low and high dose respectively. ALT (22.35–32.88 U/L) and AST (34.31–45.91 U/L) activities, Total Bilirubin (0.27–0.34 mg/dl) and serum Globulin (30.39–37.60 g/l) levels were not significantly different ($p>0.05$) in the LDE and HDE groups when compared to the PC group, while Total protein (54.05–67.78 g/l) levels were significantly different ($p<0.05$) in the LDE and HDE groups when compared to the PC groups. ALP (207.69–351.21 U/L) activity and Albumin (23.66–33.50 g/l) levels in the LDE group was not significantly different ($p>0.05$), while in the HDE groups they were significantly different ($p<0.05$) when compared to the PC group. The efficacy of the extract in balancing lipid profiles, enzymes levels and oxidative stress indices showed little or no difference to the Simvastatin used a the standard antihyperlipidaemic drug, which accounts for its antioxidant and antihyperlipidaemic potentials.

Keywords: High fat diet, Scavenging activities, Antihyperlipidaemia, Oxidative stress parameters, Lipid profiles and Liver enzymes

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

INTRODUCTION

1.1 Background of study

Cardiovascular diseases (CVD) are non-communicable diseases which are among the leading causes of mortality globally. Sa'adah *et al.* (2017) reported that the World health Organization (WHO) predicts that by 2030, an estimated number of 23.3 million human deaths would be as a result of cardiovascular diseases (Sa'adah, Purwani, Nurhayati & Ashuri, 2017).

Hyperlipidaemia is known as one of the major risk factors that adds to the occurrence and advancement of cardiovascular diseases like atherosclerosis, cardiac infractions, coronary arterial disease, hypertension etc. Hyperlipidaemia is a disorder that is characterized by an abnormal lipoprotein metabolism, which includes excessive or insufficient amounts of lipoprotein. This is evident in the clinical appearance of elevated concentrations of serum total cholesterol, triglycerides, and low-density lipoprotein (LDL) cholesterol, and a decline in high density lipoprotein (HDL) cholesterol (Rajesham & Bhikshapathi, 2018). Hyperlipidaemia encompasses of hypercholesterolemia (abnormal concentration of cholesterol in the blood), hypertriglyceridemia (abnormal concentrations of triglycerides in blood), and dyslipidaemia (irregular lipoprotein metabolism i.e overproduction or deficiency of lipoprotein) (Karan, Yang & Li, 2017). Hyperlipidaemia arises from the constant consumption of high fat-rich diets, increasing the level of cholesterol in the body, and clogging the arteries with fat deposits thus leading to atherosclerosis, hypertension, and sudden death (Rajesham & Bhikshapathi, 2018).

Grundy *et al.* (2019) recommended guidelines for the management of blood cholesterol involving lifestyle modifications like regular exercise, reduction in the intake of diets rich in high fat, and consumption of heart healthy diets like omega-3s, fiber, fruits and veggies

(Grundy *et al.,* 2019). Also, the use of medications like statin drugs: simvastatin, atorvastatin, lovastatin, and pitavastatin, and nonstatin drugs: ezetimibe, cholestyramine, niacin, and gemfibrozil have been effective in treatment of hyperlipidaemia (Grundy *et al.,* 2019). However these conventional therapeutic drugs possess certain disadvantages like being costly, and side effects like hepatotoxicity, hyperuricemia and myositis (Rajesham & Bhikshapathi, 2018).

Hence, the research for the creation of novel drugs with fewer side effects and cost effective has emerged (Etuk, 2006). The focus of the emergence of novel drugs, has been shifted to natural products especially plant derivatives because they are considered safe, non-toxic with lesser side effects (Olisa & Oyelola, 2009). Several medicinal plants have been reported by different researchers for the management of cardiovascular diseases, they include; *Vernonia amygdalina, Parinari Curatellifolia, Psidium guajava, Bryophyllum pinnatum, Persea americana, Hibsicus sabdariffa, Coriandrum sativum, Loranthus micranthus* etc (Oyewole, 2005; Taiwo, Odeigah, Jaja & Mojiminiyi, 2010; Iwalokun, Hodonu, Nwoke, Ojo & Agomo, 2011).

Diodia Sarmentosa (Sw) commonly known as Zimbabwe flora or Tropical button weed is a straggling or procumbent perennial herb. It is from the family of rubiaceae with length of 1-4m long, often with many lateral branches from the main stem. Its Stems are up to 4m long, distinctly 4-angled and hairy on the angles. The leaves are opposite and up to 6.5cm long, its colour can be green to yellowish-green, with rough tubercle based hairs. It grows in evergreen forest particularly fringing 'mushitu' edges, open riverine vegetation, bush land and also on rocky places near rivers (Umoh, Ajibesin & Ubak, 2016). The antiulcer potential of *Diodia sarmentosa* (whole plant) has been reported by Akah, Orisakwe, Gamaniel & Shittu (1998). Umoh, Ajibesin & Ubak (2016) demonstrated the anti-inflammatory and analgesic activities of

Diodia sarmentosa (Sw), Ijomone & Ekpe (2018) reported on the anti-diabetic potential and phytochemicals of *Diodia sarmentosa* (Sw), but there is no data supporting the antioxidant and antihyperlipidaemic potential of the plant.

1.2 Problem Statement

Hyperlipidaemia is an abnormal condition characterized by high levels of cholesterol in the body resulting from the consumption of diets highly rich in fat. When left untreated, cholesterol in the form of fat deposits can block the blood vessels (atherosclerosis), thereby obstructing the flow of blood to the heart causing an increase in blood pressure (hypertension), and also resulting in other cardiovascular diseases like coronary heart disease, cardiac arrest, and sudden death (Sa'adah *et al.,* 2017).

According to World Health Organization (WHO) in 2021, an estimated 17.9 million people die annually from cardiovascular diseases. This estimated figure is approximately 32% of all deaths globally, with more than 75% of cardiovascular disease-related deaths occurring in underdeveloped and developing countries, and almost 80% resulting from stroke and heart attack (WHO, 2021).

In Nigeria, hypercholesterolaemia a form of hyperlipidaemia which is a significant cause of cardiovascular disease is reported to have a prevalence of about 38% of which it is higher in women at 42% compared to men at 38%. The prevalence is at its highest in the South-South region at 53% and lowest in the South-West region at 3% (Adeloye *et al.,* 2020).

1.3 Aim and objectives of Study

The aim of this study is to determine the antioxidant and antihyperlipidaemic potentials of ethanolic leaf extract of *Diodia sarmentosa* (sw) on Wistar rats.

The specific objectives of the study

- To determine the free radical scavenging capacity of the ethanolic leaf extract of *Diodia Sarmentosa* (Sw) using hydroxyl and nitric oxide radical scavenging.
- To determine the antioxidant effect of ethanolic leaf extract of *Diodia Sarmentosa* (Sw) on Wistar rats using oxidative stress indices.
- To determine the antihyperlipidaemic potentials of ethanolic leaf extract of *Diodia Sarmentosa* (Sw) on Wistar rats using lipid profiles and liver enzymes.

1.4 Hypothesis

Null hypothesis (H0): *Diodia Sarmentosa* (Sw) has no antioxidant and antihyperlipidaemic effect.

Alternative hypothesis (H1): *Diodia Sarmentosa* (Sw) has antioxidant and antihyperlipidaemic effect.

1.5 Justification of Study

Hyperlipidaemia is a 'silent killer' that has no overt symptoms. However, familial or inherited hyperlipidaemia may be symptomatic, patients may develop yellow, fatty growth around the eyes or joints (Davis, 2019). The various risk factors associated with hyperlipidaemia includes:

obesity, diabetes, and high levels of free radicals in the body. The appropriate approaches for preventing hyperlipidaemia are lifestyle, and dietary options. These involves the consumption of healthy diets good for the heart like fish, nuts, and legumes, avoid smoking, maintaining a healthy body weight, carrying out regular exercise. All the aforementioned approaches helps in reducing low density lipoprotein cholesterol (LDL-C), and increasing high density lipoprotein cholesterol (HDL-C) in order to prevent hyperlipidaemia (Davis, 2019). Various treatments have been employed in treating hyperlipidaemia using conventional therapeutics like statins which reduces cholesterol. However, there have been reported side effects like muscle pain, and muscle damage. This has warranted to the exploration of plants with medicinal properties as cheaper and safer alternatives (Adefegha, Olasehinde, & Oboh, 2016).

1.6 Scope of Study

This research work will only investigate the antioxidant and antihyperlipidaemic potentials of *Diodia Sarmentosa* (Sw) using lipid profiles, oxidative stress markers and biochemical indices.

CHAPTER TWO

LITERATURE REVIEW

2.1 Medicinal plants

There are significant evidences showing that various plants possess antioxidant features which makes them medicinal plants. These properties help in reducing heart-related diseases or diseases leading to cardiovascular diseases like hyperlipidaemia, hypercholesterolemia, hyperglycemia, diabetes, cardiac arrest, high blood pressure, congenital heart failure etc. Plants that possess these therapeutic values include; *Allium cepa*, *Allium porrum*, *Allium sativum*, *Aloe vera*, *Asparagus officinalis*, *cistanche tubulosa, citrus species, clitoria ternatea*, *cyperus rotuntdus*, *citrullus colocynthis* etc (Al-Snafi, 2016; Nku, Archibong, Udefa, Utionkpan & Akwari, 2018; Yang, Kim, Lee, Kim & Hwang, 2019).

2.1.1 *Ixora coccinea*

The ethanolic extracts of *Ixora coccinea* possesses photochemicals such as tannins and flavonoids. These phytochemicals in the extract are antioxidants which scavenged nitric oxide (NO) radicals. The nitric oxide radicals were scavenged in a dose dependent manner, i.e increase in extract concentration caused an increase in nitric oxide radical inhibition (Banerjee *et al.*, 2011).

2.1.2 *Cistanche tubulosa*

Shimoda *et al.* (2009) reported that the ethanolic root extract of *Cistanche tubulosa* possessed anti-hypercholesterolemic effect on mice induced with high cholesterol diet. It was showed that the extract suppressed the increase of high serum cholesterol in the mice, enhanced the

mRNA expressions of apolipoprotein B, VLDL receptor, and cytochrome P450 SCC in HepG2 hepatocytes.

2.1.3 *Citrullus colocynthis*

The seeds of *Citrullus colocynthis* was reported to possess hypolipidemic qualities in patients by lowering the serum cholesterol and triglycerides concentration in the patients (Rahbar & Nabipour, 2010; Al-Snafi, 2016). Also, the oil extracted from the seeds of *Citrullus colocynthis* were used to slow down body weight gain, serum cholesterol, triglycerides, ALP level, erythrocytes count, and increased AST levels in the test rats (Amamou, Bouafia, Chabane-Sari, Meziane & Nani, 2011).

2.1.4 *Clitoria ternatea*

Al-Snafi (2016) reported the anti-hyperlipidemic effect of *Clitoria ternatea* on hyperlipidemic induced rat. In the study, the hydroalcoholic extract of the seeds and roots of *Clitoria ternatea* showed a significant reduction of serum total protein, triglycerides, very low density lipoprotein and low density lipoprotein levels. It also normalized the atherogenic index and HDL/LDL ratio in the hyperlipidemic rats (Solanki & Jain, 2010).

2.1.5 *Coriandrum sativum*

The antihyperlipidaemic activity of fresh leaves of *Coriandrum sativum* was studied against salbutamol induced cardiac injury in rabbits. The methanolic leaf extract of *Coriandrum sativum* reduced serum total cholesterol, low density lipoprotein cholesterol, very low density lipoprotein cholesterol and triglycerides (Joshi, Sharma & Sharma, 2012). Also, the extract caused an elevation of high density lipoprotein cholesterol (HDL-C), glutathione (GSH) and

catalase activity in the test rabbits. The seed extract of *Coriandrum sativum* was successful in the prevention of in vitro low density lipoprotein (LDL) oxidation, lowered lipid indices and also prevented the progression of atherosclerosis (Al-Snafi, 2016).

2.1.6 *Crotalaria juncea*

The antihypercholesterolemic effects of ethanolic extract of *Crotalaria juncea* Linn (whole plant) was studied in rats fed high-fat diet. The extract showed a decrease in the levels of total cholesterol, triglycerides, low density lipoprotein cholesterol, very low density lipoprotein and also increased high density lipoprotein in the rats in a dose-dependent manner (Kumar, David, Harani & Vijay, 2014).

Also, the antihyperlipidaemic activity of alcoholic and methanol extract of leaves of *Crotolaria juncea* (CJ) was investigated against Triton induced hyperlipidaemia in mice. The both extract of *Crotolaria juncea* significantly decreased the levels of serum total cholesterol, triglyceride, low density lipoprotein, very low density lipoprotein and elevates high density lipoprotein cholesterol (Harikumar, Niveditha, Kumar, Monica & Gajendra, 2012).

2.1.7 *Aloe vera*

Aloe vera gel was investigated to have hepatoprotective effect and potential to treat hyperlipidaemia induced by high salt intake in rats. It was observed that *aloe vera* gel reduced triglycerides, total cholesterol, low density lipoprotein cholesterol and increased Aspartate aminotransferase and Alanine transaminase elevations (Nku *et al.,* 2018).

2.1.8 *Ricinus comminus*

Study showed that seeds of *Ricinus comminus* have antihyperglycaemic potential when used to treat alloxan induced diabetic rats. It reduced blood glucose level, total cholesterol, triglyceride and increased high density lipoprotein cholesterol levels.

2.1.9 *Garcinia daedalanthera*

The result from the study of the antihyperlipidaemic property of extract *Garcinia daedalanthera* on hyperlipidemic rats showed it reduced total cholesterol, low density lipoprotein cholesterol, triglycerides and also increased high density lipoprotein cholesterol levels in the hyperlipidemic induced rats. Thus, it can serve as a natural product for the prevention of hyperlipidemia (Najib, Fachri, Sauriasari, Elya & Tjandrawinata, 2018).

2.1.10 *Tulbaghia violacea rhizomes*

A study by Olorunnisola, Bradley & Afolayan (2012) showed that *Tulbaghia violacea* rhizomes possesses antihyperlipidemic potential by reduction of serum levels of enzymes such as alanine transaminase, aspartate aminotransferase, alkaline phosphate, lactate dehydrogenase and total bilirubin in high cholesterol induced rats. It also prevented the reduction in total protein and albumin in the treated rats.

2.1.11 *Cymbopogon citratus*

The extract of *Cymbopogon citratus* possessed an antioxidant property, by showing high scavenging activity of hydroxyl radicals with increasing concentration of the extract (Keshari, Srivastava, Upadhayaya & Srivastava, 2018).

2.1.12 *Salvadora persica*

Aqueous leaf extract of *Salvadora persica* was reported to possess antioxidant, antihyperlipidaemic and antihyperglycemic abilities in alloxan induced diabetic male rats. Its antioxidant qualities showed that it increased the activities of catalase, superoxide dismutase, glutathione-S-transferase, and decreased lipid oxidation (as determined by Malondialdehyde levels) (El Rabey *et al.*, 2018). Elevated lipid profiles and reduced lipid profile due to induced diabetes were reduced and increased respectively after treatment.

2.2 Free Radicals

A study by Denham Harman in the year 1956 on the role of free radicals in the aging process gave rise to the sudden interest into the research of free radicals, and their effects on the biological system (Obeagu, 2018). In 1969, the discovery of superoxide dismutase by McCord & Fridovich provided evidence of the relevance of free radicals in the biological system (Obeagu, 2018). The third major breakthrough in the research of free radicals was in the year 1977, when Mittal & Murad proved that hydroxyl radical (OH-) stimulates the activation of guanylate cyclase and formation of the “second messenger” cyclic guanosine monophosphate (cGMP) (Obeagu, 2018). Afterwards different evidence has shown that free radicals coexists with the biological system, and its use in different psychological functions (Lushchak, 2014; Obeagu, 2018).

A free radical is known as any molecular specie that contains an unpaired electron in an atomic orbital, and capable of existing on its own. The existence of these unpaired electrons in the atomic orbital leads to some common properties that are shared by most radicals. Most radicals are very reactive and unstable, they can also be electron donors or electron acceptors which

make them behave as oxidants or reductants (Obeagu, 2018). Radicals are highly reactive species like oxygen singlet, peroxynitrite radicals, superoxide anion radical, hydrogen peroxide, nitric oxide radical, hypochlorite and hydroxyl radical. These aforementioned radicals are the major oxygen-containing free radicals. They play a role in various disease state found in the nucleus and membranes of cells, causing cell damage and homeostatic disruption to relevant biological molecules like DNA, carbohydrates, proteins and lipids (Mohammed, Kadhim, Jassimand, & Abbas, 2015).

2.2.1 Formation of Free Radicals

Free radicals are formed when a weak bond split or divides leaving a molecule with an odd and an unpaired electron which normally does not happen. Free radicals are very unstable and react quickly with other compounds in other to gain electrons needed to make them stable. When these molecules are attacked, they lose their electrons and become free radicals. They begin a chain reaction which results to a sequence which takes nanoseconds leading to disruption of a living cell (Khan, Garg, Singh & Kumar, 2018). Some free radicals may arise in the immune system's cells to neutralise viruses and bacteria which normally the body can handle during metabolism, but when the free radical production becomes excessive or antioxidants are unavailable, damage can occur in the living cell (Khan *et al.,* 2018). Redox regulation is a mechanism in the living cell that balances the beneficial and harmful effect of free radicals thereby maintaining a redox homeostasis (Obeagu, 2018).

2.2.1.1 Steps involved in Free Radical Production

In chemistry, free radicals take part in radical addition and radical substitution as reactive intermediates. Chain reactions involving free radicals can usually be divided into three different processes:

- Initiation,
- Propagation and
- Termination.

Initiation Phase are those reactions that results in a net increase in the number of free radicals. In most cases, this is a homolytic cleavage event that rarely takes place, due to the high energy barriers involved which often requires heat, UV radiation or a metal-containing catalyst to overcome such energy barriers.

Propagation Phase involves free radicals generated reacting with stable molecules to form new free radicals. These new free radicals go on to form new free radicals and the chains go on. This is the 'chain part' of the chain reactions. This propagation phase often involves hydrogen addition of the radicals to double bonds.

Termination reactions are those reactions which results in a net decrease in the number of free radicals. This is the reaction of two free radical species to form a stable, non-radical molecule (Khan *et al.,* 2018). It is a very rare due to the low concentration of radical species and the small probability of two radicals colliding with each other.

2.2.2 Importance of Free Radicals

- Free radicals carry out various critical functions in the body; controlling the flow of blood through the arteries, fighting various infections in the body, and keeping the brain in check.
- Free radicals are produced and mobilized by phagocytic cells involved in body defense to destroy bacteria and other cells of foreign matter which they ingest.
- Some free radicals at low levels in the body are signaling molecules which are responsible for turning on and off of genes having similar functions with antioxidants in the body.
- Free radicals such as nitric oxide and superoxide are produced in very high amount by immune cells to poison viruses and bacteria.
- Some free radicals kill cancer cells that are why certain cancer drugs are produced with the aim of increasing the free radical amount in body (Khan *et al.,* 2018).

2.2.3 Sources of Free Radicals

There are two main sources of free radicals, they include;

- Exogenous sources
- Endogenous sources

Exogenous sources include the following; xenobiotics, radiation, drugs, toxins etc (Van den Ende, Peshev & De Gara, 2011).

Endogenous sources of free radicals has an intracellular origin mainly derived from signaling pathways and metabolic and/or inflammation processes or mechanisms in the body such as few to be discussed below briefly

2.2.3.1 Mitochondrial Respiration

Mitochondrial Respiration is the most vital source of reactive oxygen species (ROS) production that is non-regulated. During the mitochondrial electron transport system of ATP generation through aerobic respiration, four-electron of oxygen is reduced (Mohammed *et al.,* 2015; Obeagu, 2018). It is estimated that 2-3% of O_2 consumed by the mitochondria is incompletely reduced, due to the leakage of some electrons yielding ROS, and 1-5% leading to hydrogen peroxide (H_2O_2) production by the antioxidant superoxide dismutase (SOD), because of the inability of superoxide anion radical to pass through the lipid membrane (Mohammed *et al.,* 2015; Obeagu, 2018). H_2O_2 produced sometimes leads to creation of more chemical reactive molecule like hydroxyl radical, when H_2O_2 reacts with iron in the Fenton reaction (Victor, Rocha & De la Fuente, 2004; Mohammed *et al.,* 2015; Obeagu, 2018).

2.2.3.2 Cellular Oxidase

Superoxide, this free radical specie can also be produced by one-electron reduction of oxygen by Xanthine oxidase (XO), a non-heme enzyme that is usually found in the cytosol especially during hypoxic conditions. It transfers reducing equivalents to oxygen and have the capability of producing superoxide (O_2^-), and hydrogen peroxide by using oxygen as an electron acceptor. It can also exist as Xanthine Oxidoreductase (XOR) in dehydrogenase form under physiological conditions that utilizes NAD or oxygen as the final electron acceptor (Obeagu, 2018).

2.2.3.3 Hydroxyl Radical

The hydroxyl radical ($OH\cdot$) is a neutral forms of hydroxide ion which possess very high reactivity, making it a very deleterious radical (Obeagu, 2018). They are short lived radical species with half life of 10^{-9} making them unstable, unlike superoxide radicals that are relatively stable. Hydroxyl radical is produced by two major biochemical reaction called Haber-Weiss reaction and Fenton reaction (Obeagu, 2018).

$O^{2-} + H_2O_2 = HO\cdot + OH^- + O_2$

(Haber-Weiss reaction)

However, this reaction in order to produce HO requires a metallic constant (CU^{2+} or CU^{3+}) and is a combination of transition metal mediated chemical reactions called Fenton reaction (Obeagu, 2018).

$Fe^{3+} + .O_2^- = Fe^{2+} + O_2$

$Fe^{2+} + H_2O_2 = Fe^{3+} + OH^- + HO^{\circ}$

(Fenton reaction)

2.2.4 Reactive Oxygen Species (ROS)

Oxygen is used to carry out metabolism in the body, but can sometimes react with a metabolic compound to form a free radical which causes biological damage in a living cell (Manisha, Hasan, Rajak & Jat, 2017). Oxygen is a stable product, but when it gains or losses an electron in oxygen molecule or its compound it becomes a reactive oxygen species. Oxygen in form of free radical are produced in large amounts in the body, they act as a substance or molecule

which can cause oxidative stress, thereby causing disease able effects in a living cell (Bhattacharya, 2015).

Reactive oxygen species (ROS) are very small, highly reactive molecules with unpaired valence shell electrons. Reactive oxygen species include singlet oxygen, superoxide, hydroxyl radical, hydrogen peroxide, hydroperoxyl radical, ozone etc (Manisha *et al.,* 2017). ROS is formed as a natural secondary product of the normal metabolism of oxygen which plays important roles in cell signaling. However, ROS levels can increase excessively during environmental stress leading to significant damages in cell structures (Khan *et al.,* 2018). ROS helps in wound repair and blood homeostasis by addition of platelets to already existing platelets in the sites of injury. Also, the harmful effects of reactive oxygen species on the cell are in form of DNA damage, oxidations of polydesaturated fatty acids in lipids, oxidations of amino acids in proteins, oxidatively inactivates specific enzymes by oxidation of co-factors (Khan *et al.,* 2018).

2.2.5 Reactive Nitrogen Species

Reactive nitrogen species (RNS) are free radicals containing nitrogen with high oxidizing ability. Hence, it reacts with reactive oxygen species to induce oxidative stress (Ahmadinejad, Moller, Hashemzadeh-Chaleshtori, Bidkhori & Jami, 2017). ROS and RNS collectively are called reactive oxygen and nitrogen species (RONS). These types of free radicals are mainly produced by cellular enzymes such as NADPH-oxidase, myeloperoxidase and nitric oxide synthase (NOS) (Ahmadinejad, Moller, Hashemzadeh-Chaleshtori, Bidkhori & Jami, 2017; Obeagu, 2018). In animal cells, production of RNS starts with the reaction of superoxide with ($O_2^{\bullet-}$) with nitric oxide ($NO^{\bullet}$) to form peroxynitrite ($ONOO^-$), which is a highly reactive

species capable of causing damage to lipids, DNA bases, proteins, thiols etc (Blaise, Gauvin, Gangal & Authier, 2005).

2.2.6 Nitric Oxide

Nitric oxide is a small molecule that contains an unpaired electron on its anti bonding, which makes it a radical. It is soluble in aqueous and lipid media making it able to readily diffuse through cytoplasm and plasma membrane (Habib & Ali, 2011). Nitric oxide is generated in biological tissues through a tightly regulated process by specific enzyme known as nitric oxidesynthases (NOSs). Nitric oxide exists in three isoforms namely; Neuronal NOS (nNOS), inducible NOS (iNOS) and endothelial NOS (eNOS) (Blaise *et al.,* 2005).

During inflammatory process, cells of the immune system produce both nitric oxide and superoxide through a process known as oxidative burst. During this process, nitric oxide and superoxide anion react together to produce significant amounts of a much more oxidatively active molecule known as peroxynitrite anion ($ONOO^-$), which is a potent oxidizing agent that can cause DNA fragmentation and lipid oxidation (Habib & Ali, 2011).

$NO\cdot \quad + \quad O_2\cdot^- \quad = \quad ONOO^-$

Nitric oxide + Superoxide Peroxynitrite

2.3 Oxidative Stress

Oxidative stress can be defined as an imbalance or inequality between the production of reactive oxygen species or reactive nitrogen species, and the levels of antioxidants used as protective or defensive mechanisms (Amiri, 2018; Khan *et al.,* 2018). Oxidative stress can also be as a result of the fast production of reactive forms of oxygen than they can be neutralized

by antioxidants, i.e free radicals exceeding the oxidant system of the body. Hence, increasing RONs and decreasing or lack of antioxidants induces oxidative stress (Amir, 2018). Any prolonged imbalance results to oxidative damage to cells, tissues and organs.

Generally, oxidative stress are caused by series of activities like alcohol consumption, use of drugs (anti-inflammation, anti-analgesic, anti-cancer and anti-depressants), environmental pollution by pollutants like mercury chloride, lead and other factors like mobile phone/radio frequency radiation exposure, UV radiation, x-Ray exposure, temperature (cold stress), maternal high-fat diet, pesticides etc (Amiri, 2018).

Oxidative stress is said to be the cause of cancer, Alzheimer's diseases, Parkinson's disease, amyotrophic lateral sclerosis, and various lifestyle-related disorders like arterial sclerosis, coronary heart diseases, atherosclerosis, stroke, high blood pressure, myocardial infarction, cerebral apoplexy, dementia, diabetes, cataract, asthma, obesity, lung cancer etc (Halder & Bhattacharya, 2014; Amiri, 2018). Oxidative stress is one of the major causes of cardiac diseases like congestive heart failure, hypertension, and atherosclerosis (Sugamura & Keaney, 2011). The smooth muscles and cardiac muscles during the regulation of metabolic process release free oxygen radicals as a form of the byproducts of this process, which interrupts the supply of blood to the heart, which sometimes leads to myocardial infarction (Cardiac infarction, heart attack) of the heart (Manisha *et al.,* 2017). Some phagocytic cells utilize the low density lipoprotein (LDL) cholesterol and oxidize the LDL, thus leading to the formation of artery clogging plaque responsible for stroke. Various studies have reported that heart tolerance to oxidative stress is dependent on age. This means that heart tolerance to oxidative stress reduces with age, because of the decrease in the concentrations of the antioxidant

enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), giving rise to the development of Cardiovascular (CV) alterations (Liguori *et al*., 2018).

Research links atherosclerosis (Clogging of blood vessels or arteries caused by fatty acid deposits usually cholesterol) with oxidized LDL-cholesterol (oxLDL). This compound is mainly responsible for this cardiac disease, and is usually common among elderly people (Gradinaru, Borsa, Ionescu & Prada, 2015). As a matter of fact, various studies showed a significant relationship between oxLDL and higher arterial stiffness, independent of other traditional cardiovascular disease (CVD) risk factors.

Oxidative stress also plays some beneficial roles in the body system, such as assisting in birth delivery by inducing apoptosis to prepare the birth canal for delivery. It also plays a role in strengthening of the biological defense mechanisms during ischemia and physical exercise (Yoshikawa & Naito, 2002). Oxidative stress activates the aging process, because aging is known to be the progressive loss of tissue and organ function over a period of time (Liguori *et al*., 2018).

2.3.1 Oxidative stress targets

Oxidative stress basically targets three (3) main cellular components namely,

- Proteins,
- Lipids and
- DNA

2.3.1.1 Oxidative damage to proteins

Direct damage to proteins can be caused by free radicals, ROSs or RNSs which causes adverse effects on many kinds of protein by interfering with enzyme activity and the function of structural proteins (Khan *et al.,* 2018). Oxidative damage to proteins can oxidatively alter or change the protein in three processes; oxidative alteration of a particular amino acid, peptide cleavage caused by free radicals, and development of protein cross-linkage owing to reaction with lipid peroxidation products. Proteins having amino acids such as methionine, cystein, arginine, and histidine are known to be best susceptible to oxidation (Mohammed *et al.,* 2015). Protein alteration assisted by free radicals increases its vulnerability to enzyme proteolysis. Enzyme activity, receptors and membrane transport are likely to be affected as a result of oxidative damage to protein products. This oxidatively damaged protein products might have very reactive groups like peroxyl radical that may assist in membrane damage and many cellular functions. ROS can cause harm to proteins by producing carbonyls and other amino acids alteration like formation of methionine sulfoxide, protein peroxide and protein carbonyls. Protein oxidation also alters enzyme activity, signal transduction mechanism, heat stability and proteolysis susceptibility leading to aging (Mohammed *et al.,* 2015).

2.3.1.2 Oxidative damage on lipids

Adverse effect of oxidative stress (Free radicals) on lipids gives rise to lipid peroxidation. Lipid peroxidation is a free radical mediated process that causes biochemical lesions. It originates when a source of secondary free radical progressively acts as a secondary messenger or directly react with other biomolecules (Niki, Yoshida, Saito & Noguchi, 2005). The products of lipid peroxidation play a role as biomarkers for oxidative stress, cellular regulators and signaling messengers. Lipid peroxidation takes place on polysaturated fatty acid located on the cell

membranes, and then progresses with radical chain reaction. Hydroxyl radical is assumed to initiate ROS by removing hydrogen atom, thus lipid radical is formed which is changed into diene conjugate overtime. Peroxy radical on the other hand is a highly reactive radical which is formed by addition of oxygen. Peroxy radical then attacks another fatty acid producing lipid hydroperoxide (LOOH) and another radical giving rise to lipid peroxidation. During lipid peroxidation, a number of compounds are produced which serves as markers in lipid peroxidation assay like alkanes, malanoaldehyde and isoprotanes. The presence of these compounds has been proved in many diseases like diabetes, hyperlipidaemia, neurogenerative diseases, ischemic reperfusion injury, hypertension etc (Mohammed *et al.,* 2015).

Lipid Peroxidation also occurs on polyunsaturated fatty acids (PUFA) such as linoleates, arachidonates and cholesterol. The free radical-mediated peroxidation of PUFA progresses by five basic reactions. First, there is a hydrogen atom transfer from the PUFA to the chain initiating radical or chain carrying peroxyl radicals to give a Pentadienyl carbon-centered lipid radical. Secondly, the lipid radical reacts with molecular oxygen to give a lipid peroxyl radical. The third reaction is the fragmentation of the lipid peroxyl radical to give oxygen and a lipid radical (a reverse reaction of the second reaction), the fourth reaction is the rearrangement of the peroxyl radical, and the last reaction is the cyclization of the peroxyl radical which is only important for PUFA possessing more than three double bonds, and does not take place during the oxidation of linoleates (Niki *et al.,* 2005).

There are various stress markers produced by oxidation of proteins/nitrogen and one of the most important compounds of them is ‘lipoxidation products’. The detection and identification of lipid peroxidation products are easier and more reliable carried out with powerful tools such as Coordination ion- spray mass spectrometry (CIS-MS) and electrospray ionization (ESI) or

matrix-assisted laser desorption and ionization time-of-flight (MALDI-TOF) mass spectrometry.

2.3.1.3 Oxidative damage to DNA

Various studies has shown that nucleic acids like DNA and RNA are vulnerable to oxidative damage especially Mitochondrial DNA, where DNA is the major target with evidence seen in aging and cancer (Mohammed *et al.,* 2015). Oxidative nucleotides like glycol, dTG, and 8-hydroxy-2-deoxyguanosine can be used as biological marker for oxidative stress, but 8-hydroxy-2-deoxyguanosine is the most commonly used DNA damage marker (Mohammed *et al.,* 2015).

Free radical attacks DNA causing DNA fragmentation, thus leading to activation of the poly (ADP-ribose) synthetase enzyme which splits Nicotinamide-adenine dinucleotide (NAD^+) that aids in the repair of DNA. However, if the damage is extensive, NAD^+ levels may become depleted causing the cell to no longer be able to function effectively and eventually dies (Khan *et al.,* 2018). The site of tissue damage by free radicals depends on the tissue and the free radical species involved. Widespread damage can lead to death of the cell which can be by necrosis or apoptosis depending on the type of cellular damage. When a cell membrane or an organelle membrane is damaged by free radicals, it loses its protective properties thereby putting the health of the entire cell at risk (Khan *et al.,* 2018). Free radicals such singlet oxygen can cause deleterious effect to DNA, particularly through oxidation of guanine residues leading to G:C to T:A transverse during replication and then to mutations (Obeagu, 2018).

2.4 Antioxidants

Various studies has reported that Oxidative stress (OS) is connected in the pathophysiology of many neurological, particularly neurodegenerative diseases. Due to the oxidation of vital cellular components such as lipids, proteins, and DNA by reactive oxygen species, oxidative stress can cause cellular damage and cell death overtime. However, therapy using free radical scavengers (antioxidants) has the ability to prevent, delay, or improve many neurologic disorders. The major aim of antioxidants is to balance/neutralise out free radicals produced during different metabolic processes, inflammation and injury in the cell (Rahman, 2007). However, antioxidant therapeutic options sometimes vary and need to be tailored for various individual diseases due to its biochemistry and complexity. In most cases, therapeutic administration with antioxidants may need to be given early in chronic neurologic disorders to achieve a better and more effective result (Zadak, Hyspler & Ticha, 2009). Similar to free radicals, antioxidants can also be produced endogenously like superoxide dismutase (SOD) and reduced glutathione (GSH). They can also be introduced to the biological system exogenously, usually through diet such as Vitamin C, Carotenoids and Vitamin E (Zadak *et al.,* 2009; Amber, Shiman & Badiavas, 2013).

The defense mechanism of antioxidants is carried out in three main ways;

- By separating proteins from transition metals. Hence, preventing their availability for reaction with free radicals, thereby inhibiting their deleterious effect.
- Provision of small molecules that have the ability to scavenge or remove free radicals.
- Using specific mechanisms for the correction of ROS-induced DNA damage (Foyer, 2005).

2.4.1 Classification of Antioxidants

Antioxidants are classified in various ways such as; Based on their location and based on their nature and action.

Based on their location includes the following;

- Plasma antioxidants: Uric acid, Ascorbic acid, Bilirubin, Transferrin, Ceruloplasmin.
- Cell membrane antioxidants: α- tocopherol (membranous chain breaking antioxidant)
- Intracellular antioxidants: SOD (Superoxide dismutase), Catalase, Glutathione peroxidase, Glutathione reductase (Manisha *et al.,* 2017).

Based on their nature and action includes;

- Enzymatic antioxidants: SOD, Catalase, Glutathione peroxidase and Glutathione reductase.
- Non-enzymatic antioxidants and Nutrient Antioxidants: Beta-carotene, α- tocopherol, Ascorbic acid.
- Metabolic antioxidants: Glutathione, Bilirubin, Uric acid, Transferrin, Albumin, Ceruloplasmin, haptoglobin (Manisha *et al.,* 2017).

Table 2.1: Reactive oxygen species (ROS) and their corresponding neutralising antioxidants and also additional antioxidants

Reactive Oxygen Species (ROS)	Antioxidants (Endogenous)		Antioxidants (Exogenous)
	Direct role	Indirect role	
Hydrogen Peroxidase	Catalase (Cofactor)	Transferin (Iron), Vitamin C, Ferritin (Iron), beta-carotene, myoglobin (Iron)	Vitamin C, beta-carotene, lipoic acid
Hydroxyl Radical	Glutathione peroxidase (cofactor selenium)		Vitamin C, Lipioic acid
Lipid peroxidase	Glutathione peroxidase (cofactor selenium)		Vitamin E, beta-carotene
Superoxide radical	Superoxide dismutase (Cofactor Cu/Zn/Mn)	Ceruloplasmin (Metallothionin) Albumin	Vitamin C
Pro-oxidants/ Antioxidant equilibrium	Thiols (GSH, Lipoic acid, N-acetyl cysteine) NADH and NADPH ubiquinone	Bilirubin, uric acid	Flavonoids

(Khan, Garg, Singh & Kumar, 2018)

2.4.1.1 Superoxide dismutase (SOD)

SODs are known as a group of strongly related enzymes, that catalyse the disintegration of superoxide anion present in almost all aerobic cells and extracellular fluids into oxygen and hydrogen peroxide (Rahman, 2007). SOD enzymes consists three (3) families depending on the metal co-factor; Iron(Fe), Copper(Cu)/Zinc(Zn) and Manganese (Mn). Also, Nickel (Ni)SOD is another type which binds nickel. In Eukaryotes, Mn-SOD has been found mainly in the mitochondria and peroxisomes, Fe-SOD can be found in peroxisomes and CuZn-SOD in peroxisomes and cytosol (Valko *et al*., 2007).

SOD are said to be present in three forms namely; SOD1 which is found in the cytoplasm, SOD2 found in the mitochondria and SOD3 is found outside the cell. SOD1 and SOD3 contains copper and zinc as their reactive points, and SOD2 contains manganese as its reactive point (Obeagu, 2018). Reports has been suggested that an inadequate dietary intake of these trace minerals can compromise the efficiency of these antioxidant defense mechanisms (Yadav *et al*., 2016).

Equation showing the reaction of SOD

$$O_2^- + O_2^- \xrightarrow{[2H+]\ SOD} H_2O_2 + O_2$$

Superoxide. Hydrogen peroxide + Oxygen (Jain & Jain, 2016).

2.4.1.2 Catalase (CAT)

Catalase is common in cells exposed to oxygen, and is regularly used to catalyse the breakdown or degradation of hydrogen peroxide (by product of a range of normal metabolic processes) to oxygen and water using either manganese or an iron as a Cofactor (Rhee, Chae & Kim, 2005). Catalase has one of the highest effective rates for all enzymes; with one molecule of catalase being able to convert approximately 6million molecules of hydrogen peroxide to water and oxygen per minute. It can be found in all organs but predominantly in the liver having only hydrogen peroxide as its substrate (Sweeti & Nidhi, 2016).

$$2H_2O_2 \xrightarrow{CAT} 2H_2O + O_2$$

Hydrogen peroxide Water Oxygen

2.4.1.3 Glutathione System

The glutathione system consists of four main classes;

- Glutathione (GSH),
- Glutathione reductase,
- Glutathione peroxidases (GP_x) and
- Glutathione S-transferases (GST).

2.4.1.3a Glutathione (GSH)

They are synthesized from three key amino acid such as Cysteine, Glycine and Glutamic acid (Dolas & Gotmare, 2015). Glutathione is a tripeptide antioxidant that exists in the cytoplasm, mitochondria and chloroplast in a reduced form throughout the cells, and helps in the detoxification of harmful materials like xenobiotics and heavy meatals (Obeagu, 2018). Its

cysteine moiety contains a thiol group that is a reducing agent, which can be reversibly oxidized and also reduced (Obeagu, 2018). In the glutathione redox cycle, glutathione serves as a primary substrate for glutathione peroxidase and glutathione s-transferase. Deficiency of glutathione causes severe degeneration of the epithelial cells of the jejunum and intestinal colon. The ratio of reduced glutathione to oxidized glutathione in a cell is a measure of cellular oxidative stress (Obeagu, 2018).

2.4.1.3b Glutathione reductase

Glutathione is maintained or preserved in the cell as Glutathione reductase. It subsequently reduces other metabolites and enzyme systems, while still capable of reacting with oxidants. It plays a pivotal role in maintaining cells redox state, hence recognized as one of the most important cellular antioxidant (Obeagu, 2018).

2.4.1.3c Glutathione peroxidase (GP_x)

Glutathione peroxidase is an antioxidant enzyme that contains four selenium-cofactors which catalyzes the degradation of hydrogen peroxide to water, and organic hydroperoxides to alcohols. It is also the major source of protection against low levels of oxidative stress (Sweeti & Nidhi, 2016; Obeagu, 2018). At least four different glutathione peroxidase isozymes can be found in animals (Sweeti & Nidhi, 2016). Glutathione peroxidase 1 can be found in large quantities in the cytoplasm, and is a very efficient remover or scavenger of hydrogen peroxide, while glutathione peroxidase 4 is found at low levels and highly active, but shows higher activity with lipid hydroperoxides. Glutathione peroxidase 2 is extracellular enzyme found in the intestine, and glutathione peroxidase 3 also an extracellular enzyme, but abundant in the plasma (Obeagu, 2018).

2.4.1.3d Glutathione S-transferases (GST)

Glutathione S-transferases are a different class of glutathione-dependent antioxidant enzymes, that possesses high activity with lipid peroxides. They have shown to be at high levels in the liver, and also helps in detoxification metabolism (Traber & Atkinson, 2007).

2.4.1.4 β-Carotene

Beta-carotene possesses antioxidant properties that helps neutralize free radicals (reactive oxygen) molecules, which has the potential or ability to damage lipids in cell membranes and genetic materials, leading to the development of cardiovascular diseases and cancer (Yadav *et al*., 2016). In vitro studies have shown that carotenoids can inhibit the oxidation of fats under certain conditions. They may have anti-atherosclerotic potential, but their effects in humans appear to be more complex.

2.4.1.5 Vitamin C

Vitamin C or Ascorbic acid is a monosaccharide fat soluble vitamin that can only be derived from diet, and cannot be synthesized in humans. Vitamin C is able to prevent formation of nitrosamines, neutralize reactive oxygen species like H_2O_2, thus boosting immunological response. Its intake has shown protective effects against various types of cancer such as cancer of the lung, breast, pancreas, stomach, cervix, rectum and oral cavity (Sweeti & Nidhi, 2016). Vitamin C is usually maintained in the reduced form in the body, and its reaction with glutathione can be catalysed by protein. It can act as co anti-oxidants, by generation of α tocopherol from α tocopherol radical, resulting in ascorbate radical due to its metal reducing capacity in presence of copper and Fe generating OH° (Yadav *et al*., 2016).

$$\text{Ascorbate} + Cu^{2+} + O_2 + 2H^+ \longrightarrow \text{Dehydroascorbate} + Cu^+ + H_2O_2 + OH^- + OH^{o} + Cu^{++}$$

2.4.1.6 Vitamin E

Vitamin E is a generic term used for natural occurring Tocopherol and Tocotrienols. Tocopherols are known as Vitamin E, and Tocotrienols are ProVitamin E. They are stored with fat in the liver and other tissues. They are regarded as fat soluble vitamins that exist in eight different forms and possess antioxidant properties. Of all of the different forms, α-tocopherol has been reported to be most researched as it has the greatest bioavailability, because it is a major membrane bound antioxidant used by the cell with the body choosing to absorb and metabolise it (Yadav *et al*., 2016). The main function of α-tocopherol is protecting the cell from lipid peroxidation, it reacts with lipid radicals in the reaction by removing or scavenging free radicals intermediates to prevent propagation reaction (Sweeti & Nidhi, 2016). This reaction forms α –tocopherol radicals (oxidized α-tocopheroxyl radicals) which is reduced back to his active reduced form by ascorbic acid, retinol or ubiquinol (Obeagu, 2018). It possesses a quality as a chain-breaking antioxidant. Therefore, it transfers its phenolic H-atom to a lipid peroxyl radical, converting it to a lipid hydroperoxide and a Vitamin E radical. They are responsible for protecting poly unsaturated fatty acid (PUFA) and low density lipoprotein (LDL) in the cell membrane against lipid peroxidation (Yadav *et al*., 2016).

2.4.1.7 BioFlavonoids

Flavonoids possess antioxidant properties by promoting antioxidant activity, cellular health, normal tissue growth, and restoration in the body. Flavonoids are a class of secondary plant phenolics with significant antioxidant and chelating properties. They also work with ascorbic acid to reduce oxidative stress, i.e inhibit lipid peroxidation for the water based portion of the cell, and may slow down some of the effects of aging (Sunil, 2014). The antioxidant activity of phenolic compounds are determined by their structure, particularly the tendency at which

hydrogen atom from an aromatic hydroxyl group can be given to a free radical. Their cardioprotective effects ranges from the ability to inhibit lipid peroxidation, chelate redox-active metals, and reduce other processes involving reactive oxygen species (Sweeti & Nidhi, 2016).

2.4.1.8 Uric acid

Uric acid is responsible for 50% of the antioxidant ability in plasma. Uric acid is thought to have been used as an alternative to ascorbate in evolution. As similar to ascorbate, uric acid is capable of initiating production of free radical species (Obeagu, 2018).

2.5 LIPIDS

Lipids are water insoluble organic compounds that are important in carrying out various normal functions in living organisms. They are important components of the cell membranes that are used in storing of energy, play a significant role as enzyme co-factors, hormones, and intracellular messengers (Xenoulis & Steiner, 2010). Since, lipids are water-insoluble molecules they cannot be transported in aqueous solutions like plasma, they are therefore transported in plasma as macromolecular complexes known as lipoproteins (Karan *et al.,* 2017). Lipoproteins are spherical structures consisting of a hydrophobic core containing lipids, and an amphophilic (i.e. both hydrophobic and hydrophilic) outer layer of phospholipids, free cholesterol, and proteins called apolipoprotein forming a protective envelope surrounding the lipid core (Karan *et al.,* 2017).

2.5.1 Lipid Profile

A lipid profile or lipid panel is a panel of blood tests, that serves as an initial clear medical screening tool used for the determination of abnormalities in lipids like cholesterol and triglyceride. The results obtained from these tests can identify certain genetic diseases, and can estimate the possibility of risks for cardiovascular diseases, pancreatic diseases and other kinds of diseases. Thus, providing a development of treatment plan and follow up (Onwe *et al*., 2015). A lipid profile measures total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol and triglyceride. Lipid panels are usually carried out as part of a physical examination. Treatments options sometimes include; changes in lifestyle like diet, regular exercise and lipid-reducing medications such as statins (Onwe *et al*., 2015). A lipid profile should be carried out after nine to twelve hours fast without food, liquids or medication. In cases where fasting is not possible, the values for total cholesterol and high density lipoprotein cholesterol may still be useful (Sidhu & Naugler, 2012). Continuous intake of diet containing high lipids can cause hyperlipidemia (Sa'adah, Purwani, Nurhayati, Ashuri, 2017). This is a condition characterized by an elevation in total cholesterol, triglycerides, low density lipoprotein-cholesterol (LDL-C), and a decrease in high density lipoprotein-cholesterol (HDL-C) (Vafa, Haghighatjoo & Ziaee, 2011). Hyperlipidemia when not treated, speed up the occurrence of atherosclerosis, one of the major factors that triggers cardiovascular disease like hypertention, coronary heart disease and stroke (Sa'adah *et al.,* 2017). Atherogenic index shows the measure of the potential occurrence of atherosclerosis. Clinical investigations have revealed that the higher the atherogenicity risk predictor indices and biomarkers, the higher the risk of developing atherosclerosis and coronary heart disease (Ranjit, Guntuku & Pothineni, 2015). Studies have shown that serum lipid ratios and atherogenic coefficient/indices are better predictors of cardiovascular disease than isolated serum lipid parameters (Ranjit *et al.,* 2015;

Chikezie, Ojiako, Emejulu & Chikezie, 2018). Additionally, atherogenic dyslipidemia is defined in terms of the ratio of serum LDL-C concentration to that of HDL-C concentration referred to as Castelli's risk index II (CRI-II), and the ratio of serum TC/HDL-C which denotes Castelli's risk index I (CRI-I). Estimates of CRI-II and CRI-I are useful diagnostic indices for determining the degree of cardiovascular risk (Chikezie *et al.,* 2018).

2.5.2 Basic classification of Lipids

Lipids can be classified as total cholesterol (TC) and its derivates like low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), very low density lipoprotein cholesterol (VLDL-C) and triglycerides (TG) (Onwe *et al*., 2015)

2.5.2.1 Total cholesterol (TC)

From the Guidelines of National Cholesterol Education Program USA (NCEP), it is stated that TC concentrations below 200 mg/dL in adults, and less than 180 mg/dL in children have been regarded as safe, but concentrations greater than 240 mg/dL are referred to as Hyperlipidemic. Furthermore, epidemiological evidence suggests that the risk of cardiac occurrence decreases as TC levels falls to 150 mg/dL (Fryar, Hirsch, Eberhardt, Yoon & Wright, 2010).

2.5.2.2 Triglyceride (TG)

Triglycerides are another kind of fat transported in the blood by lipoproteins. Excess calories, alcohol or sugar in the body is converted into triglycerides and stored in the lipocytes throughout the body (Smelt, 2010). Normal triglyceride concentration ranges below 150mg/dL, whereas concentrations ranging from 200-499mg/dL are regarded as high. Triglyceride

concentrations ranging from 500 mg/dL and above are considered dangerous because of development and progression of various cardio-vascular diseases (CVDs) (Onwe *et al*., 2015).

2.5.2.3 Low density lipoprotein cholesterol (LDL-C)

LDL-C is known as the 'bad cholesterol'. It is produced by the liver and transported to different areas of the body like muscles, tissues, organs and heart. High levels of LDL-C show high amounts of cholesterol in the blood stream than normal which increases the risk of heart related diseases (Costet, 2010). According to NCEP guidelines, LDL cholesterol concentrations ranging below 100mg/dL i.e as low as 50 to 70mg/dL are considered optimal and normal, while concentrations ranging from 160-189mg/dL are considered high. As the concentration of LDL cholesterol decreases, risks of CVDs decreases (Onwe *et al*., 2015). Low Density Lipoprotein (LDL) is pro-atherogenic, therefore high levels of LDL increases the risk of coronary heart disease (CHD) (Karam *et al.,* 2017).

2.5.2.4 High density lipoprotein cholesterol (HDL-C)

HDL-C is commonly known as 'good cholesterol'. It is generated in the liver and intestine. It transports cholesterol and other lipids from tissues back to the liver for degradation (Ridker *et al*., 2010). A healthy heart shows high levels of HDL cholesterol with an optimal concentration of HDL-C of 60mg/dL, while HDL concentrations below 40 mg/dL indicates a major risk factor for CVDs. High density lipoprotein (HDL) is anti-atherogenic therefore low levels of HDL also increases CHD risk (Karam *et al.,* 2017).

2.5.2.5 Very low density lipoprotein cholesterol (VLDL-C)

VLDL-C is similar to LDL-C because it contains mostly fat and not much protein. It is synthesized in the liver. VLDL cholesterol is the lipoproteins which transport cholesterol from the liver to organs and tissues in the body formed by the combination of cholesterol and triglycerides (Sundaram &Yao, 2010). VLDLs are heavier than LDL and associated with atherosclerosis and coronary heart disease (Sundaram & Yao, 2010).

2.6 The Liver

The liver is the second largest organ (after the skin) and largest gland in the human body weighing about 1500g. The liver has the shape of a prism or wedge with its base to the right and its apex to the left. It is the organ responsible for maintaining the body's internal environment by the metabolism and detoxification of drugs, toxins and metabolic waste products (Klaassen, 2007). The liver carries out various functions which includes glucose storage and synthesis, plasma protein synthesis, bile formation, decomposition of red blood cells and hormone production (Yang, Schnackenberg, Shi & Salminen, 2014).

Anatomically, the liver lies slightly under the diaphragm and anterior to the stomach, a position that eases the maintenance of metabolic homeostasis of the body. The liver receives blood through two different blood supplies namely the portal vein and hepatic artery. The portal vein feed the liver by carrying blood containing digested nutrients from the gastrointestinal tract, spleen and pancreas to the liver, while the hepatic artery carries oxygenated blood from the lungs to the liver (Yang *et al.,* 2014). The human liver is made up of four lobes, and each lobe is made up of many lobules under microscopic examination. Blood enters the lobules from the portal vein and hepatic artery, and then flows down the different regions of the lobule. The

lobule is divided into three regions: The Periportal (Zone 1), which is the closest to the entering blood supply having the highest oxygen tension, Midzonal (Zone 2) is intermediate and Centrilobular (Zone 3) adjacent to the central vein and has the poorest oxygenation (Yang *et al.,* 2014).

2.7 Liver Toxicity

The liver is the first internal organ to encounter certain harmful effects as a result of the blood flow from the stomach and intestine. These effects are caused by factors such as ingested metals, drugs, alcohol, toxins, chemicals etc (Klaassen, 2007). As a result of these exposures which can be either chronic or acute, liver functions are altered. When drugs are orally consumed in high concentrations, it gives rise to drug-induced liver injury (DILI). For example, an analgesic and antipyretic drug known as Acetaminophen (APAP) can cause fatal acute liver failure when therapeutic doses are exceeded, due to the production of a highly reactive hepatotoxic metabolite in the liver (Lee, 2004). DILI is one of the major limiting factor in the pharmaceutical industry and public health, because DILI is a common cause of drug development termination, drug restrictions, and post-marketing drug withdrawal (Kaplowitz, 2005).

Drug-Induced liver injury is classified as regards to clinical liver diseases which are Hepatocellular, Cholestatic, or Mixed hepatocellular/cholestatic.

Hepatocellular involves cellular damage on the hepatocytes. Example, the centrilobular necrosis caused by APAP. This kind of damage is usually characterized with elevated serum alanine aminotransferase (ALT) levels, caused by leakage from damaged hepatocytes (Yang *et al.,* 2014).

Cholestatic injury usually involves damage to some part of the bile processing or excretion apparatus thereby resulting in impaired bile processing or excretion. This kind of injury is often characterized with elevated serum bilirubin and alkaline phosphatase (ALP), thus showing alterations in bile homeostasis and/or bile duct injury (Yang *et al.,* 2014).

Mixed injury is a mixture effect of both types of injuries. Sadly, drugs hardly produce a single clear clinical picture, making the diagnosis of DILI difficult. For example, amoxicillin/clavulanic acid usually causes cholestatic injury, but can also produce acute hepatocellular injury or a mixed type injury (Stirnimann, Kessebohm & Lauterburg, 2010; Yang *et al.,* 2014).

2.8 Liver Function Tests

Liver function tests (LFT) are also known as liver profile or liver panel. They are helpful screening tool, which are effective diagnostic method of detecting hepatic dysfunction. Since the liver performs different functions, a single biochemical test is not enough to provide complete estimate of liver functions (Thapa & Walia, 2007; Yap & Aw, 2010). Furthermore, clinical history, physical examination, radiological imaging and specific disease markers play a key role in interpretation of these functions.

Liver function tests have various uses which includes the following; screening, pattern of disease, assess severity and Follow up. Liver function tests are non-invasive, but are sensitive screening and diagnostic tools for liver dysfunction. They are useful in recognizing the pattern of liver disease by differentiating between acute hepatitis, and various cholestatic disorders and chronic liver diseases. Liver function tests are helpful in estimating, predicting the degree and

outcome of certain liver diseases, and also evaluating their response to therapy like autoimmune hepatitis (Thapa & Walia, 2007).

Liver function tests has limitations like lack of sensitivity, and lack of specificity despite its advantages. Liver function tests are not sensitive or specific to a particular disease. Example, serum albumin might be reduced in chronic disease and also in nephrotic syndrome. Also, Aminotransferases may be increased in cardiac diseases and hepatic diseases. In terms of sensitivity, liver function tests may be normal in certain liver diseases such as cirrhosis, congenital hepatic fibrosis etc (Thapa & Walia, 2007).

2.8.1 Alanine aminotransferase (ALT)

Alanine aminotransferase (ALT) is the most generally used clinical biomarker, which is regularly used for the non-invasive monitoring of liver diseases in experimental species and in humans (Ozer, Ratner, Shaw, Bailey & Schomaker, 2008). ALT is an enzyme found in higher concentration in the liver with lower activities found in skeletal muscle and heart tissues. It causes the metabolism (transamination) of the amino acid alanine and plays an important role in gluconeogenesis. During hepatocellular injury, ALT escapes from the liver into the extracellular space, and enters the blood causing high serum ALT levels compared to control subjects, thus showing liver cell damage (Ozer *et al.,* 2008; Yang *et al.,* 2014). Unfortunately, Extrahepatic injury like muscle injury or cardiac injury can also lead to increase in ALT, making ALT not entirely hepato-specific.

ALT: Alanine + α ketoglutarate= Pyruvate+ Glutamate (Thapa & Walia, 2007).

Regardless of the fact that extrahepatic injury can cause increase in ALT, serum ALT remains the most widely used and generally accepted biomarker for DILI. It is considered to be the

clinical chemistry gold standard for DILI detection (Ozer *et al.,* 2008). Also, newer biomarkers of liver injury with greater specificity to liver can be used in addition with ALT, for safe evaluation of developing compounds in the pharmaceutical industry.

Recent studies have showed that measuring the ALT isozymes, ALT1 and ALT2 helps to differentiate the source of injury. ALT1 is located in human hepatocytes, renal tubular epithelial cells, and in the salivary gland epithelial cells, while ALT2 is localized to human adrenal gland cortex, neuronal cells bodies, cardiac myocytes, skeletal muscle fibers, and in the endocrine pancreas. When compared to ALT1, ALT2 was found to add less to the total serum ALT activity and was probably a reflection of mitochondrial damage (Yang *et al.,* 2014). A novel immunoassay has been developed to discriminate human ALT1 and ALT2 activities and might improve the ALT assay.

2.8.2 Aspartate aminotransferase (AST)

Based on the same explanation as ALT, recent studies has shown aspartate aminotransferase (AST) as a standard biomarker for DILI accepted by clinicians (Ozer *et al.,* 2008; Shi, Hong, Senior & Tong, 2010). AST are found in heart, brain, skeletal muscle and liver tissue. Similar to ALT, AST is responsible for the metabolism (transamination) of the amino acid aspartate. Despite the fact that the sensitivity of AST test is known to be lower than that of ALT, it is still a generally used liver biomarker. Owing to its widespread expression in extrahepatic organs, like the heart and muscle, AST is important but has less specificity than ALT in detecting DILI.

AST: Alanine + α ketoglutarate = Oxaloacetate + Glutamate (Thapa & Walia, 2007).

The ratio of serum ALT and AST activity is important in differentiating DILI from extrahepatic organ injury, It also helps in the diagnosis of acute hepatitis and cirrhosis with an AST/ALT

ratio at 2:1. AST have been discovered to have at least two isoenzymes, one is cytosolic AST and another is mitochondrial AST (mAST). It is reported that mitochondrial isoenzyme is responsible for an estimated 80% of AST activity in human liver, whereas most of the circulating AST activity in normal people is gotten from cytosolic isoenzyme (Thapa & Walia, 2007). It has not been fully determined that cytosolic AST or mAST adds to serum AST elevation, and also if these AST isoenzymes are affected by drug-driven induction or inhibition (Yang *et al.,* 2014).

Table 2.2: Current clinical biomarkers of liver toxicity

Biomarker	Tissue Localization	Injury	Specific Damage Marker	Comments
ALT	Primarily localized to liver	Elevated in blood due to liver necrosis and with heart and skeletal muscle injury (necrosis)	Hepatocellular necrosis	Commonly used to assess hepatocellular injury
AST	Localized in heart, brain, skeletal muscle and liver	Elevated in blood due to liver or extrahepatic tissue injury	Hepatocellular Necrosis	Less specific than ALT
TBL	Taken up, conjugated in liver, and secreted into bile	Marker of hepatobiliary injury and liver function; also increased due to hemolysis	Cholestasis, biliary; Liver function	Conventional biliary injury; in conjunction with ALT, better indicator of disease severity in humans
ALP	Broad tissue localization	Marker of hepatobiliary injury	Cholestasis	Conventional biliary injury; associated with drug-induced cholestasis in humans
GGT	Activity localized to Kidney>liver, pancreas	Marker of hepatobiliary injury	Cholestasis, biliary	Conventional biliary injury; high sensitivity in humans, elevation can be caused by alcohol or heart disease

Albumin	Main constituent of serum total protein	Decreased inblood with chronic liver disease	Liver function	Liver fails to synthesize enough protein, especially albumin
Ammonia	Converted to less toxic urea in liver Injury	Elevation in blood to liver injury	Liver function	Failure of the conversion in severe liver disease
Cholesterol/ **Triglycerides** **Clotting time**		Increased in blood due to the failure of bile elimination Increased with severe liver injury	Liver function Liver function	Liver fails to remove them to bile ducts Liver fails to produce coagulation factors, increased clotting time; international normalized ratio equivalent to prothrombin time
Urobilinogen		Low level in urine due to biliary obstruction	Liver function	Colorless product of bilirubin reduction, similar role to bilirubin

(Yang *et al.,* 2014)

2.8.3 Alkaline phosphatase (ALP)

Alkaline phosphatases are a family of zinc metaloenzymes, with serine a non-essential amino acid found at the active center. They discharge inorganic phosphate from various organic orthophosphates, hydrolyze monophosphatases at an alkaline pH and are present in nearly all tissues (Yap & Aw, 2010). Alkaline phosphatase (ALP) is an enzyme found in the liver, and its concentration in serum increases as a result of blocked bile ducts (Yang *et al.,* 2014). ALP is another diagnostic clinical biomarker generally used for cholestatic DILI. It is noted that other conditions such as bone disease and pregnancy are associated with increase in ALP levels, therefore ALP should not be regarded as a specific biomarker of cholestatic DILI. The unique advantage of ALP is that it is at least partially predictive of biliary obstructive types of liver injury when used together with other DILI biomarkers (Ozer *et al.,* 2008).

ALP is found in the liver, bone, kidney, intestine and placenta. Serum ALP is a mixture of different ALP isoenzymes which can be separated by electrophoresis. In normal serum, ALP comprises the liver and bone moieties, bone ALP is unstable to heat, while liver ALP found on canalicular surfaces is increased during biliary obstruction (intrahepatic and extrahepatic). In hepatocyte injury, ALP is often normal or slightly increased. This characteristic serves as guide to differentiate liver parenchymal disease from biliary dysfunction (Yap & Aw, 2010). Alkaline phosphatase from the liver, bone and kidney are thought to be from the same gene, while that from intestine and placenta are derived from different genes.

In humans with primary biliary cirrhosis, a chronic cholestatic liver disease of unknown cause, tests reveal increase in serum alkaline phosphatase activity (20-fold) and GGT activity with or without elevated ALT levels. In humans, increased alkaline phosphatase levels have been associated with drug- induced cholestasis (Wright & Vandenberg, 2007). In rats, GGT activity

is considered a reliable marker for cholestasis compared to alkaline phosphatase activity (Ozer *et al.,* 2008).

2.8.4 Total bilirubin (TBL)

Bilirubin is an endogenous anion derived when hemoglobin degrades from the red blood cells (Thapa & Walia, 2007). Serum bilirubin is a mixture of α, β, γ and δ fragments which are unconjugated, singly conjugated, doubly conjugated and covalently bound to albumin, respectively. Even though δ bilirubin measurement is available, it is not widely used because total bilirubin assay is sufficient for LFT, but separation by fractionation may be required in isolated increases in bilirubin and neonatal jaundice (Yap & Aw, 2010).

Serum bilirubin is helpful in differentiating the causes of jaundice. In the case of pre-hepatic jaundice caused by haemolysis, unconjugated bilirubin is increased with little or no increase in conjugated bilirubin, while in hepatic and post-hepatic jaundice, there is increased conjugated and δ bilirubins (Yap & Aw, 2010). Therefore, serum bilirubin concentration is a liver function biomarker, which measures the ability of liver to clear bilirubin from the blood as it circulates through the liver.

Bilirubin can be classified into direct bilirubin and indirect bilirubin according to original vander Bergh method of measuring bilirubin. Direct bilirubin (DB) refers to the conjugated bilirubins or water-soluble fractions that react directly with the diazo reagent, while indirect bilirubin is a derived value obtained from the difference of the total bilirubin and DB.

Total bilirubin (TBL) on the other hand is a composite or mixture of unconjugated (extrahepatic) and conjugated (hepatic) bilirubin. Total bilirubin is the factor which helps to diagnose Gilbert syndrome in males easily. Increased TBL causes a morbid condition known

as jaundice, and liver metabolism problems like reduced hepatocyte uptake, impaired bilirubin conjugation, or reduced bilirubin secretion (Wintrobe & Greer, 2009). In acute human hepatic injury, total bilirubin can be a better indicator of disease severity compared to ALT (Ozer *et al.,* 2008).

2.8.5 Albumin (ALB)

Albumin is measurable the most significant protein in plasma synthesized by the liver, and is a useful indicator of hepatic function. The serum albumin level is not a good indicator of hepatic protein synthesis in acute liver disease, because serum albumin changes slowly due to its long half life of about 20 days (Thapa & Walia, 2007; Yap & Aw, 2010). Albumin synthesis only takes place in the liver, and is affected not only in liver disease, but also by nutritional status, hormonal balance and osmotic pressure. The serum levels are typically down in patients with cirrhosis and ascites. In patients with or without ascites, the serum albumin level corresponds with prognosis also the rate of albumin synthesis has been shown to correlate with the Child- Turcotte or Child- Pugh score (Thapa & Walia, 2007). The serum albumin levels tend to be normal in diseases like acute viral hepatitis, drug-related hepatotoxicity and obstructive jaundice. Albumin is decreased by trauma, inflammatory conditions and malnutrition (Yap & Aw, 2010).

2.8.6 Gamma-glutamyl transferase activity (GGT)

Gamma-glutamyl transferase (GGT) is membrane bound glycoprotein, whose activity is found in the liver, kidney, and pancreas tissues. However, its enzyme concentration in liver is lower compared to kidney (Ozer *et al.,* 2008).The gene for gamma-glutamyl transpeptidase is on chromosome 22 (Thapa & Walia, 2007). GGT has various functions like catalytic transfer of

gamma-glutamyl groups to amino acids, short peptides and water, including the hydrolysis of glutathione to a gamma-glutamyl moiety and cysteinlyglycine (a naturally occurring dipeptide) in glutathione (Csanaky & Gregus, 2005). GGT also assist in the pancreatic transport of amino acids across cell membranes and breaks the gamma-glutamyl linkage of leukotriene C4 (Ozer *et al.,* 2008). In liver disease, gamma-glutamyl transpeptidase activity correlates well with the activity of alkaline phosphatase to verify that elevations of alkaline phosphatase is as a result of biliary injury. Gamma-glutamyl activity is a marker of hepatobiliary injury, especially cholestasis and biliary effects. However, in rare cases, gamma-glutamyl transpeptidase activities may be normal in intra hepatic cholestasis and shows high sensitivity with a low false negative rate in humans (Thapa &Walia, 2007; Ozer *et al.,* 2008). Other conditions that cause high levels of gamma-glutamyl transpeptidase are high alcohol intake, uncomplicated diabetes mellitus, acute pancreatitis and myocardial infarction. Drugs like phenobarbitone, phenytoin, paracetamol, tricyclic antidepressants may also cause elevated levels of gamma-glutamyl transpeptidase (Thapa & Walia, 2007). Rat GGT is expressed in a tissue-specific manner, but the rat GGT assay detects bile duct hyperplasia and necrosis (Ozer *et al.,* 2008).

2.8.7 Lactate dehydrogenase (LDH)

Lactate dehydrogenase (LDH) is an oxidoreductase enzyme (EC 1.1.1.1.27) found in different types of human tissues. Despite LDH being a tetrameric enzyme, only two subunits have been determined which are denoted as H for heart and M for muscle (AL-Janabi, Ali & Noree, 2015). The main duty of LDH is the conversion of pyruvic acid, the final product of glycolysis to lactic acid and vice versa in muscle through production of cellular energy. LDH is localized in most of the active organs in the body in small amounts. Therefore, high level of this enzyme may indicate unusual conditions that can result from liver, muscular disorder (e.g. acute

myocardial infarction) and even from cancer disease (AL-Janabi *et al.,* 2015). Moreover, total LDH activity may increase in the blood of patients suffering from disease like allergy, but not in patients with chronic obstructive pulmonary disease (AL-Janabi *et al.,* 2015). Elevated activities of LDH is a biomarker for liver disease.

2.8.8 Urobilinogen

Urobilinogen is a colorless product of bilirubin reduction which an increase in the urine serves as a sensitive indicator of Hepatocellular dysfunction. Urobilinogen is a good indicator of alcoholic liver damage, cirrhosis or malignant disease of the liver. It shows early in urine in the case of viral hepatitis, and also increases in hemolysis (Thapa & Walia, 2007). Urobilinogen level has an identical role as bilirubin in the indication of liver dysfunction. Low urobilinogen may result from biliary obstruction or complete obstructive jaundice. For instance, in cholestatic jaundice, urobilinogen disappears from urine, but at times present in case of gallstones (Yang *et al.,* 2014). Since urobilinogen is formed in the intestine by bacteria, broad-spectrum antibiotic treatment can automatically decrease its level due to the damage of intestinal bacterial flora (Wintrobe & Greer, 2009). When Urobilinogen reacts with Ehrlich's aldehyde reagent it gives a purple pigment which is carried out using freshly voided urine (Thapa & Walia, 2007).

2.8.9 Cholesterol/ Triglycerides

After the liver takes up lipoprotein cholesterol (insoluble), a portion is enzymatically converted to bile salt (soluble). The liver cell or hepatocyte has cholesterol 7α-hydroxylase, which is the rate-limiting enzyme for the multiple process conversion (Yang *et al.,*2014). Triglycerides may get elevated during acute hepatic necrosis as a result of hepatic lipase deficiency. Cholesterol

and triglycerides can accumulate in the blood as low-density lipoprotein cholesterol when bile cannot be eliminated. Hence, since the liver is responsible for the production of blood coagulation factors, the clotting time will be increased due to the impaired synthesis in the liver. However, it is not a sensitive biomarker because it only happens at the late stage of liver disease (Yang *et al.,* 2014).

2.8.10 Ammonia

Ammonia is one of the major products of nitrogen metabolism, moved from the muscle and other peripheral tissues to the liver, where it is converted to urea by the urea cycle, and excreted by the kidney in form of urine (Yang *et al.,* 2014). This urea is less toxic in the body, but in a case where there is liver dysfunction, i.e inability of the liver to carry out this conversion, there is high elevation of ammonia in the body (Yang *et al.,* 2014). Hence, the liver has a high functional reserve for ammonia conversion, so it often takes a significant amount of injury to alter ammonia levels. Increasing ammonia indicates end-stage liver disease and a high risk of hepatic coma (Yang *et al.,* 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant materials

Fresh samples of *Diodia sarmentosa* (Sw) leaves were collected from farm lands and from natural vegetation within Ihiagwa environment. The plant was identified by Prof. I.I Iloegbulam of Crop Science department in Federal University of Technology, Owerri.

Figure 3:1 Photograph showing leaves of *Diodia sarmentosa*

3.2 Chemicals and reagents

Analytical grade chemicals and reagents were used for this study.

3.3 Experimental animals

Male wistar rats weighing between 150-200g were used for this study. The animals were purchased from Department of Biochemistry, University of Port Harcourt, Rivers State, Nigeria.

3.4 Measurement of heart rate

Pulse rate of the rats were measured using a stethoscope and number of beats per minute recorded, by counting the number of beats per minute.

3.5 Preparation of plant extract

Fresh leaves of *Diodia sarmentosa* (Sw) were air-dried at room temperature and then ground into fine powder using laboratory mortar and pestle. This leaves now in fine powder were soaked in 80% ethanol for a period of one week then filtered using Whatman filter paper No. 42 to get the plant extract.

3.6 Experimental site

The animals were acclimatized in an animal house at Biochemistry department in Federal University of Owerri, under room temperature and relative humidity of 40-65% with a 12h natural light-dark cycle. The animals were granted free access to water and rats chew in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for animal experiment.

3.7 Grouping of animals and treatment

30 wistar rats (male) used for this experiment were grouped into five (5) groups, six (6) for each group;

Group NC: Animals received only standard pellet diet and water and, served as Negative control.

Group PC: Untreated hyperlipidaemia rats on high fat diet served as Positive control.

Group LDE: Hyperlipidaemic rats treated with low dose at 250mg/kg body weight of ethanolic leaf extract of *Diodia sarmentosa* (Sw).

Group HDE: Hyperlipidaemic rats treated with high dose at 500mg/kg body weight of ethanolic leaf extract of *Diodia sarmentosa* (Sw).

Group SAD: Hyperlipidaemic rats treated with standard antihyperlipidiaemic drug (simvastatin) at 5mg/kg body weight of simvastatin.

According to the Ezejiofor & Okoroafor (2019), the median lethal dose (LD_{50}) of ethanolic extract of *Diodia sarmentosa* was established as 1600 mg/kg. Therefore, safe doses of 250 mg/kg and 500 mg/kg were adopted for this study.

3.8 Preparation of hyperlipidaemic rats and treatment phase

The rats were placed on high-fat diet [Ghee or clarified butter and Coconut oil in the ratio of 3:1] for a period of six (6) weeks adapted from a previous study by Munshi, Joshi & Rane (2014) except the negative control (NC) group. All animals were allowed access to food and water and body weight of rats were taken weekly.

After the 3rd week, the blood samples were collected through ocular puncture and a confirmatory test was carried out to determine if the hyperlipidemic induction was successful. At the start of the 4th week to the end of the 6th week, group PC continued on the high fat diet, while groups LDE, HDE and SAD continued on high fat diet and their respective drugs, and group NC remained on normal rat chew. The appropriate dosages of the *Diodia sarmentosa* (Sw) were administered to the animals orally once daily by intubation using intravenous cannula tube.

The rats were allowed to fast for twenty-four hours after the last treatment, and then anesthetized with chloroform vapour and blood samples were collected through cardiac puncture. The heart was homogenated, and all samples put inside labeled tubes for biochemical analyses.

3.9 Screening of ethanolic solvent of the leaf for antioxidant activity

3.9.1 Hydroxyl radical scavenging assay

Principle: Free radical dependent 2-deoxyribose degradation was studied using the Fenton oxidant reaction mixture of Fe^{3+}/ascorbic acid and H_2O_2 as described by Halliwell, Guttridge & Aruoma (1987), as reported by Alisi, Ojiako, Osuagwu & Onyeze (2011). Fenton oxidant reaction mixture releases hydroxyl radicals which attack and degrade 2-deoxyribose into fragments. These fragments react with thiobarbituric acid on heating at low pH to form a pink product (thiobarbituric acid reacting substances (TBARS), whose optical density was read at 532 nm.

Procedure: The reaction mixture contained: Deoxyribose (2.8mM), $FeCl_2$ (0.1mM), EDTA (0.1mM), H_2O_2 (1mM), ascorbic acid (0.1mM), KH_2PO_4–KOH buffer (20mM, pH 7.4), and the extract (10-3000µg/ml) in a final volume of 1.0ml. After incubation for 1 hr at 37^{o}C, 1.5ml of 20% acetic acid (pH 3.5), 1.5ml of 0.8% thiobarbituric acid (TBA), 0.2ml of 8.1% sodium dodecyl sulphate (SDS) was added, and the incubation mixture heated at 100^{o}C for 1hr, cooled and 2ml of trichloroacetic acid added. The mixture was vortexed vigorously and centrifuged for 10 minutes and the absorbance of the supernatant read at 532nm wavelength. Inhibition of deoxyribose degradation was expressed in percentage, and hydroxyl radical scavenging was calculated relative to control using the equation:

$$\% \text{ Inhibition} = 100 - \frac{\text{Test}}{\text{Control}} \times 100$$

3.9.2 Nitric oxide scavenging assay

Principle: Based on the principle that the compound sodium nitroprusside (SNP) is known to decompose in aqueous solution at physiological pH (7.2) producing $NO^{\cdot}$. Under aerobic conditions, $NO^{\cdot}$ reacts with oxygen to produce stable products: nitrate and nitrite, the quantities of nitrate and nitrite can be determined using Griess reagent. The nitric oxide scavenging activity of *Diodia sarmentosa* (Sw) was estimated according to the earlier method described by Marcocci, Packer, Droy-Lefaix, Sekaki & Gardes-Albert (1994), as reported by Lalhminghlui & Jagetia (2018).

Procedure: Sodium nitroprusside (25mM) in phosphate buffered saline was mixed with different concentrations of ethanol extract of *Diodia sarmentosa* (Sw) and incubated at 29°C for 120 min. An aliquot (2ml) of the incubation solution was then mixed with Griess reagent (1% sulfanilamide, 5% H_3PO_4 and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride). A chromophore was formed during diazotization of nitrite with sulfanilamide and subsequent coupling with NED, and its absorbance read at 550nm wavelength. The inhibition of nitrite formation by extract or the standard antioxidant (Quercetin) were calculated relative to control using the equation:

$$\% \text{ Inhibition} = 100 - \frac{\text{Test}}{\text{Control}} \times 100$$

3.10 Biochemical analyses

3.10.1 Serum lipid profiles

3.10.1.1 Determination of total cholesterol concentration

The enzymatic (cholesterol esterase/oxidase/peroxidase) method of Allain, Poon, Chan, Richmond & Fu (1974) was used for determination of total cholesterol as reported by Saidu, Bilbis, Muhammad & Nasir (2012)

Principle: Free and esterifed cholesterol in the sample originates, by means of the coupled reactions described below, to form a coloured complex that absorbs at 500nm

Cholesterol ester + H_20 $\xrightarrow{\text{Cholesterol esterase}}$ cholesterol + fatty acid

Cholesterol + ½ O_2+ H_20 $\xrightarrow{\text{cholesterol oxidase}}$ cholesterol + H_20_2

$2H_20_2$ + 4 – amino antipyrine + phenol $\xrightarrow{\text{Peroxidase}}$ Quinoneimine + $4H_20$

Procedure: The reagents were exposed to room temperature and pipetted into labeled test tubes as follows:

Reagents	Blank	Standard	Sample
Cholesterol	-	10µl	-
Sample	-	-	10µl
Reagent A (35mmol/l sodium cholate, 28mmol/lphenol, cholesterol esterase >0.2U/ml, cholesterol oxidase >0.4U/l, Peroxidase >0.8U/ml ; 0.5mmol/l 4-aminoantipyrene, pH 7.0)	1.0ml	1.0ml	1.0ml

The test tubes were mixed thoroughly and incubated for 10mins at room temperature

The absorbance (A) of samples or standard was read against the reagent blank at 500nm in a spectrophotometer (turner® 390).

Calculations: The cholesterol concentration in the sample was calculated as follows

$$C_{sample}\ (mg/dl) = \frac{A_{sample}}{A_{standard}} \times C_{standard}\ (mg/dl)$$

3.10.1.2 Determination of triglyceride (TG) concentration

The glycerol phosphate oxidase/peroxidase method as described by Bucolo & David (1973) as reported by Saidu *et al.* (2012).

Principle: Triglyceride in the sample originates, by means of the coupled reactions described below, to form a coloured complex that can be measured by spectrophotometry.

Triglycerides + H_20 $\xrightarrow{\text{Lipase}}$ Glycerol + fatty acid.

Glycerol + ATP $\xrightarrow{\text{Glycerol kinase}}$ Glycerol – 3 – phosphate + ADP

Glycerol - 3 - phosphate + O_2 $\xrightarrow{\text{Glycerol – 3 – phosphate oxidase}}$ Dihydroxy- acetonephosphate + H_2O_2

$2H_20_2$ + 4 - aminoantipyrine + Chlorophenol $\xrightarrow{\text{peroxidase}}$ Quinoneimine + $4H_2O$

Procedure: The reagents were exposed to room temperature and reagents were pipetted into labeled test tubes as follows:

Reagents	Blank	Standard	Sample
TG standard (glycerol 200mg/dl)	-	10µl	-
Sample (serum)	-	-	10µl
Reagent A (45mmol/l glycerol kinase>1.5µmol/ml, glycerol 3-phosphate oxidase>4µmol/ml, peroxidase>0.8 µmol/ml, 4-aminoantipyrene, 0.75mmol/l, ATP 0.9 µmol/ml, pH 7.0	1.0ml	1.0ml	1.0ml

The test tubes were mixed thoroughly by vortexing and incubated for 15mins at room temperature. The absorbance (A) of samples or standard was read against the reagent blank at 500nm in a spectrophotometer (turner® 390).

Calculations: The Triglyceride concentration in the sample was calculated as follows

$$C_{sample}\ (mg/dl) = \frac{A_{sample}}{A_{standard}} \times C_{standard}\ (mg/dl)$$

3.10.1.3 Determination of high density lipoprotein (HDL)-Cholesterol Concentration.

The serum HDL-cholesterol concentration was measured directly in the serum by using the phosphotungstate/Mg-cholesterol oxidase and peroxidase method as described by Grove (1979) and Burstein, Scholnick & Morfin (1980) as reported by Ojiako *et al*. (2018)

Principle: The apoB containing lipoproteins in the specimen was precipitated with phosphotungstate and magnesium ions. The supernatant contains HDL. The HDL cholesterol was measured spectrophotometrically by means of the coupled reactions described below.

Cholesterol esters + H_20 $\xrightarrow{\text{cholesterol esterase}}$ cholesterol + fatty acid

Cholesterol + ½ O_2 + H_2O $\xrightarrow{\text{cholesterol oxidase}}$ cholestenone + H_2O_2

$2H_2O_2$ + 4-aminoantipyrine + cholorophenol $\xrightarrow{\text{peroxisase}}$ Quinoneimine + $4H_2O$

Procedure: The reagents were exposed to room temperature and were pippetted into labelled test tubes as follows:

Sample (Serum)	0.2ml
Reagent-A (0.4mmol/l phosphotungstste and 20mmol/l magnesium chloride)	0.5ml

The test tubes were mixed thoroughly by vortexing and stood for 10mins at room temperature. They were centrifuged at 4000rpm for 10mins; Supernatants were carefully separated from the precipitate and pippeted into labeled test tubes as follows:

Reagents	Blank	Standard	Sample
Distilled water	50µl	-	-
HDL Cholesterol standard (S)	-	50µl	-
Sample supernatant	-	-	50µl
Reagent-B (35mmol/l phosphate buffer, 0.5mmol/l sodium cholate, cholesterol esterase >0.2U/ml, cholesterol oxidase >0.1U/l, Peroxidase >1.0U/ml ; 0.5mmol/l 4-aminoantipyrene, 4mmol/l dichlorophenolsulphanate), pH 7.0	1.0ml	1.0ml	1.0ml

The test tubes were mixed thoroughly and incubated for 30mins at room temperature. The absorbance (A) of samples or standard was read against the reagent blank at 500nm in a turner 390 spectrophotometer.

Calculations: The HDL cholesterol concentration in the sample was calculated as follows;

$$C_{sample} = \frac{A_{sample} \times C_{standard}}{A_{standard}} \text{ (mg/dl)}$$

3.10.1.4 Determination of low density lipoprotein cholesterol (LDL-C) concentration

LDL in the sample was determined according to the method of Assman, Jabs, Kohnert, Nolte & Schriewer (1984) as reported by Ojiako *et al*., (2018).

Principle: LDL in the sample Precipitates with polyvinyl sulphate. Their concentration is calculated from the difference between the total cholesterol and the cholesterol in the supernatant after centrifugation. The cholesterol concentration was measured colorimetrically by means of the coupled reactions described below.

Cholesterol esters + H_20 $\xrightarrow{\text{Cholesterol esterase}}$ Cholesterol + Fatty acid

Cholesterol + ½ 02 + H_20 $\xrightarrow{\text{Cholesterol oxidase}}$ Cholestenone + H_20_2

$2H_20_2$ + 4 – aminoantipyrine + Phenol $\xrightarrow{\text{Peroxidase}}$ Quinoneimine + $4H_20$

Procedure: The reagents were exposed to room temperature and reagents were pipetted into labeled test tubes as follows:

Sample	0.4ml
Reagent (A) (3g/l polyvinyl sulphate, 3g/l polyethyleneglycol).	0.2ml

The test tubes were mixed thoroughly by vortexing and stood for 15mins at room temperature, thereafter they were centrifuged at 4000rpm for 15mins. Supernatants were carefully separated from the precipitate and pippeted into labeled test tubes as follows;

Reagents	Blank	Standard	Sample
Distilled water	20µl	-	-
Cholesterol standard	-	20µ	-
Sample Supernatants	-	-	20µ
Reagent-A(35mmol/l sodium cholate, 28mmol/lphenol, cholesterol esterase >0.2U/ml, cholesterol oxidase >0.4U/l, Peroxidase >0.8U/ml ; 0.5mmol/l 4-aminoantipyrene), pH 7.0	1.0ml	1.0ml	1.0ml

The test tubes were mixed thoroughly by vortexing and incubated for 30mins at room temperature. The absorbance (A) of samples or standard was read against the reagent blank at 500nm in a turner 390 spectrophotometer.

Calculations: The cholesterol concentration in the sample was calculated as follows;

$$C_{supernatant} = \frac{A_{sample}}{A_{standard}} \times C_{standard} \times \text{Dil Factor (mg/dL)}$$

3.10.1.5 Determination of very low density lipoprotein cholesterol (VLDL-C) concentration

VLDL-cholesterol was determined using the formula of Friedewald, Levy & Fredrickson (1972) as reported by Chikezie *et al*. (2018).

VLDL-C = Triglyceride/5

Values are expressed in mg/dL.

3.10.1.6 Atherogenic Ratios and Indices

The Atherogenic indices were calculated as

Castelli's Risk Index I (CRI-I) = LDLC/HDLC

Castelli's Risk Index II (CRI-II) = TC/HDLC

Atherogenic Coefficient (AC) = (TC– HDLC)/HDLC

Atherogenic Index of Plasma (AIP) = log (TG/HDLC)

$$\%\text{Protection of drug} = \frac{AC_{positve\ control} - AC_{treated\ grp}}{AC_{positve\ control}} \times 100$$

(Chikezie *et al*., 2018)

Where AC= Atherogenic Coefficient

3.10.2 Oxidative Stress Markers

3.10.2.1 Tissue malondialdehyde (Lipid peroxidation)

Principle: Lipid peroxidation in the supernatant fractions was determined spectrophotometrically by assessing the concentration of thiobarbituric acid reactive substances (TBARS). This method was described by Buege & Aust (1978), as reported by Nair, Abraham & Jaya (2008). The results were expressed in malondiadehyde (MDA) formed relative to an extinction coefficient of 1.56 x 10^6mol/cm. Small quantities of MDA are produced during lipid peroxidation. These react with Thiobarbituric acid (TBA) to generate a pink coloured complex which in acid solution absorb light at 532nm and flourescences at 532nm and is readily extractable into organic solvents such as butan-1-ol.

Procedure: About 1.5mL of Acetic acid (20%; pH 3.5), 1.5mL of thiobarbituric acid (0.8%) and 0.2mL of sodium dodecyl sulphate (8.1%) was added to 0.1mL the samples and heated at 100 ◦C for 60 min. Mixture was cooled and 5mL of *n*-butanol-pyridine (15:1) mixture, 1mL of distilled water was added and vortexed vigorously. After centrifugation at 1200×*g* for 10 min, the organic layer was separated and absorbance measured at 532 nm using a spectrophotometer. Malondialdehyde (MDA) was calculated using a molar extinction coefficient of $1.56\times10^5 M^{-1}$ cm^{-1} and expressed as nanomoles of MDA.

3.10.2.2 Assay of superoxide dismutase (SOD) activity

This assay was carried out according to the procedure of Das, Samanta & Chainy (2000).

Procedure: About1.4mL aliquot of the reaction mixture comprising of 1.1mL of 50nM phosphate buffer (pH=7.4), 0.075mL of 20mML methionine, 0.4ml of 1% (v/v) Triton X-100, 0.075mL of 10mM hydroxylamine and 0.1mL of 50mM EDTA. This aliquot (1.4mL) was added to 0.1ml of the sample and incubated at 30^0C for 5mins. This was followed by the

addition of 80µl of 50µM riboflavin, and these tubes were exposed for minutes to 200 watts Philip lamps. After the exposure time, 1mL of Greiss reagent (mixture of equal volume of 1% sulphanilamide in 5% phosphoric acid) was added and the absorbance of the colour formed was measured at 543nm. One unit of enzyme activity was measured at the amount of SOD capable of inhibiting 50% of nitrate formation under the assay condition.

3.10.2.3 Assay of catalase (CAT) activity

The catalase enzyme activity in tissues was assayed following the procedure of Aebi (1984), as reported by Hassan, Ahmed, Hassanein & Waly (2016).

Principle: The method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2, with the formation of perchromic acid as an unstable intermediate. The chromic acetate thus produced is measured calorimetrically at 570-610nm. The catalase preparation is allowed to split H_2O_2 for different periods of time. The reaction is stopped after 5mins by the addition of dichromate/acetic acid mixture, and the remaining H_2O_2 is determined by measuring chromic acetate colorimetrically after heating the reaction mixture.

Procedure: Samples were incubated with H_2O_2 (5mM, 2.0 mL) in the presence of 2.5ml 0.01 M phosphate buffer (pH 7.4). About 1.0 ml portion of the reaction mixture was withdrawn and blown into 2 ml of dichromate/acetic acid reagent at 60 sec intervals. Samples were incubated in boiling water for 15 min. After cooling at room temperature, the optical density was measured at 570nm. The upper layer of the mixture was taken and the absorbance read at 570 nm.

The rate of decomposition of H_2O_2 was measured using the equation for a first-order reaction.

$$K = \frac{1}{t} \text{Log}_{10} \frac{So}{S}$$

Where S_O is the initial H_2O_2 concentration and S is the H_2O_2 concentration at a particular time interval given as t (minutes). The values of K are plotted against t, and the velocity constant K(0) at 0 minute determined by extrapolation (that is the intercept on the vertical axis). The catalase activity of the sample was expressed in terms of katalase unit per g protein

Kat. = K (0) / g protein = Unit/gprotein

3.10.2.4 Determination of glutathione-s-transferase activity

Glutathione-S-transferase (GST) in tissue was measured by the method of Habig *et al*., (1974) as reported by Sasi Bhusana Rao *et al*., (2016). The assay mixture consists of 1.7 ml of sodium phosphate buffer (0.14 M, pH 6.5), 0.2 ml of GSH (30 mM), and 0.04 ml of the samples. The reaction was initiated by addition of 0.06 ml of 1-chloro-2, 4-dinitrobenzene (CDNB) (0.01 M dissolved in 50% ethanol). The activity was calculated by using mill molar extinction coefficient of CDNB-GSH conjugate as 9.6. The activity was expressed as μmoles of CDNB-GSH conjugate formed/min/mg protein.

3.10.2.5 Determination of glutathione peroxidase (GPx) activity

Glutathione peroxidase (GPx) in tissue was measured by the method of Rotruck *et al*. (1973) as reported by Sajeeth *et al.* (2011). Briefly, the reaction mixture which consists of 0.5 ml of phosphate buffer (0.2 M, pH 7.0 containing 0.4 mM EDTA, and 10 mM sodium azide), 0.2 ml of tissue homogenate, 0.2 ml of GSH (2 mM) and 0.1 ml of H_2O_2 (0.2 mM). The reaction mixture was incubated for 10 min at room temperature along with blank containing all reagents

except the samples. The reaction was stopped by the addition of 0.5 ml of TCA (10%), centrifuged at 4000 rpm for 5 min and GSH content in 0.5 ml of supernatant was estimated by following the method of Ellman (1959). The activity was expressed as μg of GSH consumed/min/mg protein.

3.10.3 Liver Enzymes

3.10.3.1 Assay of serum alanine aminotransferase (ALT) activity

Principle: This was carried out based on the method of Reitman & Frankel (1957), as reported by George, Osioma, Okpoghono & Aina (2011). The principle of the test is that ALT catalyses the transfer of the amino group from L-alanine to α-ketoglutarate resulting in the formation of pyruvate and L-glutamate. ALT is measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenylhydrazine.

Reagent Composition: Reagent1: 100mmol/l phosphate buffer pH 7.4, 100mmol/l L-alanine and 2mmol/l α-oxoglutarate.

Reagent 2: 2mmol/l 2,4-dinitrophenyl hydrazine

Procedure: The reagents were pipetted into labeled test tubes as follows: 0.5ml of reagent 1 was placed into tubes labeled reagent blank and sample. Then 0.1ml of sample was pipetted into sample tubes, while 0.1ml of distilled water was pipetted into the blank and thoroughly mixed. The setup was incubated at 37^0C for exactly 30 minutes. Then 0.5ml of reagent 2 was added into both the blank and sample tubes, mixed thoroughly and incubated further for exactly twenty (20) minutes at room temperature. Into each of the tubes, 5.0ml of 0.4M NaoH was added into both blank and sample tubes, mixed thoroughly and allowed to stand at room

temperature for 5 minutes. The optical density of the sample was measured against the reagent blank at 546nm in a spectrophotometer. The activity of ALT in the serum was read from the ALT calibration curve in U/L (see Appendix V)

3.10.3.2 Assay of serum aspartate aminotransferase (AST) Activity

Principle: Determination of AST activity was based on the method of Reitman & Frankel (1957), as reported by George *et al.* (2011). AST catalyzes the transfer of the alpha-amino group from L-aspartate to α-ketoglutarate resulting in the formation of oxaloacetate and L-glutamate. AST is measured by the reaction of oxaloacetate with 2, 4–Dinitrophenylhydrazine (DNPH) to form 2,4 – dinitrophenylhydrazone in an alkaline medium.

α-ketoglutarate + L-aspartate ⟶ L-glutamate + oxaloacetate.

Oxaloacetate + 2, 4-dinitrophenylhydrazine ⟶ 2,4 – dinitrophenylhydrazone

Reagent Composition: Reagent1: 100mmol/l phosphate buffer pH 7.4, 100mmol/l L-aspartate and 2mmol/l α-oxoglutarate.

Reagent 2: 2mmol/l 2,4-dinitrophenyl hydrazine

Procedure: The reagents were pipetted into labeled test tubes as follows: 0.5ml of reagent 1 was placed into tubes labeled reagent blank and sample. Then 0.1ml of sample was pipetted into sample tubes, while 0.1ml of distilled water was pipetted into the blank and thoroughly mixed. The setup was incubated at 37^0C for exactly 30 minutes. Then 0.5ml of reagent 2 was added into both the blank and sample tubes, mixed thoroughly and incubated further for exactly twenty (20) minutes at room temperature. Into each of the tubes, 5.0ml of 0.4M NaoH was added into both blank and sample tubes, mixed thoroughly, and allowed to stand at room

temperature for 5 minutes. The optical density of the sample was measured against the reagent blank at 546nm in a spectrophotometer. The activity of AST in the serum was read from the AST calibration curve in U/L (see Appendix V)

3.10.3.3 Assay of serum alkaline phosphatase (ALP) activity

ALP activity was determined according to the method described by Englehardt (1970), as reported by Ujowundu, Igwe, Agha & Okechukwu (2014).

Principle: ALP catalyses the hydrolysis of p-nitrophenylphosphate to p-nitrophenol with the simultaneous release of phosphate group. The rate of reduction of p-nitrophenylphosphate, measured by determining the rate of increase in absorbance at 405nm, is directly proportional to the ALP activity.

p-nitrophenylphosphate + H_2O ALP $\longrightarrow$ phosphate+ p-nitrophenol

Reagent Composition: Buffer (1M diethanolamine buffer, 0.5M $MgCl_2$), Subtrate (10M p-nitrophenylphosphate)

Procedure: The reagents were exposed to room temperature, and reagents were pipetted into labeled cuvettes as follows: 1.0ml of the ALP reagent was pipetted into a cuvette serving as blank and sample cuvette. Into the blank, 0.02ml of distilled water was added and used to zero the spectrophotometer. Into another cuvette 1.0ml of ALP reagent was pipetted and 0.02ml of serum was added, mixed and initial absorbance read. The timer was started simultaneously and the absorbance read after 1minute, 2 and 3minutes interval respectively.

The ALP activity was calculated as follows:

Calculations:

ALP activity (U/L) = (ΔA 405nm/min) X 2760

Where; ΔA= Change in absorbance, min = minute.

3.10.3.4 Determination of total protein concentration

The Biuret method as described by Gornall, Bardawill & David (1949), as reported by Hassan *et al.* (2007) was employed for the determination of protein concentration.

Principle: Copper (II) ions, in an alkaline medium, interact with protein peptide bonds resulting in the formation of a coloured complex. The test was carried out using a protein test-kit (Biosystems, USA) that utilizes the Biuret method for protein determination.

Reagent Composition: Solution 1 (Biuret Reagent) 6mmol/l of copper (II) acetate, 12mmol/l of potassium iodide, 1.15mmol/l of sodium hydroxide, and detergent. Standard is 66g/l bovine serum albumin.

Procedure: The reagents were pipetted into labeled test tubes as follows: 1.0ml of reagent A was placed into tubes labeled reagent blank, standard and sample. Then 0.02ml of distilled water, standard and sample was pipetted into reagent blank, standard and sample tubes respectively. The set-up was thoroughly mixed and incubated at room temperature for 30 minutes. The absorbance of the sample and standard was measured against the reagent blank at 546nm in a spectrophotometer.

Calculations:

The total protein concentration in the sample was calculated as follows

$$C_{sample} = \frac{A_{sample}}{A_{standard}} \times C_{standard}\,(g/l)$$

3.10.3.5 Determination of serum albumin concentration

This was determined by method employing bromocresol green as described by Doumas, Watson & Briggs (1971), as reported by Adedeji *et al.* (2012).

Principle: Albumin, in the sample, reacts with bromocresol green in an acid medium forming a coloured complex that absorbs maximally at 630nm.

Reagent Composition: Reagent A: Bromocresol reagent (100mmol/l acetate buffer, 0.27mmol/l bromocresol green, detergent), pH 4.1.

Procedure: The reagents were pipetted into labeled test tubes as follows: 1.0ml of reagent A was placed into tubes labeled reagent blank, standard and sample. Then 0.01ml of distilled water, standard and sample was pipetted into reagent blank, standard and sample tubes respectively. The set-up was thoroughly mixed and incubated at room temperature for 1 minute. The absorbance of the sample and standard was measured against the reagent blank at 630nm in a spectrophotometer.

Calculations:

The albumin concentration in the sample was calculated as follows:

$$C_{sample} = \frac{A_{sample}}{A_{standard}} \times C_{standard}\,(g/l)$$

3.10.3.6 Determination of serum globulin concentration

Serum globulin concentration was calculated as the difference between serum total protein and serum albumin as reported by Nku *et al.* (2018).

Serum Globulin = Serum Total Protein – Serum Albumin

3.10.3.7 Determination of serum total bilirubin concentration

The method that exploits the use of diazotized sulphanilic acid as described by Pearlman & Lee (1974) as reported by Ojo, Opara & Babatunde (2015).

Principle: Total (conjugated and unconjugated) bilirubin couples with a diazo reagent in the presence of a surfactant to form azobilirubin. The diazo reaction is accelerated by the addition of surfactant as a solublizing agent.

Reagent Composition: Bilirubin Standard (4.3g/l), Reagent AT- (29mmol/lsulphanilic acid, 0.2mmol/l HCl and 50mmol/l cetrimedin).Working reagent (11.6mmol/l sodium nitrite and 4ml of reagent AT).

Procedure: The reagents were exposed to room temperature, and test tubes were labeled test tubes as follows: Reagent blank, Sample blank, sample and standard. About 1.0ml of reagent AT was placed into tubes labeled sample blank, and equivalent volume of working reagent was pipetted into the tubes labeled reagent blank, sample and Standard. Furthermore, 0.1ml distilled water was added into the reagent blank, 0.1ml standard to standard tube, while 0.1ml sample was added to the sample blank and samples respectively. The set-up was vortexed and incubated at room temperature for 2 minutes. The absorbance (A) of samples blanks was read in a spectrophotometer at 540nm against distilled water. The absorbance (A) of samples and standard were read in a spectrophotometer against the reagent blank at 540nm.

The total bilirubin concentration was calculated as follows:

$$C_{sample} = \frac{A_{sample} - A_{sample\ blank}}{A_{standard}} \times C_{standard}\ (g/l)$$

3.10.3.8 Determination of serum lactate dehydrogenase(LDH):

Serum LDH was determined according to the method of Wroblewski & La Due (1975) as reported by Oloyede & Sunmonu (2008).

Principle: Lactate dehydrogenase catalyzes the reduction of pyruvate with NADH to form NAD^+. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance, which is proportional to the LDH activity in the sample.

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{L-Lactate} + NAD^+$$

Procedure: Reagents used were from Agappe diagnostic kit. 0.01ml of serum was added to 1 ml of working reagent prepared by mixing 4 volume of Reagent 1 [Tris buffer (pH 7.4, 80mmpl/L),Pyruvate(1.6mmol/L), sodium chloride (200mmol/L)], with 1 volume of reagent 2 [NADH (240 µ mol/L)] provided in the kit. Mixed well and incubated for 1 minute at 37º C. The change in absorbance was measured per minute for 3 minutes at 340nm.

LDH activity (U/L) = (Δ OD / min) x 16030

Data Analysis

Data were analyzed using appropriate software (Microsoft Excel 2013). Results were presented as mean ± Standard deviation of four determinations and statistically analyzed using one-way analysis of variance on statistical computer software program (SPSS 21). The degree of statistical difference was accepted as significant at $p < 0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

RESULTS

4.1.1 Results of free radical scavenging activities

The result of nitric oxide scavenging activities of *Diodia sarmentosa* and Quercetin dihydrate is as presented in Fig 4.1. From the result, the IC_{40} of *Diodia sarmentosa* and Quercetin dihydrate was 578.46µg/ml ± 37.13 and 35.53µg/ml ± 3.43 respectively.

The result of hydroxyl radical scavenging activities of *Diodia sarmentosa* and Quercetin dihydrate is as presented in Fig 4.2. According to the result, the IC_{40} of *Diodia sarmentosa* and Quercetin dihydrate was 794.74µg/ml ± 26.63 and 157.39µg/ml ± 34.38 respectively.

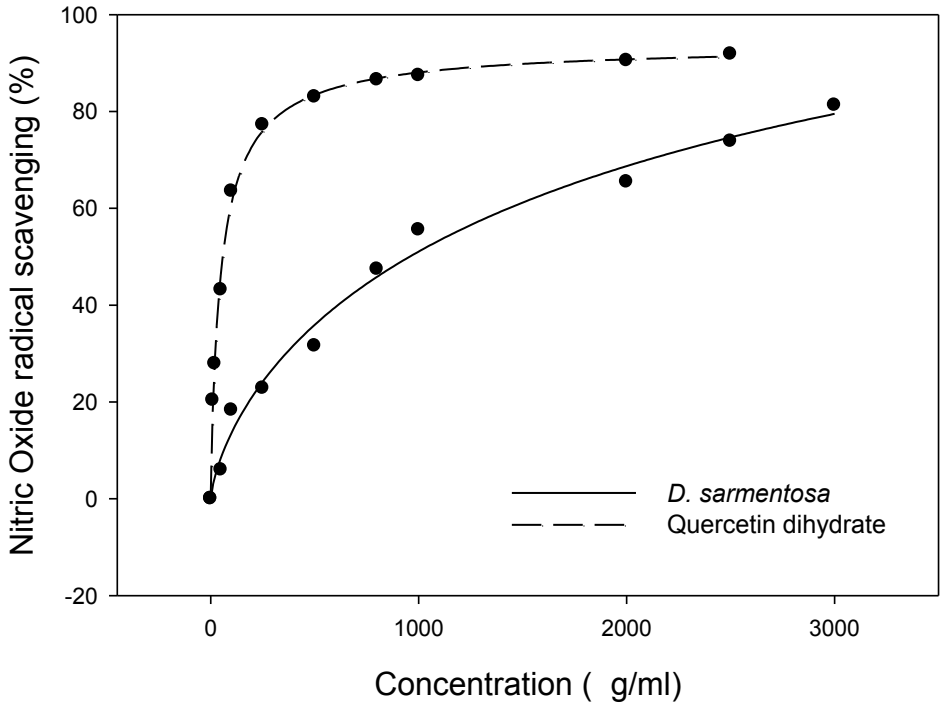

Fig 4.1: Graded concentrations of ethanolic extract of *Diodia sarmentosa* and Quercetin dihydrate on scavenging of nitric oxide radicals.

IC_{50} of *Diodia sarmentosa* = 907.17 µg/ml ± 45.36

IC_{50} of Quercetin dihydrate = 55.37 µg/ml ± 4.43

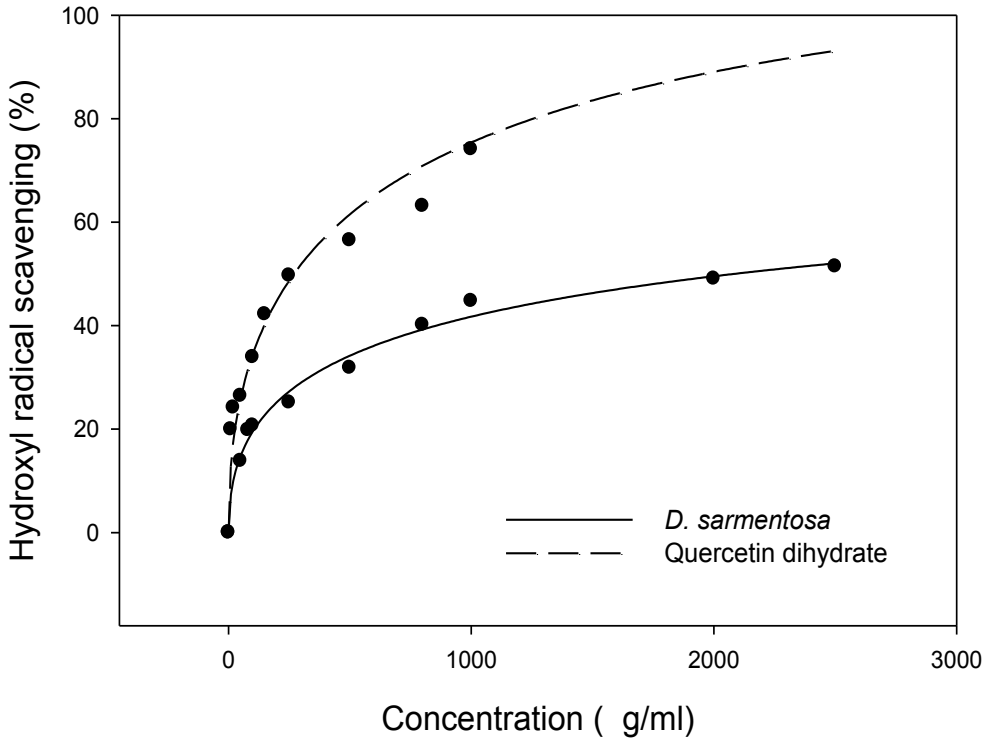

Fig 4.2: Graded concentrations of ethanolic extract of *Diodia sarmentosa* and Quercetin dihydrate on scavenging of hydroxyl radicals.

IC_{50} of *Diodia sarmentosa* = 2173.44 µg/ml ± 100.11

IC_{50} of Quercetin dihydrate = 322.92 µg/ml ± 25.83

4.1.2 Results of Oxidative Stress Indices

The results of superoxide dismutase (SOD) activities in the heart tissue and serum of the different study groups with ranges between 19.37 ± 3.43 — 29.71 ± 2.77 (IU/mg Protein) in the heart tissue, and 20.51 ± 1.13 — 28.49 ± 1.05 (IU/mg Protein) in the serum is as presented in Table 4.1.

The results of glutathione peroxidase (GPx) activities in the heart tissue and serum of the various study groups is as presented in Table 4.2. GPx in the heart tissue between 2.30 ± 0.20 — 3.85 ± 0.20 (mg GSH/min/mgprotein), and 1.52 ± 0.27 — 3.54 ± 0.27 (mg GSH/min/mgprotein) in the serum.

Table 4.3 shows the results of glutathione s-transferase (GST) activities in the heart tissue and serum of the various study groups. GST ranges between 0.82 ± 0.05 — 1.66 ± 0.22 (µmol GSH-CDNB/min/mgprotein) in the heart tissue, and 0.78 ± 0.13 — 1.03 ± 0.14 (µmol GSH-CDNB/min/mgprotein) in the serum.

Table 4.1: Superoxide dismutase (SOD) activities in the heart tissue and serum of the various study groups.

	Groups	Heart tissue	Serum
SOD (IU/mg Protein)x10^-6	NC	26.29±1.65[b]	28.49±1.05[c]
	PC	19.37± 3.43[a]	20.51± 1.13[a]
	LDE	29.71± 2.77[b]	25.73± 0.85[b,c]
	HDE	26.70± 2.56[b]	28.59± 1.86[c]
	SAD	24.62± 2.19[a]	24.95± 1.54[b]

Values are mean ± standard deviation (n=4)

Groups with different alphabets are significantly different ($p<0.05$), while groups with similar alphabets are not significantly different.

NC: Negative control

PC: Positive control

LDE: Low dose of the extract

HDE: High dose of the extract

SAD: Standard antihyperlipidaemic drug

Table 4.2: Glutathione peroxidase (GPx) activities in the heart tissue and serum of the various study groups.

	Groups	Heart tissue	Serum
GPx(mg GSH/min/mgprotein)	NC	3.85 ± 0.20^{d}	3.54 ± 0.27^{c}
	PC	2.30 ± 0.20^{a}	1.52 ± 0.27^{a}
	LDE	$3.54 \pm 0.08^{c,d}$	2.65 ± 0.28^{b}
	HDE	$3.27 \pm 0.31^{b,c}$	2.53 ± 0.29^{b}
	SAD	2.88 ± 0.20^{b}	$2.06 \pm 0.27^{a,b}$

Values are mean ± standard deviation (n=4)

Groups with different alphabets are significantly different ($p<0.05$), while groups with similar alphabets are not significantly different.

NC: Negative control

PC: Positive control

LDE: Low dose of the extract

HDE: High dose of the extract

SAD: Standard antihyperlipidaemic drug

Table 4.3: Glutathione S-transferase (GST) activities in the heart tissue and serum of the various study groups.

	Groups	Heart tissue	Serum
GST (µmol GSH-CDNB /min/mgprotein)x10^-6	NC	0.82± 0.05[a]	0.80± 0.08[a]
	PC	1.66± 0.22[c]	1.03± 0.14[a]
	LDE	1.43± 0.24[b,c]	0.79± 0.11[a]
	HDE	1.07± 0.27[a,b]	0.78± 0.13[a]
	SAD	1.16± 0.20[a,b]	0.83± 0.15[a]

Values are mean ± standard deviation (n=4)

Groups with different alphabets are significantly different ($p<0.05$), while groups with similar alphabets are not significantly different.

NC: Negative control

PC: Positive control

LDE: Low dose of the extract

HDE: High dose of the extract

SAD: Standard antihyperlipidaemic drug

The results of catalase (CAT) activities in the heart tissue and serum of the different study groups with ranges; heart tissue: 5.03 ± 0.32 — 7.18±0.62 (μMH2O2/min/mgprotein), and serum: 12.19 ± 0.85 — 16.35 ± 0.63 (μMH2O2/min/mgprotein) is as presented in Table 4.4.

The results of lipid peroxidation expressed as Malondialdehyde (MDA) levels in the heart tissue and serum of the various study groups in the heart tissue; 0.25 ± 0.02 — 0.64 ± 0.06 (nmol/mgprotein), and serum; 0.16 ± 0.01 — 0.38 ± 0.02 (nmol/mgprotein) is as presented in Table 4.5.

Table 4.4: Catalase (CAT) activities in the heart tissue and serum of the various study groups.

	Groups	Heart tissue	Serum
CATALASE (µM H2O2/min/mgProtein)x10^-6	NC	6.08± 0.33^{a,b,c}	16.02± 0.90^{c}
	PC	5.03± 0.32^{a}	12.19± 0.85^{a}
	LDE	5.83± 0.60^{a,b}	14.12± 0.59^{b}
	HDE	6.84± 0.57^{b,c}	15.43± 1.13^{b,c}
	SAD	7.18± 0.62^{c}	16.35± 0.63^{c}

Values are mean ± standard deviation (n=4)

Groups with different alphabets are significantly different ($p<0.05$), while groups with similar alphabets are not significantly different.

NC: Negative control

PC: Positive control

LDE: Low dose of the extract

HDE: High dose of the extract

SAD: Standard antihyperlipidaemic drug

Table 4.5: Lipid peroxidation expressed in malondialdehyde (MDA) levels in the heart tissue and serum of the various study groups.

	Groups	Heart tissue	Serum
MDA (nmol/mgprotein)	NC	0.25 ± 0.02^{a}	0.16 ± 0.01^{a}
	PC	0.64 ± 0.06^{c}	0.38 ± 0.02^{c}
	LDE	0.37 ± 0.03^{b}	0.23 ± 0.02^{b}
	HDE	$0.32\pm 0.02^{a,b}$	0.23 ± 0.02^{b}
	SAD	0.28 ± 0.03^{a}	0.20 ± 0.02^{b}

Values are mean ± standard deviation (n=4)

Groups with different alphabets are significantly different ($p<0.05$), while groups with similar alphabets are not significantly different.

NC: Negative control

PC: Positive control

LDE: Low dose of the extract

HDE: High dose of the extract

SAD: Standard antihyperlipidaemic drug

4.1.3 Results of lipid profiles

The result of the total cholesterol (TC) levels across the various study groups with range of 80.54 ± 7.14 — 177.25 ± 2.93 (mg/dl) is as presented in Fig 4.3. The levels of TC across the study groups in descending order is as follows; PC > LDE > HDE > NC > SAD.

The result of the triglyceride (TG) levels of the various study groups with range of 45.64 ± 1.78 — 159.74 ± 4.69 (mg/dl) is as presented in Fig 4.4. The levels of TG across the study groups in descending order is as follows; PC > NC > LDE > HDE > SAD.

The result of the High density lipoprotein cholesterol (HDL-C) levels of the various study groups, with range of 35.19 ± 3.82 — 57.81 ± 4.31 (mg/dl) is as presented in Fig 4.5. The levels of HDL-C across the study groups in descending order is as follows; SAD > HDE = NC > LDE > PC.

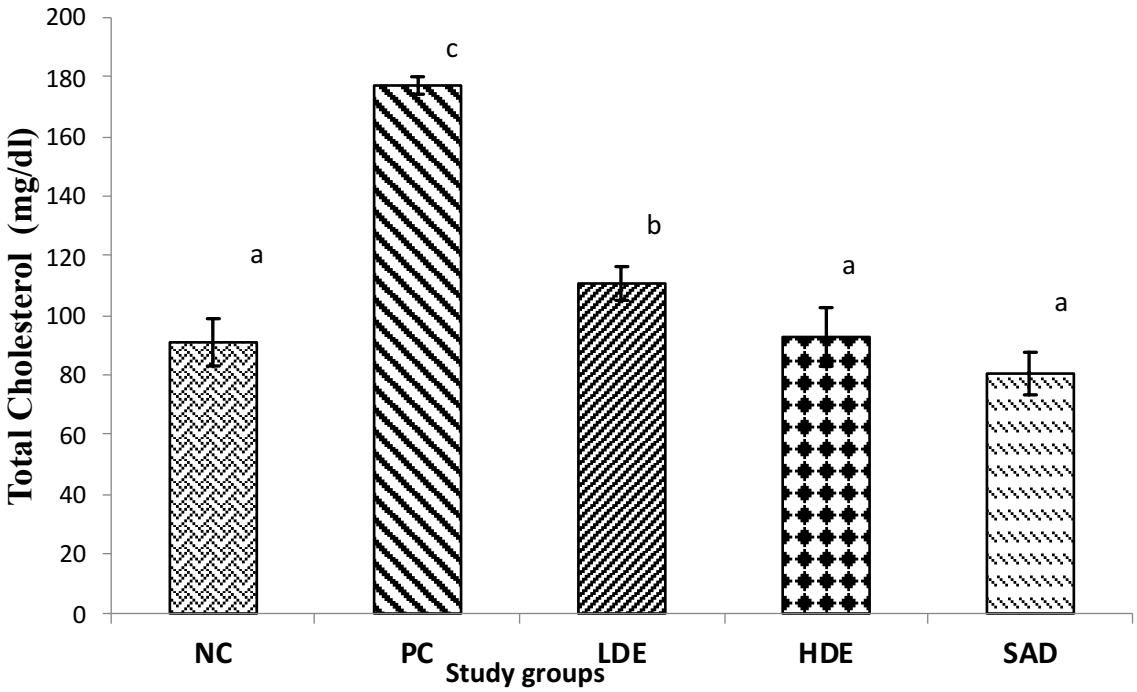

Fig 4.3: Total cholesterol (TC) levels across the various study groups.

Values are the means ± SD (n=4).

Groups with different alphabets are significantly different ($p<0.05$), while groups with similar alphabets are not significantly different.

NC: Negative control

PC: Positive control

LDE: Low dose of the extract

HDE: High dose of the extract

SAD: Standard antihyperlipidaemic drug

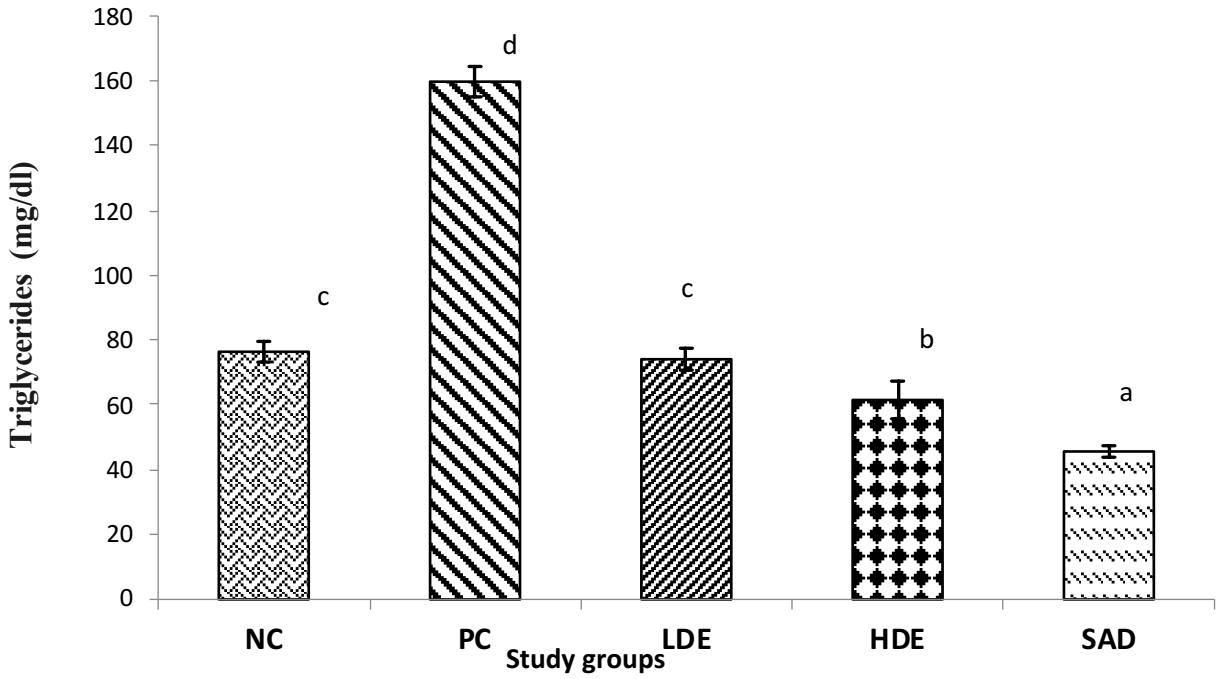

Fig 4.4: Triglycerides (TG) levels across the various study groups.

Values are the means ± SD (n=4).

Groups with different alphabets are significantly different ($p<0.05$), while groups with similar alphabets are not significantly different.

NC: Negative control

PC: Positive control

LDE: Low dose of the extract

HDE: High dose of the extract

SAD: Standard antihyperlipidaemic drug

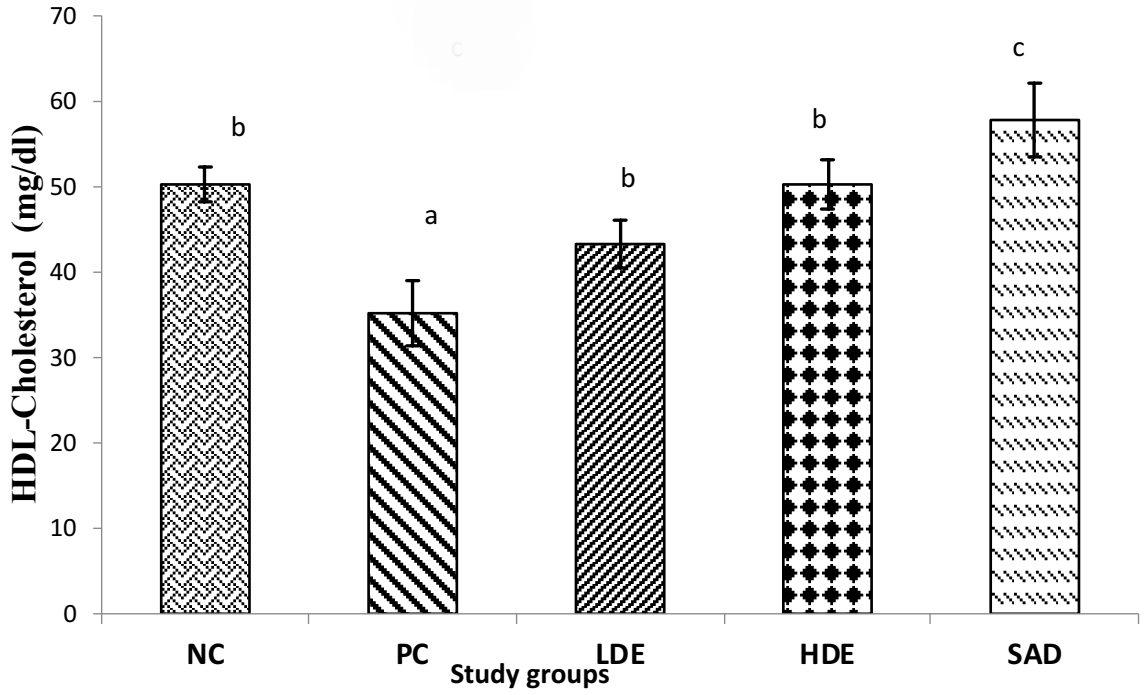

Fig 4.5: High density lipoprotein cholesterol (HDL-C) levels across the various study groups.

Values are the means ± SD (n=4).

Groups with different alphabets are significantly different ($p<0.05$), while groups with similar alphabets are not significantly different.

NC: Negative control

PC: Positive control

LDE: Low dose of the extract

HDE: High dose of the extract

SAD: Standard antihyperlipidaemic drug

The result of the low density lipoprotein cholesterol (LDL-C) levels of the various study groups with range of 18.09 ± 2.80 — 36.77 ± 1.60 (mg/dl) is as presented in Fig 4.6. The levels of LDL-C across the study groups in descending order is as follows; PC > LDE > HDE > SAD > NC.

The result of the very low density lipoprotein cholesterol (VLDL-C) levels of the various study groups with range of 9.13 ± 0.36 — 31.59 ± 0.74 (mg/dl) is as presented in Fig 4.7. The levels of VLDL-C across the study groups in descending order is as follows; PC > NC > LDE > HDE > SAD.

The result of Castelli's risk index I (CRI-I) values of the various study groups with range of 0.33 ± 0.03 — 1.05 ± 0.11 is as presented in Fig 4.8. The values of CRI-I across the study groups in descending order is as follows; PC > LDE > HDE > NC > SAD.

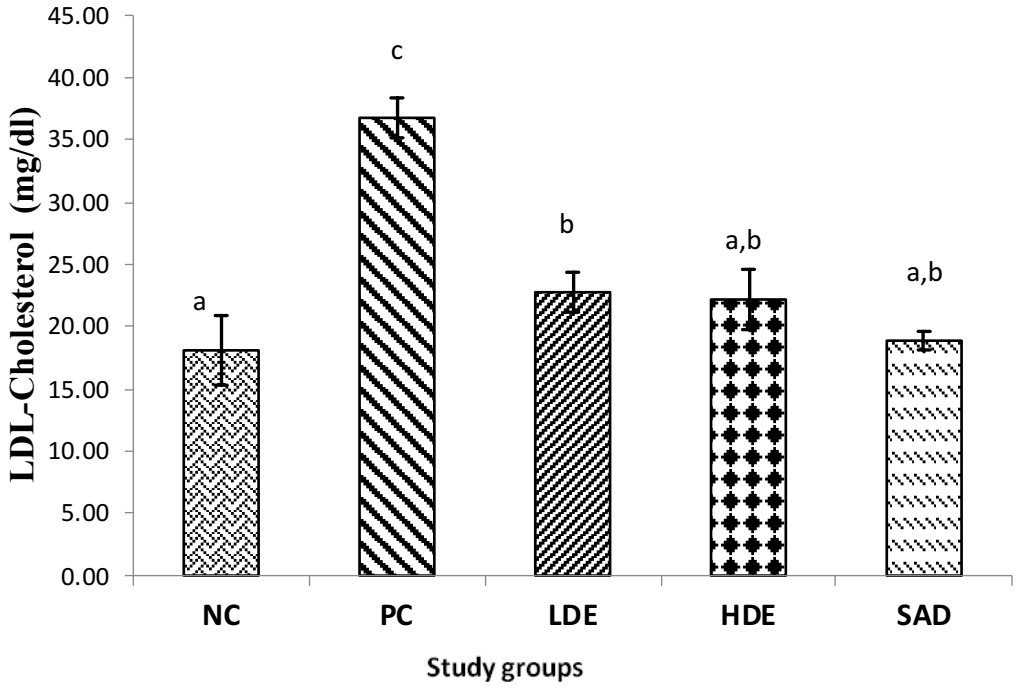

Fig 4.6: Low density lipoprotein cholesterol (LDL-C) levels across the various study groups.

Values are the means ± SD (n=4).

Groups with different alphabets are significantly different ($p<0.05$), while groups with similar alphabets are not significantly different.

NC: Negative control

PC: Positive control

LDE: Low dose of the extract

HDE: High dose of the extract

SAD: Standard antihyperlipidaemic drug

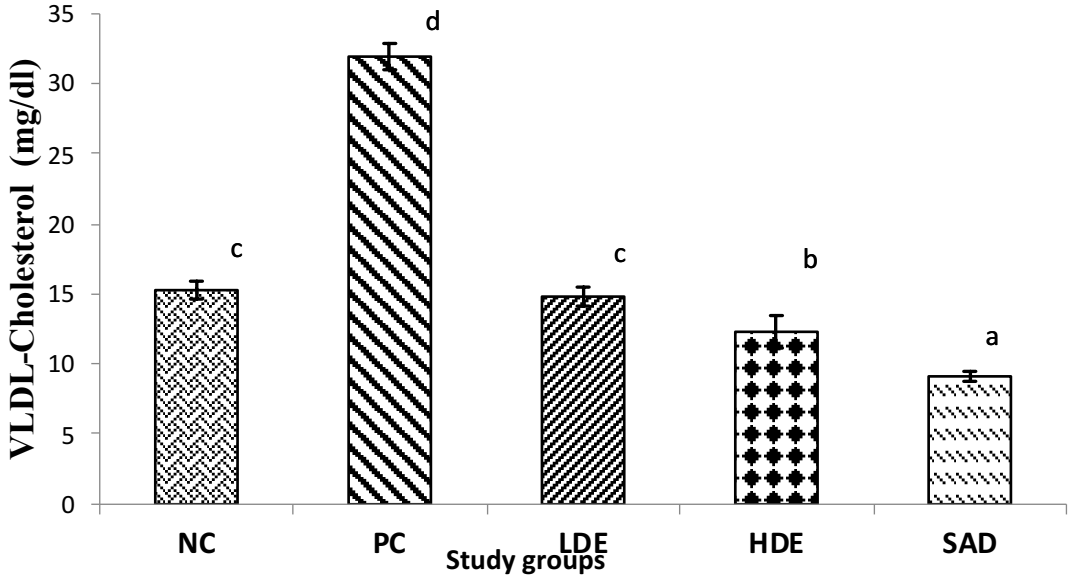

Fig 4.7: Very low density lipoprotein cholesterol (VLDL-C) levels across the various study groups.

Values are the means ± SD (n=4).

Groups with different alphabets are significantly different ($p<0.05$), while groups with similar alphabets are not significantly different.

NC: Negative control

PC: Positive control

LDE: Low dose of the extract

HDE: High dose of the extract

SAD: Standard antihyperlipidaemic drug

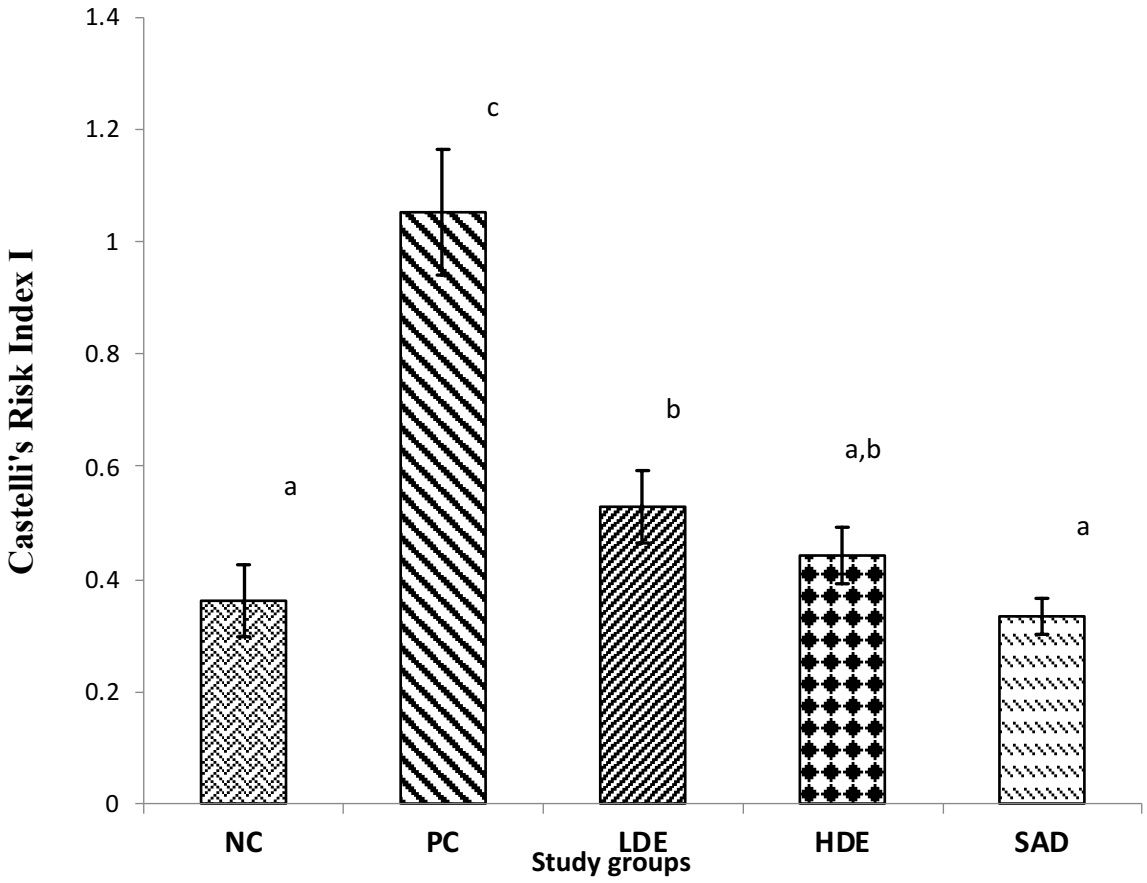

Fig 4.8: Castelli's Risk Index I levels across the various study groups.

Values are the means ± SD (n=4).

Groups with different alphabets are significantly different ($p<0.05$), while groups with similar alphabets are not significantly different.

NC: Negative control

PC: Positive control

LDE: Low dose of the extract

HDE: High dose of the extract

SAD: Standard antihyperlipidaemic drug

The result of Castelli's risk index II (CRI-II) values of the various study groups with range of 1.39 ± 0.08 — 5.08 ± 0.53 is as presented in Fig 4.9. The values of CRI-II across the study groups in descending order is as follows; PC > LDE > HDE > NC > SAD.

The result of Atherogenic Coefficient (AC) values of the various study groups with range of 0.39 ± 0.08 to 4.08 ± 0.53 is as presented in Fig 4.10. The values of AC across the study groups in descending order is as follows; PC > LDE > HDE > NC > SAD.

The result of Atherogenic index of plasma (AIP) values of the various study groups, with range of -0.10 ± 0.02 to 0.66 ± 0.06 is as presented in Fig 4.11. The values of AIP across the study groups in descending order is as follows; PC > LDE > NC > HDE > SAD.

Table 4.6 shows the percentage protection of the treatment groups. The treatments groups are low dose extract (LDE), high dose extract (HDE) and standard antihyperlipidaemic drug (SAD). The highest percentage protection was in SAD at 90.44%, the lowest percentage was in LDE at 61.76%, and in HDE at 79.17%

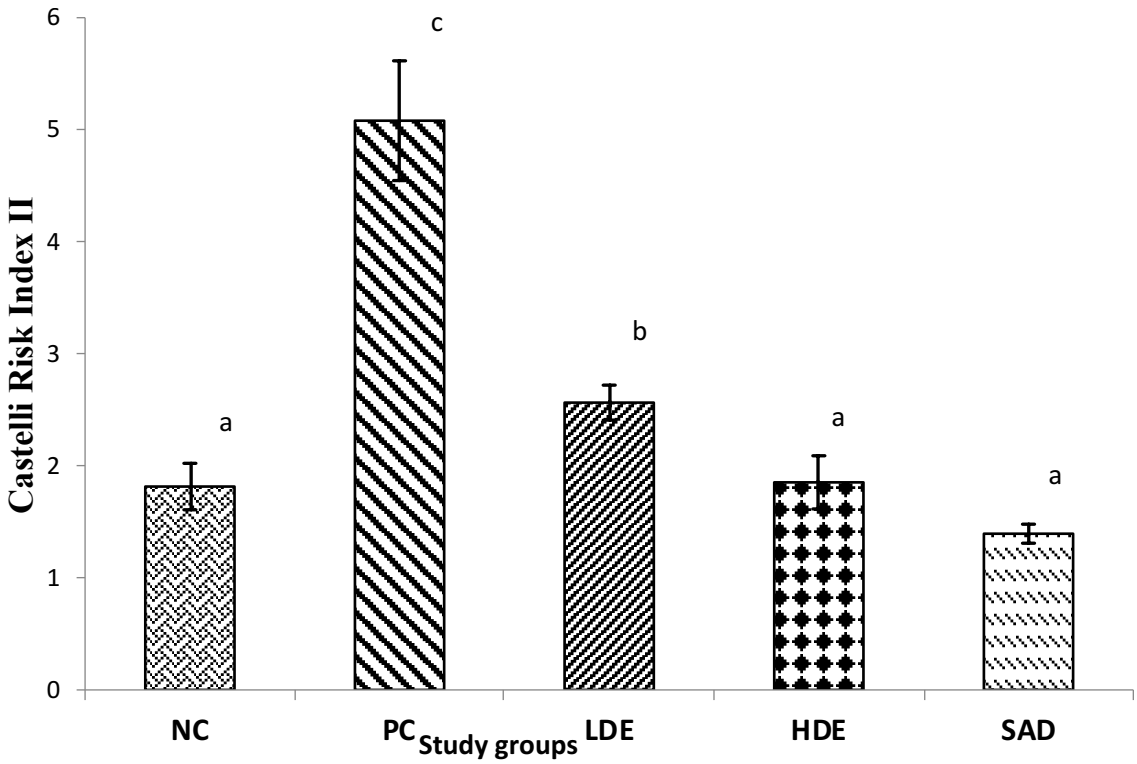

Fig 4.9: Castelli's Risk Index II levels across the various study groups.

Values are the means ± SD (n=4).

Groups with different alphabets are significantly different ($p<0.05$), while groups with similar alphabets are not significantly different.

NC: Negative control

PC: Positive control

LDE: Low dose of the extract

HDE: High dose of the extract

SAD: Standard antihyperlipidaemic drug

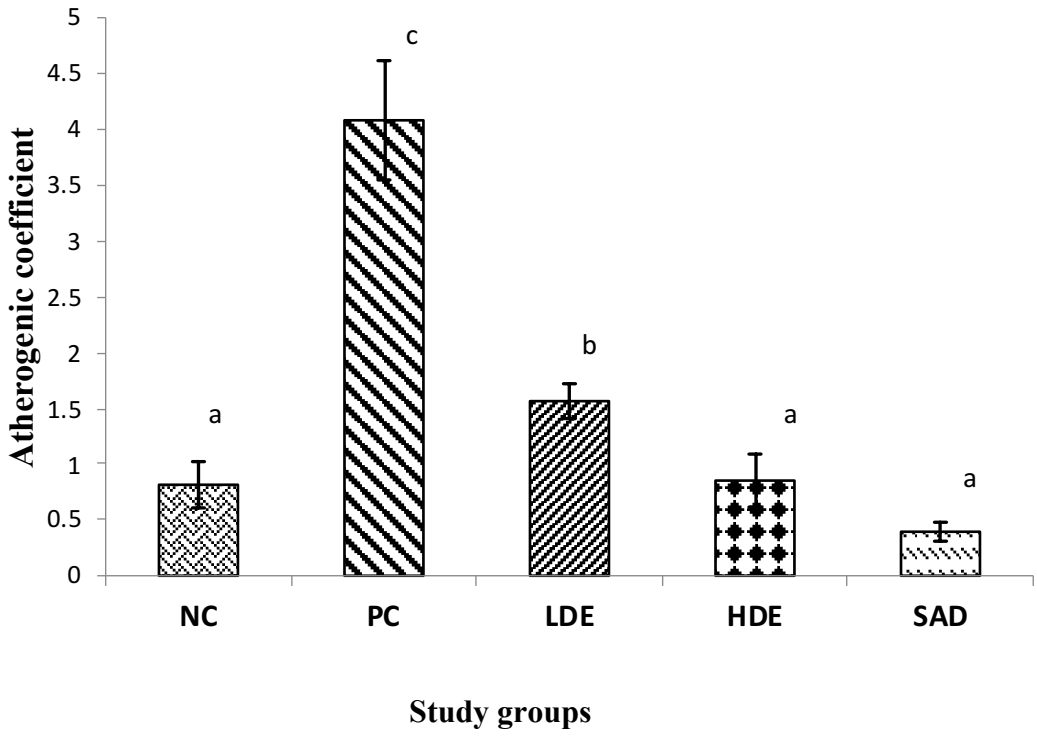

Fig 4.10: Result of Atherogenic Coefficient levels across the various study groups.

Values are the means ± SD (n=4).

Groups with different alphabets are significantly different ($p<0.05$), while groups with similar alphabets are not significantly different.

NC: Negative control

PC: Positive control

LDE: Low dose of the extract

HDE: High dose of the extract

SAD: Standard antihyperlipidaemic drug

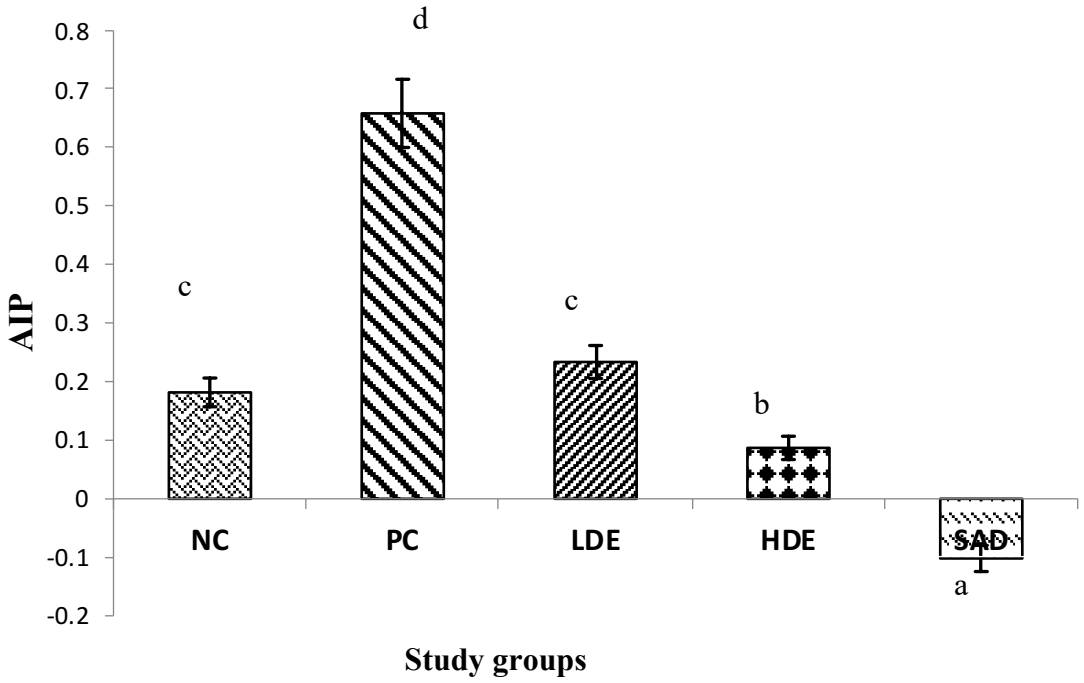

Fig 4.11: Result of Atherogenic Index of Plasma levels across the various study groups

Values are the means ± SD (n=4).

Groups with different alphabets are significantly different ($p<0.05$), while groups with similar alphabets are not significantly different.

NC: Negative control

PC: Positive control

LDE: Low dose of the extract

HDE: High dose of the extract

SAD: Standard antihyperlipidaemic drug

Table 4.6: Percentage protection of test drugs compared with standard antihyperlipidaemic drug (Simvastatin)

Groups	%Protection
LDE	61.76
HDE	79.17
SAD	90.44

NC: Negative control

PC: Positive control

LDE: Low dose of the extract

HDE: High dose of the extract

SAD: Standard antihyperlipidaemic drug

4.1.4 Result of enzyme activities

The result of Alanine transaminase (ALT) activity of the various study groups with range of 22.35 ± 2.58 — 32.88 ± 1.31 (U/L) is as presented in Fig 4.12. The activities of ALT across the study groups in descending order is as follows; PC > LDE > HDE > SAD > NC.

Fig 4.13 shows the result of the Aspartate transaminase (AST) activity of the various study groups with range of 34.31 ± 4.87 — 45.91 ± 2.28 (U/L). The activities of AST across the study groups in descending order is as follows; LDE > PC > HDE > SAD > NC.

The result of Alkaline phosphatase (ALP) activity of the various study groups with range of 207.69 ± 53.33 — 351.21 ± 29.74 (U/L) is as presented in Fig 4.14. The activities of ALP across the study groups in descending order is as follows; PC > LDE > HDE > SAD > NC.

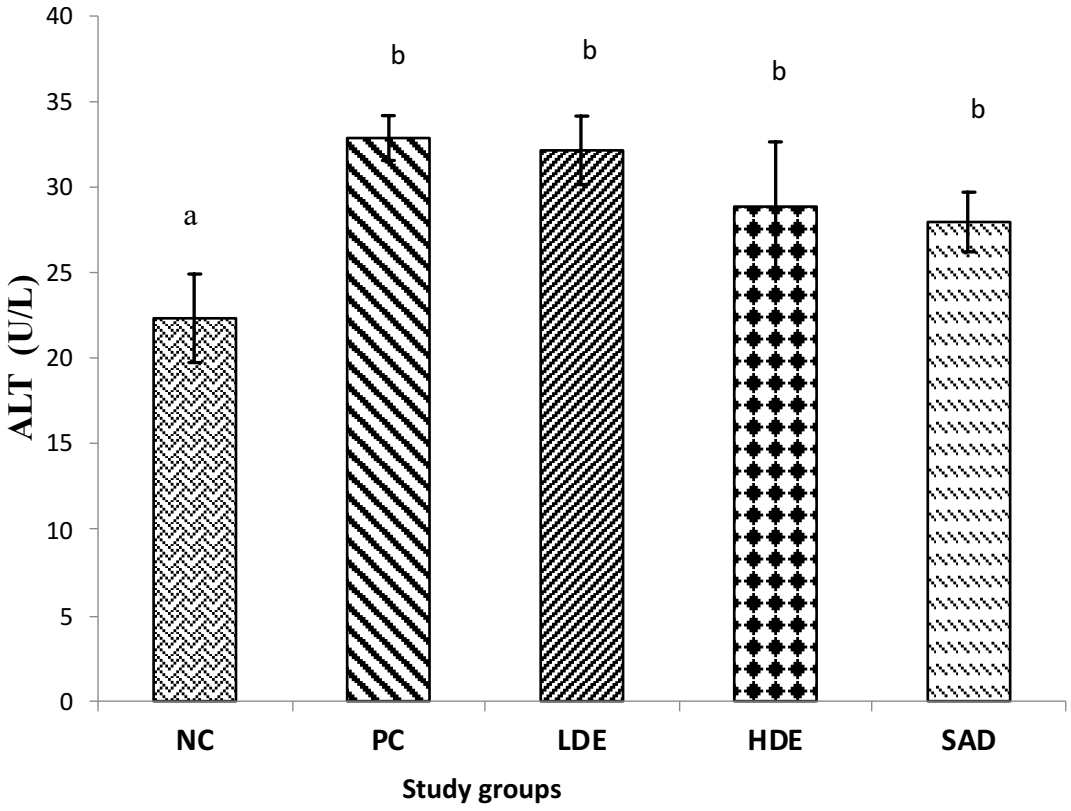

Fig 4.12: Alanine transaminase (ALT) activity across the various study groups.

Values are the means ± SD (n=4).

Groups with different alphabets are significantly different ($p<0.05$), while groups with similar alphabets are not significantly different.

NC: Negative control

PC: Positive control

LDE: Low dose of the extract

HDE: High dose of the extract

SAD: Standard antihyperlipidaemic drug

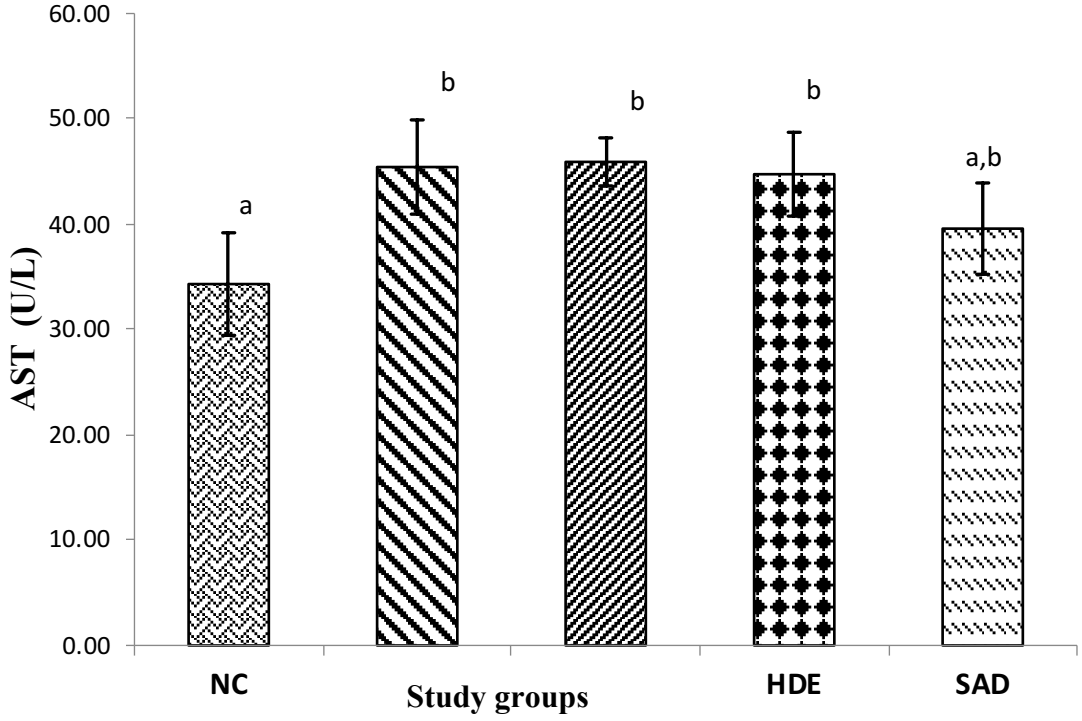

Fig 4.13: Aspartate aminotransferase (AST) activity across the various study groups.

Values are the means ± SD (n=4).

Groups with different alphabets are significantly different ($p<0.05$), while groups with similar alphabets are not significantly different.

NC: Negative control

PC: Positive control

LDE: Low dose of the extract

HDE: High dose of the extract

SAD: Standard antihyperlipidaemic drug

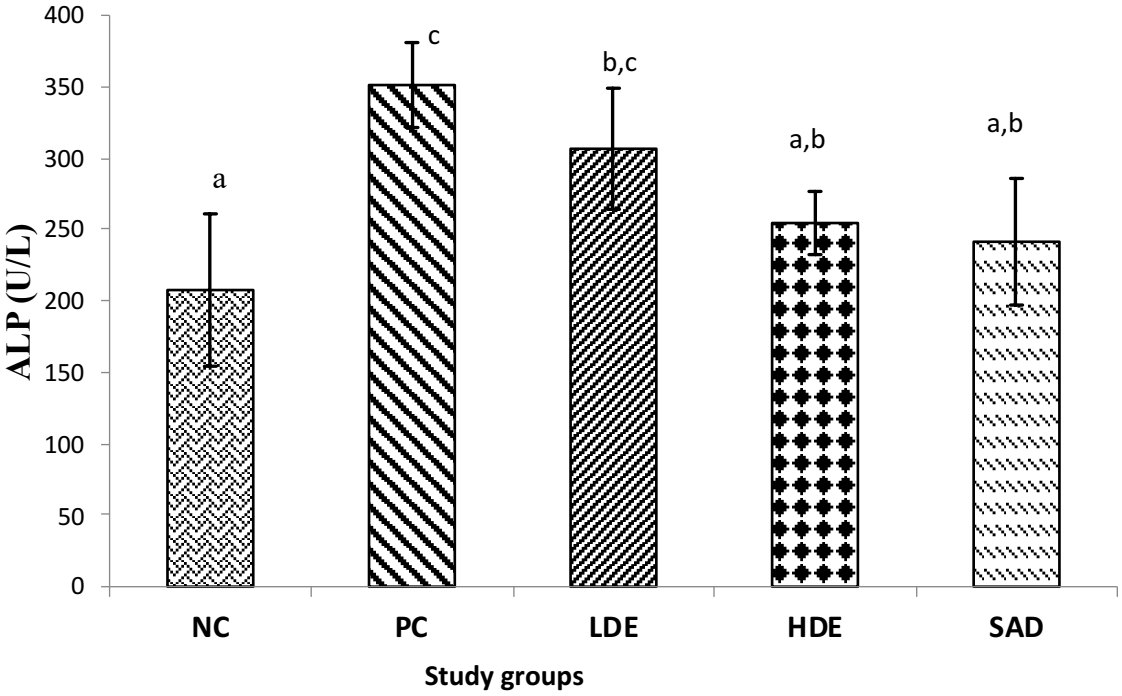

Fig 4.14: Alkaline Phosphatase (ALP) activity across the various study groups.

Values are the means ± SD (n=4).

Groups with different alphabets are significantly different ($p<0.05$), while groups with similar alphabets are not significantly different.

NC: Negative control

PC: Positive control

LDE: Low dose of the extract

HDE: High dose of the extract

SAD: Standard antihyperlipidaemic drug

The total bilirubin levels of the various study groups with range of 0.27 ± 0.02 — 0.34 ± 0.03 (mg/dl) is as presented in Fig 4.15. The level of total bilirubin across the study groups in descending order is as follows; PC > LDE > HDE > SAD > NC.

The serum total protein level of the various study groups with range of 54.05 ± 2.67 — 67.78 ± 5.08 (g/l) is as presented in Fig 4.16. The levels of total protein across the study groups in descending order is as follows; SAD > NC > HDE > LDE > PC

Fig 4.17 shows the result of the serum albumin levels of the various study groups with range of 23.66 ± 0.33 — 33.50 ± 2.92 (g/l). The levels of serum albumin across the study groups in descending order is as follows; SAD > HDE > NC > LDE > PC

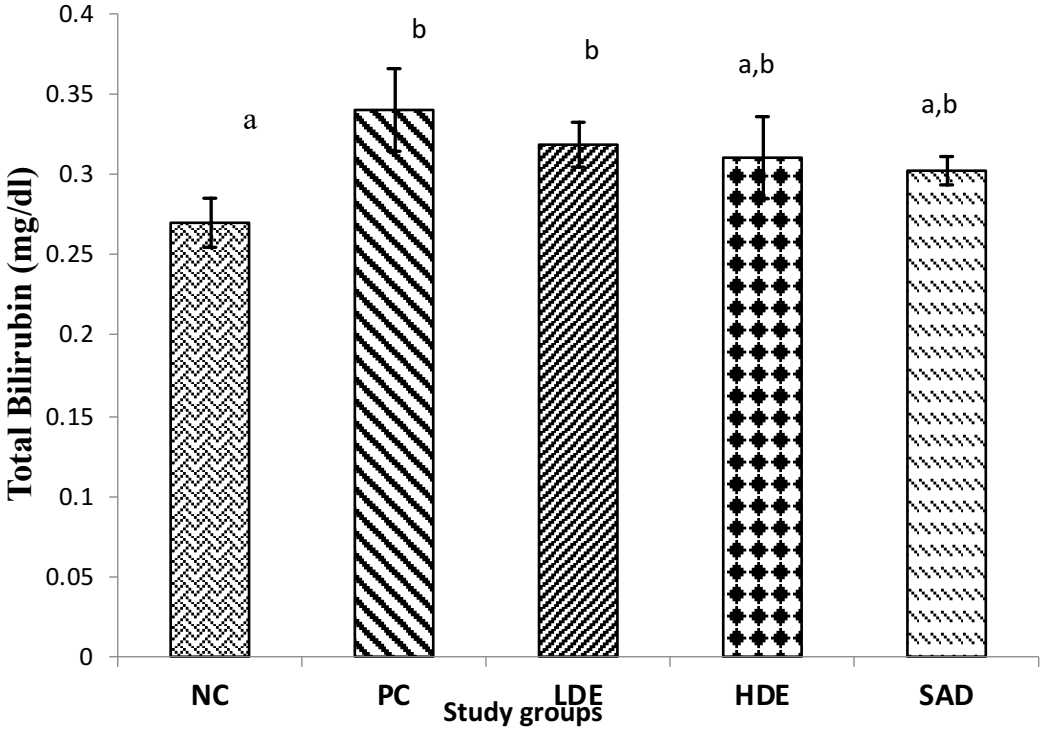

Fig 4.15: Total Bilirubin (TB) levels across the various study groups.

Values are the means ± SD (n=4).

Groups with different alphabets are significantly different ($p<0.05$), while groups with similar alphabets are not significantly different.

NC: Negative control

PC: Positive control

LDE: Low dose of the extract

HDE: High dose of the extract

SAD: Standard antihyperlipidaemic drug

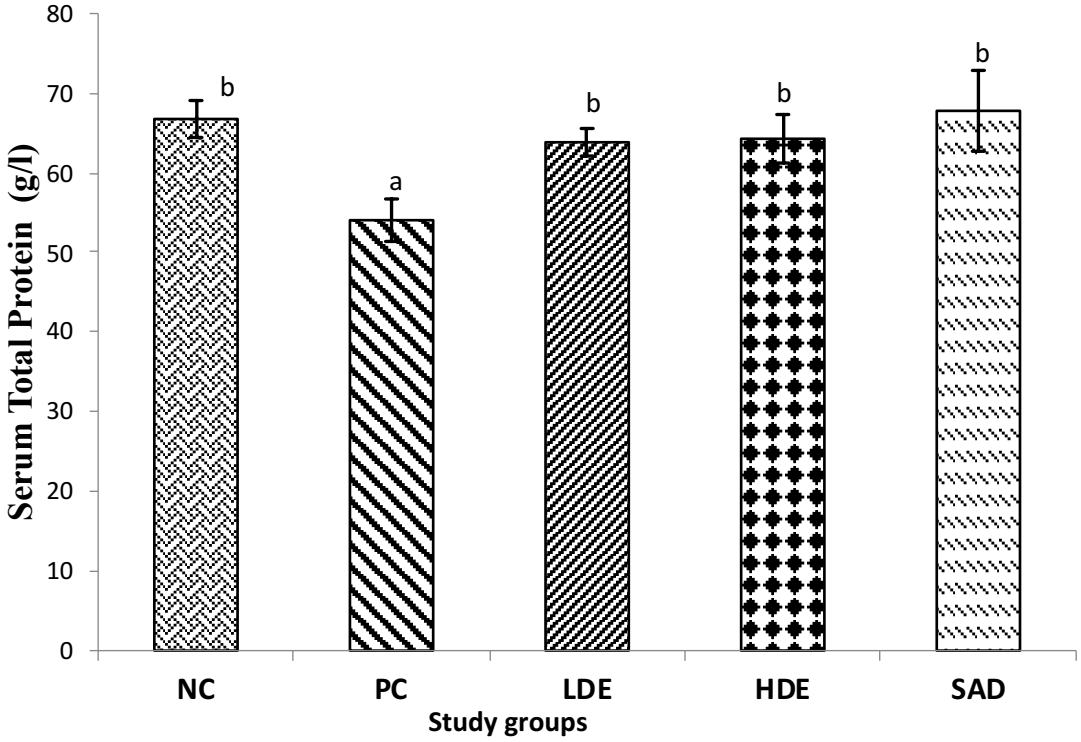

Fig 4.16: Serum total protein levels across the various study groups.

Values are the means ± SD (n=4).

Groups with different alphabets are significantly different ($p<0.05$), while groups with similar alphabets are not significantly different.

NC: Negative control

PC: Positive control

LDE: Low dose of the extract

HDE: High dose of the extract

SAD: Standard antihyperlipidaemic drug

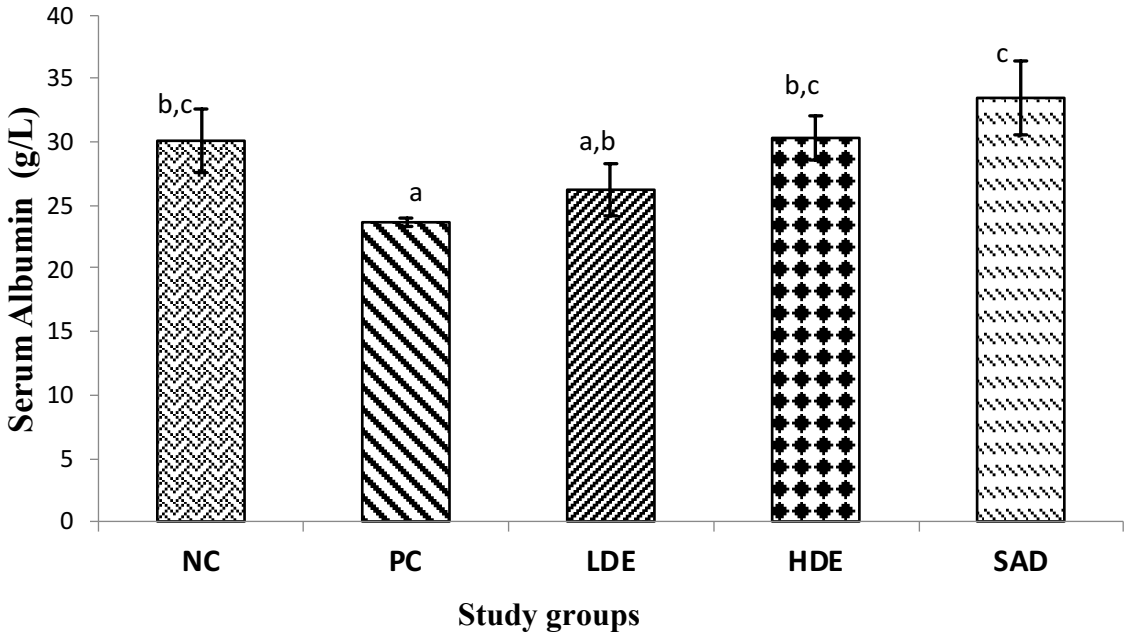

Fig 4.17: Serum albumin levels across the various study groups.

Values are the means ± SD (n=4).

Groups with different alphabets are significantly different ($p<0.05$), while groups with similar alphabets are not significantly different.

NC: Negative control

PC: Positive control

LDE: Low dose of the extract

HDE: High dose of the extract

SAD: Standard antihyperlipidaemic drug

The result of the serum globulin levels of the various study groups with range of 30.39 ± 2.82 — 37.41 ± 2.40 (g/l) is as presented in Fig 4.18. The levels of serum globulin across the study groups in descending order is as follows; LDE > NC > SAD > HDE > PC

Fig 4.19 shows the result of the serum lactate dehydrogenase activity of the various study groups with range of 13.38 ± 1.66 — 22.94 ± 2.08 (U/L). The activities of serum lactate dehydrogenase across the study groups in descending order is as follows; PC > LDE > HDE > SAD > NC.

The result of lactate dehydrogenase activity in the heart tissue of the various study groups with range of 72.65 ± 8.45 — 97.50 ± 6.70 (U/L) is as presented in Fig 4.20. The activities of lactate dehydrogenase in the heart tissue across the study groups in descending order is as follows; SAD > NC > HDE > PC > LDE.

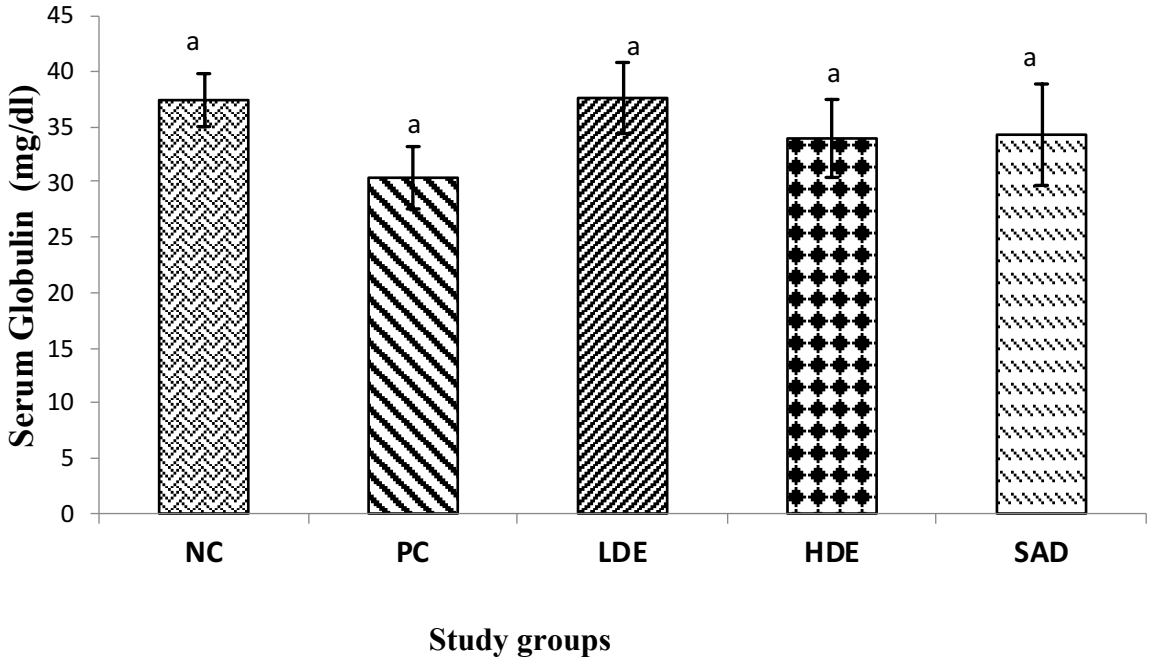

Fig 4.18: Serum globulin levels across the various study groups.

Values are the means ± SD (n=4).

Groups with different alphabets are significantly different ($p<0.05$), while groups with similar alphabets are not significantly different.

NC: Negative control

PC: Positive control

LDE: Low dose of the extract

HDE: High dose of the extract

SAD: Standard antihyperlipidaemic drug

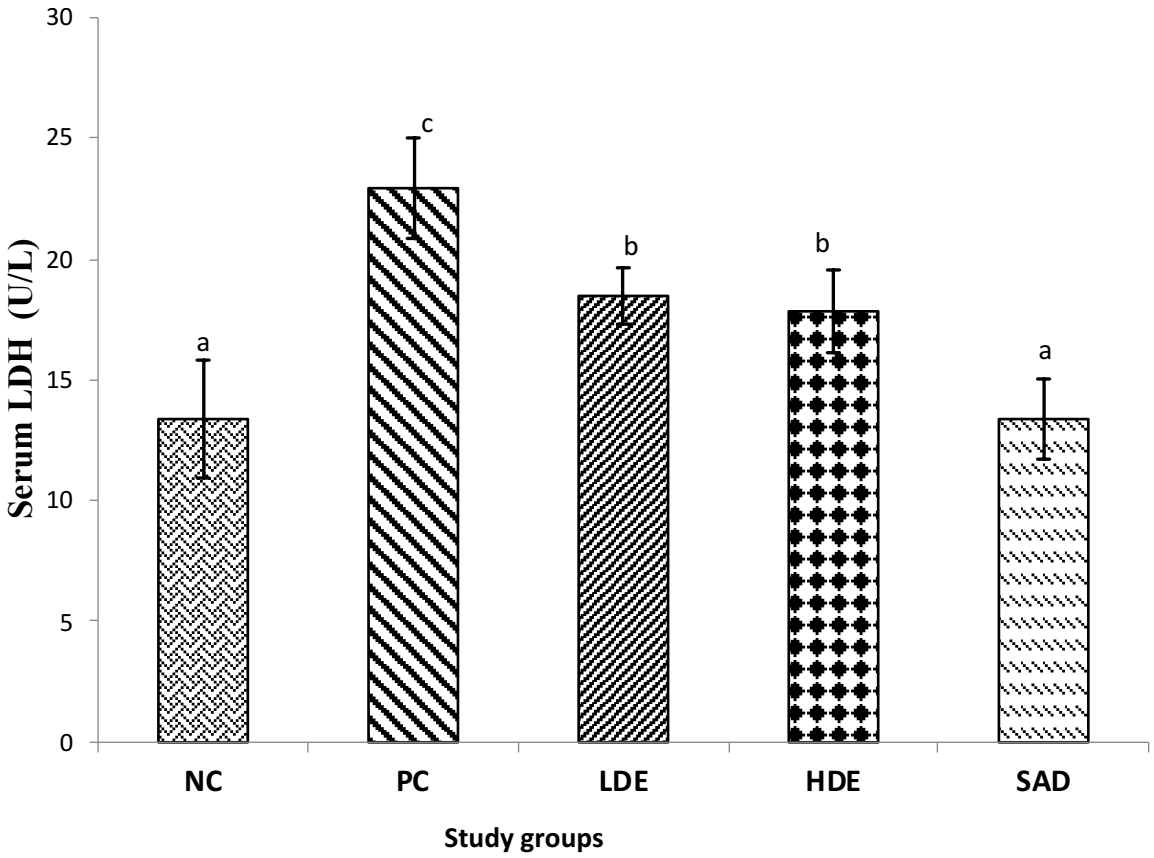

Fig 4.19: Serum lactate dehydrogenase activity across the various study groups.

Values are the means ± SD (n=4)

Groups with different alphabets are significantly different ($p<0.05$), while groups with similar alphabets are not significantly different.

NC: Negative control

PC: Positive control

LDE: Low dose of the extract

HDE: High dose of the extract

SAD: Standard antihyperlipidaemic drug

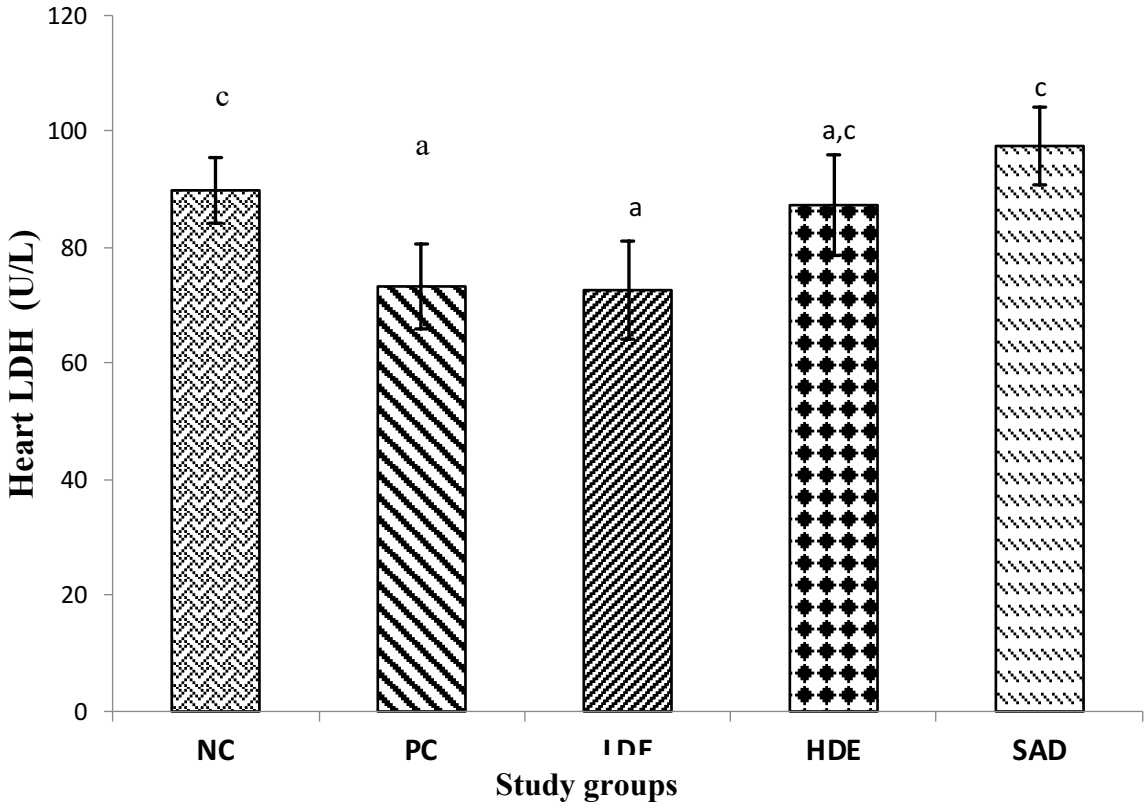

Fig 4.20: Lactate dehydrogenase activity in the heart tissue across the various study groups

Values are the means ± SD (n=4)

Groups with different alphabets are significantly different ($p<0.05$), while groups with similar alphabets are not significantly different.

NC: Negative control

PC: Positive control

LDE: Low dose of the extract

HDE: High dose of the extract

SAD: Standard antihyperlipidaemic drug

4.1.5 Result of Heart rate

Table 4.7 shows the results of the weekly heart rate of all the study groups. The different groups are Negative control (NC), Positive control (PC), Low dose extract (LDE), High dose extract (HDE) and standard antihyperlipidaemic drug (SAD).

Table 4.7: Result of weekly heart rate of all experimental groups.

	Heart Rate/Min (bpm)					
Groups	1st week	2nd week	3rd week	4th week	5th week	6th week
NC	135.33 ± 3.01	141.4 ± 13.01	155.4 ± 10.86	168.17 ± 5.46	179 ± 10.12	199.02 ± 3.94
PC	131.33 ± 3.01	157.6 ± 7.83	179.33 ± 7.34	188.0 ± 6.82	200.6 ± 4.08	207.83 ± 6.01
LDE	134.0 ± 4.20	155.29 ± 5.16	175.33 ± 3.93	180.67 ± 4.68	187.50 ± 10.23	197.33 ± 4.32
HDE	135.33 ± 4.68	152.0 ± 9.12	176.0 ± 16.35	180.67 ± 5.89	188.0 ± 10.63	190.33 ± 5.32
SAD	132 ± 5.06	153.33 ± 5.89	174.0 ± 11.31	179.17 ± 4.40	186.0 ± 3.79	190.0 ± 4.34

Values were determined by calculating how many beats per minute

Values are mean ± standard deviation of four determinations.

4.2 Discussion

4.2.1 Free radical scavenging capacity

4.2.1.1 Nitric Oxide Scavenging capacity

Nitric oxide (NO) is a radical that has the ability to cause damage to the function and structure of many cellular components. The toxic effect of NO increases when reacted with superoxide to form peroxynitrite anion (.ONOO-), peroxynitrite anion is a strong oxidant that breaks down to form .OH and NO_2 (Awah & Verla, 2010). From the result of this study (Fig 4.1), it shows the ethanolic extract of *Diodia sarmentosa* scavenged nitric oxide in vitro in a concentration dependent manner. This scavenging ability could be due to the presence of phenolic compounds like flavonoids in the plant which are antioxidants. *Diodia sarmentosa* has previously been reported by Ijonome & Ekpe (2018) to contain flavonoids after carrying out the phytochemical analysis of *Diodia sarmentosa*. The percentage nitric oxide scavenging capacity increased with increase in extract concentration. The IC_{50} of *Diodia sarmentosa* was 907.17µg/ml ± 45.36 as compared to the standard antioxidant (Quercetin dihydrate) which was 55.37µg/ml ± 4.43. IC_{50} is the measure of the concentration of the extract to give 50% inhibition of radicals. The standard antioxidant showed a 20% inhibition at a low concentration of 12.64µg/ml ± 1.01, as compared to 207.81µg/ml ± 10.39 of *Diodia sarmentosa*. Thus, showing the Quercetin dihydrate has a better scavenging capacity than the extract. The lower the concentration needed to attain the percentage inhibition, the better the antioxidant. The highest percentage inhibition i.e 80% inhibition, the extract had a concentration of 6968.20µg/ml ± 411.09, while Quercetin dihydrate had a concentration of 322.42µg/ml ± 19.35. The ability of *Diodia sarmentosa* to scavenge nitric oxide radicals is similar with the nitric oxide scavenging ability of *ocimum gratissimum* extract (Awah & Verla, 2010).

4.2.1.2 Hydroxyl Scavenging capacity

Hydroxyl radicals are known as reactive oxygen species that causes oxidation of polyunsaturated fatty acids in food causing cell and tissue damage. Hydroxyl radicals are produced in vivo by the decomposition of superoxide and hydrogen peroxide catalysed by transition metals like iron and copper (Awah & Verla, 2010). The hydroxyl scavenging ability of *Diodia sarmentosa* was determined by measuring the decrease of thiobabituric reactive substance (TBARS), a product of malondialdehyde (MDA). Ethanolic extract of *Diodia sarmentosa* scavenged hydroxyl radicals in a concentration dependent manner, i.e an increase in concentration of the extract, increases the percentage inhibition of hydroxyl radicals. Fig 4.2 shows the IC_{50} of the extract was 2173.44µg/ml ± 100.11, compared to the standard antioxidant (Quercetin dihydrate) which was 322.92µg/ml ± 25.83. This shows that the standard antioxidant is a better antioxidant than the extract. At a concentration of 36392.75µg/ml ± 1820.04, the extract showed its highest percentage inhibition (70%), while at 80% inhibition, the concentration of the extract was not determined, whereas Quercetin dihydrate had a concentration of 2753.47µg/ml ± 160.21. The hydroxyl radical scavenging potential of *Diodia sarmentosa* may also be due to the presence of flavonoids. Flavonoids are known to inhibit lipid peroxidation because hydroxyl radicals are highly associated with lipid peroxidation. This finding can be attributed to the findings of Lalhminghlui & Jagetia (2018), which showed that *Schima wallichii* possessed antioxidant properties by scavenging hydroxyl radicals in a concentration dependent manner.

4.2.2 Oxidative stress indices

All these unhealthy conditions like cancer, hyperlipidaemia, hypercholesterolemia, atherosclerosis etc are as a result of oxidative stress. Oxidative stress is the imbalance or inequality in the body between the production of free radicals such as reactive oxygen and nitrogen species, and the activities of antioxidants acting as neutralizing agents (Amiri, 2018). In this study, the test rats became hyperlipidaemic by feeding with high fat diet for a period of six (6) weeks. This hyperlipidaemic condition caused an increase in the cholesterol pool in the rats, thereby altering the cell membrane, and giving rise to oxidative stress in the rats. Oxidative stress was characterized by decrease in antioxidant enzymes, and increase in free radicals. This is evident in both the serum and heart tissue of the positive control group of this study as shown in (Table 4.1 — 4.5). The other study groups were the negative control (not induced /not treated test rats), low dose extract group (treated test rats with 250mg/kg body weight of *Diodia sarmentosa*) (LDE), high dose extract group (treated test rats with 500mg/kg body weight of *Diodia sarmentosa*) (HDE), and lastly the standard antihyperlipidaemic drug group (treated test rats with 5mg/kg body weight of Simvastatin) (SAD) (Table 4.1 — 4.5). The treatment groups (LDE, HDE and SAD) increased antioxidant enzymes like superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione s-transferase (GST) and catalase (CAT) to normal levels comparable to the negative control group. Lipid peroxidation expressed as malondialdehyde (MDA) level were reduced in the treatment groups to almost the same levels with the negative control group.

Superoxide dismutase (SOD) is an antioxidant enzyme found in aerobic cells that catalyses the disintegration of superoxide anion into oxygen and hydrogen peroxide. Superoxide dismutase activity was significantly reduced ($p<0.05$) in both the serum and heart tissue of the positive

control group, when compared to the negative control group (Table 4.1). This shows the overproduction of superoxide radicals by NADPH oxidase in both serum and heart tissue causing oxidative stress. This reduction of SOD activity in the positive control groups makes it difficult for the dismutation of superoxide radical to hydrogen peroxide and oxygen. This finding is in agreement with the findings of El-Rabey *et al.* (2018), which reported a decrease in SOD activity in hyperglycemia and hyperlipidemic alloxan induced male rats. In the extract treated groups, SOD activity was significantly increased ($p<0.05$) in a dose dependent manner, when compared to the positive control group (Table 4.1). This increase in the antioxidant enzyme superoxide dismutase by the extract, reduced superoxide radicals by the dismutation of superoxide radical to form hydrogen peroxide and oxygen. The increase in SOD activity in the heart tissue and serum of both doses of the extract were comparable to the negative control group. The extract compared favorably to Simvastatin, thus showing that the extract increased SOD activity better than the standard antihyperlipidaemic drug. The efficacy of *Diodia sarmentosa* in increasing SOD activity can be likened to the efficacy of *Salvadora persica* in increasing superoxide dismutase activity in alloxan induced diabetic rats (El-Rabey *et al.,* 2018).

Glutathione peroxidase (GPx) is an enzyme that is found in almost all the tissues, though highly found in the liver (Obeagu, 2018). Glutathione peroxidase catalyses the oxidation of reduced glutathione into glutathione disulfide, degrading hydrogen peroxide and lipid hydroperoxide into harmless forms. It helps in the repair of cellular damage caused by lipid peroxidation. Table 4.2 shows glutathione peroxidase activity was significantly reduced ($p<0.05$) in both serum and heart tissue of the positive control group, when compared to the negative control group. This reduction in glutathione peroxidase activity in the positive control group caused cellular damage, due to the inability of hydroxyl radicals and lipid hydroperoxide to be

decomposed to nontoxic forms. This result is in conjunction with the findings of Sajeeth *et al.* (2011), which showed a decrease in glutathione peroxidase activity in streptozotocin induced diabetic male rats. In the extract treated groups, glutathione peroxidase activity was significantly increased ($p<0.05$) when compared to the positive control group (Table 2). This increase in GPx activity by the extract reduced cellular damage, by promoting the oxidation of reduced glutathione and degrading of hydroxyl radicals and lipid hydroperoxide to harmless forms. This increase in glutathione peroxidase activity by the extract was comparable to the negative control group. The extract compared favorably to simvastatin the standard antihyperlipidaemic drug. Thus, showing the extract being better at reducing oxidative stress, by reducing hydrogen peroxide and lipid hydroperoxide to less toxic forms. The efficacy of *Diodia sarmentosa* in the increase of GPx activity is similar to the efficacy of polyherbal formulation in increasing Glutathione peroxidase activity in streptozotocin induced diabetic male rats (Sajeeth *et al*., 2011).

Glutathione S-transferase (GST) is an antioxidant enzyme that is abundant in the liver. It catalyses the conjugation of reduced glutathione to xenobiotics for the purpose of detoxification (Traber & Atkinson, 2007). In this study, Table 4.3 shows Glutathione s-transferase activity was significantly increased ($p<0.05$) in heart tissue of the positive control group, when compared to the negative control group. However, in the serum, the increase in GST activity in the positive control group was not significantly different ($p>0.05$) compared to the other study groups (Table 4.3). This increase in GST activity in the heart tissue of the test rats could be as a result of an abnormality caused by oxidative stress from cholesterol metabolism. This result is in agreement with the findings of Rosa *et al*. (2018), which reported high GST levels impaired skin wound healing, and promoted inflammation in high fat diet and alcohol administered wistar rats. Also, Neoman *et al*. (2011) reported high GST activity in

heart tissue of high fat diet obesity in rats. In the extract treated groups (Low and high dose), glutathione s-transferase (GST) activity in the heart tissue was significantly reduced ($p<0.05$), when compared to the positive control group. GST activity was brought to normal levels with the negative control groups in the heart tissue by the extract, because they were not significantly different ($p>0.05$). When compared to the standard antihyperlipidaemic drug group, GST activity in the extract group were not significantly different ($p>0.05$) in the heart tissue. This observation might be because the drugs altered the expression of glutathione s-transferase as a result of toxic metabolites produced by the drugs in the body system. In the serum, GST activity was not significantly different in all the groups (Negative control, Positive control, Low and high dose and standard antihyperlipidaemic group). This result could be because the hyperlipidaemic condition and drugs did not have effect on the serum of test rats.

Catalase (CAT) is an enzyme whose activity is mostly in the liver and also found in other tissues in the body system. It catalyses the conversion of hydrogen peroxide to water and oxygen (Engwa, 2018). In this study, Table 4.4 shows the catalase activities in the heart tissue and serum of the study groups. Catalase activity was significantly reduced ($p<0.05$) in the serum of the positive control group compared to negative control group. In the heart tissue, CAT activity in the positive control group showed a decrease, but this decrease was not significantly different ($p>0.05$), when compared to the negative control group. This decrease of catalase activity in the positive control group, maybe be due to the exhaustion of the enzyme catalase, that helps in catalysing the conversion of hydrogen peroxide. This led to the overproduction of hydrogen peroxide radicals in the hyperlipidaemic state of the test rats. This imbalance induced oxidative stress in the positive control group. This finding correlates with the decrease in catalase activity in male obese rats (Bin-Meferij *et al*., 2017). In the extract treated groups (high dose and low dose), at high dose of the extract, catalase activity was

significantly increased ($p<0.05$) in the heart tissue and serum when compared to the positive control group. This increase could be due to the extract has the antioxidant potentials to reduce hydrogen peroxide radicals by increasing the enzyme catalase to catalyse the conversion of hydrogen peroxide to oxygen and water. The effect of this extract could be similar to the anti-obesity effect of *Zingiber officinale* extract in male obese rats (Bin-Meferij *et al*., 2017). The increase of catalase activity by the extract when compared to the standard antihyperlipidaemic drug group, and negative control group was not significantly different ($p>0.05$).

Lipid peroxidation is oxidative decomposition of lipids. One of the final products of lipid peroxidation is Malondialdehyde (MDA), which is produced by the overproduction of free radicals in the cell. It is a major marker of oxidative stress (Niki *et al*., 2005). In this study, Table 4.5 shows malondialdehyde level was significantly increased ($p<0.05$) in both the serum, and heart tissue of the positive control group, when compared to the negative control group. This increase in MDA levels indicates cell injury or damage caused by production of reactive oxygen species by tumornecrosis factor alpha (cytokine). Lipid peroxidation in the heart leads to cellular membrane damage, due to oxidative modification of lipids, proteins and bioavailability of free fatty acids that can ultimately lead to cardiac arrest and sudden death. This result is in concordance with Noeman *et al*. (2011), Wali *et al*. (2012) and Bin-Meferij *et al*. (2017), which all reported high levels of malondialdehyde due to lipid peroxidation in their respective research. The extract significantly reduced ($p<0.05$) MDA levels in the heart tissue and serum, when compared to the positive control group. The decrease in MDA level by the extract shows the inhibition of lipid peroxidation, by the regulation of cholesterol metabolism to reduce cellular damage. The extract is comparable to the negative control and simvastatin, because MDA levels were not significantly different ($p>0.05$) amongst themselves. The

efficacy of *Diodia sarmentosa* is similar to that of *Salvadora persica* in the reduction of lipid peroxidation (MDA activity) (El-Rabey *et al.,* 2018).

4.2.3 Lipid Profiles

Various authors have reported the decrease in high density lipoprotein cholesterol (HDL-C), and elevation of lipid profiles like total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and atherogenic indices gave rise to conditions like obesity, hypercholesterolemia, hyperlipidemia and hypertension (Ahmad *et al*., 2011; Sa'adah *et al*., 2017; El-Rabey *et al*., 2018). Also, various plants like *Clitoria ternatea, Coriandrum sativum, Citrullus colocynthis, Aloe vera* etc have shown to possess remedies against various cardiovascular related diseases (Al-Snafi, 2016). In this study, figure 4.3 — 4.11 showed the levels of all lipid profiles and atherogenic indices of all the study groups.

Total cholesterol (TC) level for the various study groups. Total cholesterol level was significantly increased ($p<0.05$) in the positive control group as compared to the negative control group (Fig 4.3). This increase in the positive control group was as a result of the high fat diet used in the study, this caused an accumulation of high saturated fatty acid which increased the cholesterol level of the test rats. High cholesterol level can lead to various cardiovascular diseases and sudden death. This finding is in agreement with the result of Neoman *et al*. (2011) and Olorunnisola *et al*. (2012), both reported different high fat diet formulation elevated total cholesterol level. The extract at both doses significantly reduced ($p<0.05$) total cholesterol levels in a dose dependent manner, when compared to the positive control group (Fig 4.3). The reduction of total cholesterol level in the high dose extract group was not significantly different ($p>0.05$) when compared to the negative control group and

standard antihyperlipidaemic drug group, showing high dose of the extract to be more efficient than low dose in reducing total cholesterol level. This shows total cholesterol level was reduced in a dose dependent manner. The plant extract has cholesterol lowering potentials, and its potency is similar to plants like *Tulbaghia violacea, Medinilla speciosa* (Olorunnisola *et al*., 2012; Sa'adah *et al*., 2017).

Triglyceride level was significantly increased ($p<0.05$) in the positive control group as compared to the negative control group (Fig 4.4). This elevation in triglycerides tends to cause cardiovascular diseases like hardening of the arteries and hypertriglyceridemia. This result is in agreement with the result of Bin-Meferij *et al*. (2017), which reported increased triglyceride level in obese rats fed with high fat diet. Both doses of the extract significantly reduced ($p<0.05$) triglyceride levels, when compared to the positive control group (Fig 4.4). The low dose of the extract brought the triglyceride level to the same level of the negative control group. However, at high dose, the reduction in triglyceride level was lower level than the negative control group, and low dose extract group. Furthermore, the antihyperlipidaemic drug group showed the lowest triglyceride level. This anti-hypertriglyceridemia potential of this extract can be explored in the management of hypertriglyceridemia induced atherogenesis. Reduced triglyceride level was demonstrated by Olorunnisola *et al*. (2012), with *Tulbaghia violacea* reducing high triglyceride level in obese rats.

High density lipoprotein cholesterol is a 'good cholesterol' which carry cholesterol and lipids from the tissue to the liver for degradation. High density lipoprotein cholesterol helps to decrease the total level of cholesterol in the blood serum.. The higher the level of HDL-C, the lower the risk of atherosclerosis. In the positive control group, High density lipoprotein cholesterol was significantly lowered ($p<0.05$) as compared to negative control group (Fig 4.5).

This was as a result of the kind of diet fed to the test rats, insufficient HDL-C leads to high level of cholesterol causing different cardiovascular diseases. This findings corresponds with the findings of Nwanjo & Oze (2006), which showed decrease in high density lipoprotein cholesterol in hyperlipidaemic treated rats. The low dose and high dose extract groups significantly increased ($p<0.05$) HDL-C levels, when compared to the positive control group (Fig 4.5). The increase in the low dose and high dose extract group were not significantly different ($p>0.05$) from the increase in the negative control group. This shows the efficacy of the extract in increasing HDL-C to normal levels at both doses. Though, simvastatin increased HDL-C to the highest level compared to other groups. This result and effectiveness of *Diodia sarmentosa* is similar to that of *Medinilla speciosa* in increasing high density lipoprotein cholesterol (Sa'adah *et al*., 2017).

Low density lipoprotein cholesterol and Very low density lipoprotein cholesterol are lipid-rich lipoprotein cholesterol. Low density lipoprotein cholesterol is a 'bad cholesterol' which transports cholesterol to the tissue. Also, very low density lipoprotein cholesterol transports triglycerides and fatty acid formed in the liver to the tissue. Low density lipoprotein cholesterol was significantly increased ($p<0.05$) in the positive control group, when compared to the negative control group (Fig 4.6). This increase is as a result of high cholesterol level from the consumption of high fat diet, and it corresponds with the findings of El-Rabey *et al* (2018). The low density lipoprotein cholesterol concentration was significantly reduced ($p<0.05$) by both doses of the extract, when compared to the positive control group (Fig 4.6). The reduction in the high dose extract group was brought to a normal level, because it was not significantly different ($p>0.05$) when compared to the negative control group. The extract has LDL-C reducing potentials like that of *Salvadora persica* (El-Rabey *et al*., 2018). Very low density lipoprotein cholesterol was significantly higher ($p<0.05$) in the positive control group when

compared to the negative control group (Fig 4.7). At both doses of the extract, VLDL-C levels were significantly reduced ($p<0.05$), when compared to the positive control group (Fig 4.7). The very low density lipoprotein cholesterol level of the low dose extract group was at normal levels with the negative control group, but was significantly different ($p<0.05$) when compared to the other treatment groups. The efficacy of *Diodia sarmentosa* in decreasing very low density lipoprotein cholesterol is similar to *Salvadora persica* (El-Rabey *et al*., 2018).

Atherogenic coefficient and indices (Castelli'srisk index I, Castelli's risk II, Atherogenic coefficient and Atherogenic index of plasma) have been reported to be better predictors of cardiovascular diseases than isolated lipid profiles. The higher the atherogenic coefficient and indices, the higher the risk of atherosclerosis and coronary heart disease and vice versa. Fig 4.8 to 4.11 shows the different Atherogenic coefficient and indices levels of the various study groups. Castelli's risk index I (CRI-I) value was significantly higher ($p<0.05$) in the positive control group compared to the negative group (Fig 4.8). The extract at both doses showed a significant reduction in ($p<0.05$) CRI-I values, when compared to the positive control group (Fig 4.8). Castelli's risk index I value in the HDE was not significantly different ($p>0.05$) when compared to the negative control group and standard antihyperlipidaemic drug group, thus showing the extract being more effective at high dose. This lower atherogenic index and coefficient shows low risk of occurrence of cardiovascular diseases, thus showing the extract and simvastatin are both effective in reducing the risk of cardiovascular diseases. Castelli's risk index II (CRI-II) was significantly higher ($p<0.05$) in the positive control group compared to negative control group (Fig 4.9). The extract at both doses showed a significantly reduction ($p<0.05$) in CRI-II values, when compared to the positive control group (Fig 4.9). The CRI-II values at the high dose extract group not significantly different ($p>0.05$) when compared to the negative control group, and standard antihyperlipidaemic drug group. Atherogenic coefficient

(AC) was significantly higher ($p<0.05$) in the positive control group, when compared to the negative control group (Fig 4.10). The extract at both doses showed a significant reduction ($p<0.05$) in AC values, when compared to the positive control group (Fig 4.10). The AC value of the high dose extract group was not significantly different ($p>0.05$), when compared to the standard antihyperlipidaemic drug group and negative control group. The effect of the extract on atherogenic coefficient was dose dependent, with the better result seen at high dose. Atherogenic index of plasma (AIP) was significantly higher at ($p<0.05$) in the positive control group compared to the other study groups (Fig 4.11). In the treatment groups, the low extract group showed a decrease in AIP similar to the negative control group, while the high dose extract group, showed a much lower AIP value than low dose extract group and negative control group. This reduction showed that the administration of the extract could reduce atherosclerosis. However, the groups administered with simvastatin (SAD group) showed a negative value, thus showing the unlikelihood of the occurrence of atherosclerosis. This lower values shows the potential of the extract to possibly reduce atherosclerosis and cardiovascular diseases, which is similar to the herbal formulations of *Acanthus montanus, Asystasia gangetica, Gongronema latifolium* and *Solanum melongena* used in reducing atherosclerosis in diabetic rats (Chikezie *et al*., 2018).

Table 4.6 shows the Percentage Protection of the test drugs and standard antihyperlipidaemic drug. The highest percentage protection of 90.4% was observed in the standard antihyperlipidaemic drug, followed by the high dose of the extract at 79.2%, and the least percentage protection was observed in the low dose extract at 61.8%. *Diodia sarmentosa* has shown to be a good lipid reducing drug, which can become a potential antihyperlipidaemic drug like simvastatin.

4.2.4 Liver Function Indices

There have been controversial reports if high cholesterol has an effect on hepatic markers like Alanine transaminase (ALT), Aspartate aminotransferase (AST) and Alkaline Phosphatase (ALP). Lu *et al*. (2007) and Olorunnisola *et al*. (2012) reported high cholesterol caused an elevation in ALT, AST and ALP levels in rats fed with high cholesterol diet. Arafa (2005) and Mabuchi *et al*. (2007) reported no change in the AST and ALP levels in hypercholesterolemia rats. Olorunnisola *et al*. (2012) reported this inconsistency might be as a result of the duration and level of cholesterol in the test rats.

In this study, there was an elevation of liver enzymes such as Alanine transaminase (ALT), Aspartate aminotransferase (AST) and Alkaline Phosphatase (ALP) in the positive control group compared to the other groups (Fig 4.12 to Fig 4.14). The increase of these liver enzymes in the positive control group maybe as a result of possible liver injury, which caused these enzymes to leak from the liver cytosol to the blood stream. Fig 4.12 shows an increase in Alanine transaminase activity in the positive control group, this elevation was significant ($p<0.05$) when compared to the negative control group, thus showing a possible liver injury in the test rats. This findings is in agreement with the results of various authors which reported an increase in Alanine transaminase in hypercholesterolemia rats (Olorunnisola *et al*., 2012; Ismail *et al*., 2015 and Adekiya *et al*., 2018). Alanine transaminase activity was reduced in the treatment groups (low dose group, high dose group and standard antihyperlipidaemic drug group), but the reduction was not significant ($p>0.05$) compared to the positive control group (Fig 4.12). This reduction by the extract group could be that *Diodia sarmentosa* has the potential to reduce liver injury. A higher dose of the extract maybe be effective to significantly reduce high ALT activities to a normal level. The efficacy of *Diodia sarmentosa* corresponds

with that of *Naringin* in the reduction of ALT activity in type 2 diabetic rats (Adelani *et al.,* 2018).

Aspartate aminotransferase is an enzyme located in the cytoplasm and mitochondrion. Majority of AST activity in the liver originates from the mitochondria. It is an indicator for hepatic injury caused by viral hepatitis, muscular damage and infraction. Fig 4.13 shows Aspartate aminotransferase activity was significantly increased ($p<0.05$) in the positive control group, when compared with the negative control group showing possible leakage of AST in the blood stream. This shows that the hyperlipidaemic condition caused an effect to the liver. Fig 4.13 shows the low dose extract group did not show a decrease in aspartate aminotransferase, but the high dose group, and standard antihyperlipidaemic drug group reduced AST activity. The decrease was not significant ($p>0.05$), thus showing at these doses, high AST activity were not reduced to normal levels.

Alkaline phosphatase (ALP) is membrane bound enzyme which determines membrane permeability of a cell. When altered, it can cause instability in the transport of metabolites in the cell. ALP serves as a biomarker to indicate liver injury because it helps to facilitate the conversion of Alanine to pyruvate and glutamate. ALP activity in the positive control group was significantly ($p<0.05$) higher, when compared to the negative control group (Fig 4.14). The treatment groups caused a decrease in ALP activities. The reduced ALP activity in the low dose group was not significantly different ($p>0.05$), but at high dose, ALP activity was significantly different ($p<0.05$), when compared to the positive control group. This shows the extract was not effective at low dose rather at high dose. The efficacy of *Diodia sarmentosa* corresponds with *Gynura procumbens* used in reducing ALP activities in hypercholesterolemia induced rabbits (Ismail *et al*., 2015).

Lactate dehydrogenase (LDH) is a biomarker for organ damage. It plays a role in the transfer of lactic acid to pyruvate and formation of ATP from ADP in anaerobic systems. Increase in lactate dehydrogenase can be as a result of prolonged exercise, or some psychological disorders like liver disease, cancers etc (Al-Janabi *et al*., 2015). In the present study, assay of LDH was carried out in both serum and heart tissue of the test rats. Lactate dehydrogenase (LDH) was significantly increased ($p<0.05$) in the serum of the positive control group, when compared to the negative control group (Fig 4.19). This increase in the enzyme lactate dehydrogenase was as a result of the oxidative stress, which caused possible liver injury from the administration of high fat diet. This findings corresponds with the findings of Jaiswal *et al*. (2013), which reported an increase in LDH in the liver of the rat induced with pesticide carbofuran. The both doses of the extract significantly reduced ($p<0.05$) lactate dehydrogenase activities in the serum, when compared to the positive control group (Fig 4.19). This shows the potency of the doses of the extract in the reduction of liver injury characterized by high LDH activity. The efficacy of *Diodia sarmentosa* is similar to that of Vitamin C in reducing carbofuran induced oxidative stress in rats (Jaiswal *et al*., 2013). Lactate dehydrogenase activity was significantly reduced ($p<0.05$) in the heart tissue of the positive control group, when compared to the negative control group (Fig 4.20). The activities of LDH is higher in the heart tissue when compared with that of the serum in the present study. Vikramathithan *et al*. (2009) and Jaiswal *et al.* (2013) reported the same observation in their respective studies. Deficiency in LDH activity affects the cardiac muscle. This is evident in the positive control group that showed low LDH activity in the heart tissue. The increased LDH activity in the high dose group was not significantly different ($p>0.05$) compared to the negative control group, thus showing that high dose of the extract was effective in balancing the declined LDH activity. The potency of

the extract is similar to Vitamin C, which increased LDH activity in the heart tissue of carbofuran induced rats (Jaiswal *et al*., 2013).

Bilirubin is a biomarker used in hepatic function. Any abnormality in the liver causes an elevation of bilirubin level. Serum total bilirubin in the positive control group was significantly increased ($p<0.05$) when compared to the negative control group (Fig 4.15). This increase may be due to severe disturbance in the hepatocellular function of the hyperlipidaemic test rats. This result corresponds to elevation of total bilirubin in hyperlipidaemic rats previously reported by Olorunnisola *et al.* (2012). The treatment groups (low dose group, high dose group and SAD group) did not show a significant different ($p>0.05$) in total bilirubin levels amongst themselves, though they all reduced total bilirubin levels in their respective groups. The efficacy of *Diodia sarmentosa* was effective in a dose dependent manner, and was similar to that of *Salvadora persica* in treating of hyperglycemia and hyperlipidaemic rats (El-Rabey *et al*., 2018).

Proteins are used as an indicator of liver injury and damage. The positive control group decreased Serum total protein level significantly ($p<0.05$) compared to negative control group (Fig 4.16). The decrease may be as a result of liver injury caused by high lipids in the test rats. This result corresponds with that of Olorunnisola *et al*. (2012), which reported decrease in serum total protein level in hypercholesterolemia rats. The extract at both doses significantly increased ($p<0.05$) serum protein levels, when compared to the negative control group (Fig 4.16). The serum total protein level in the treatment groups were not significantly different ($p>0.05$) amongst each other, and the negative control group. This means both doses of the extract increased serum protein level to normal level with the negative control group. The

potency of the extract is similar to *Tulbaghia violacea* which increased total protein in hypercholesterolemia rats (Olorunnisola *et al*., 2012).

Albumin and Globulin are types of protein that can also serve as biomarker for liver injury. Fig 4.17 showed serum albumin level in the positive control group was significantly reduced ($p<0.05$) when compared to other study groups. Fig 4.18 on the other hand shows serum globulin level was decreased in the positive control group, but its reduction was not significantly different ($p>0.05$), when compared to the other study groups. This reduction maybe attributed to the effect of the increase in poly unsaturated fatty acid in the serum causing hepatocycte injury in the test rats. This result is in agreement with the findings of some authors which reported reduction in albumin and globulin levels in high fat diet rats (Ghasi *et al.*, 2000; Olorunnisola *et al*., 2012). In the treatment groups, the low dose of the extract showed an increased albumin level that was not significantly different ($p>0.05$) when compared to the high dose and negative control group. The standard antihyperlipidaemic group showed the highest increase in albumin level, though it was not significantly different ($p>0.05$) when compared to the high dose and negative control group (Fig 4.17). The increase in globulin levels in the treatment groups were not significantly different ($p>0.05$) when compared to the negative control and amongst each other. The potency of the extract in increasing albumin and globulin levels is similar to plants like *Tulbaghia violacea* and *Moringa oleifera* lam, in increasing of serum albumin and globulin levels in hypercholesterolemia rats (Ghasi *et al.*, 2000; Olorunnisola *et al.*, 2012).

4.2.5 Heart rate

Table 4.7 shows the heart rate of all the study groups recorded weekly. From the result of this study, it was observed that the heart rate of the test rats were almost at the same level in the first week of the experiment. From the second week to the third week, the heart rate of the positive control group, low dose extract group and high dose extract groups increased when compared to the negative control group. This high heart rate known as Tachcardia (a condition in which the heart beats faster than normal while at rest) can be attributed to the high cholesterol level in the test rats. This result corresponds to Nwanjo & Oze (2006) that recorded abnormal heart rate in hyperlipidaemic rats. From the fourth week till the end of the experiment, the extract and simvastatin gradually stabilized the abnormal heart rate of the test rats. The potency of the extract is similar to aqueous garlic extract which stabilized the heart rate of hyperlipidaemic rats (Nwanjo & Oze, 2006).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The result of this study showed that the ethanolic extract of *Diodia sarmentosa* has antioxidant potentials, by its ability to in vitro scavenge for nitric oxide and hydroxyl radicals in a similar manner as the standard antioxidant (Quercetin dihydrate). Having, increased the activities of enzymatic antioxidants and reduced lipid peroxidation in vivo, the plant reduced oxidative stress. This shows the antioxidant potentials of the plant extract, though more effective at a higher dose.

Diodia sarmentosa is a plant with antihyperlipidaemic properties due to its ability to reduce lipid profiles, and increase good cholesterol, stabilize heart rate to normal levels, and reduce the risk of occurrence of cardiovascular diseases, because of its low atherogenic indices and ratios, and high percentage protection.

5.2 Recommendation

Despite the fact that this study adds to the scientific knowledge of the medicinal importance of *Diodia sarmentosa*, there is room for more research on the plant to evaluate its effect on blood pressure levels (systolic and diastolic), and also on vital renal markers to further establish if it also has antihypertensive potentials.

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APPENDIX I

Nitric Oxide radical (Extract)

Concentration (µg/ml)	Percentage inhibition (%)
43.424476236105	5
91.763851940632	10
207.81262457371	20
578.46187591553	40
907.1715259552	50
1469.302210807860	60
2660.6511640549	70
6968.195233494	80

Nitric Oxide radical (Quercetin)

Concentration (µg/ml)	Percentage inhibition (%)
2.563323546201	5
5.4907413199544	10
8.824852854013	15
12.64383457601	20
17.05359537154	25
35.528897866607	40
55.36773167550	50
87.901998311281	60
150.7933422923	70
322.4225372076	80

Hydroxyl radical (Extract)

Concentration (µg/ml)	Percentage inhibition(%)
3.8135853596032	5
17.143702693284	10
44.140771403909	15
91.148993372917	20
794.73567008972	40
2173.4412789345	50
6907.3189329356	60
36392.752360553	70
440124211.83288	80

Hydroxyl radical (Quercetin)

Concentration (µg/ml)	Percentage inhibition (%)
1.261034309864	5
5.376678109169	10
13.09510499239	15
25.443930029869	20
43.804815113544	25
157.38694190979	40
322.92160272598	50
641.44309520721	60
1289.1896404326	70
2753.4665502608	80
23148.448094726	100

APPENDIX II

Oxidative stress parameters in heart and serum

Oneway

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Serum LDH (U/L)	Between Groups	256.479	4	64.120	18.527	.000
	Within Groups	51.913	15	3.461		
	Total	308.392	19			
Heart LDH (U/L)	Between Groups	1883.851	4	470.963	8.496	.001
	Within Groups	831.492	15	55.433		
	Total	2715.343	19			
Heart SOD (IU/mg Protein)x10^-6	Between Groups	231.720	4	57.930	8.648	.001
	Within Groups	100.485	15	6.699		
	Total	332.205	19			
SOD (IU/mg Protein)x10^-6	Between Groups	174.243	4	43.561	24.334	.000
	Within Groups	26.852	15	1.790		
	Total	201.095	19			
Heart Protein (g/L)	Between Groups	3433.628	4	858.407	43.052	.000
	Within Groups	299.085	15	19.939		
	Total	3732.714	19			
Heart MDA (nmol/mgprotein)	Between Groups	.403	4	.101	78.871	.000
	Within Groups	.019	15	.001		
	Total	.422	19			
Serum MDA (nmol/mgprotein)	Between Groups	.111	4	.028	90.186	.000
	Within Groups	.005	15	.000		
	Total	.116	19			
Heart GPx (mgGSH/min/mgprotein)	Between Groups	5.863	4	1.466	33.401	.000
	Within Groups	.658	15	.044		
	Total	6.521	19			
Serum GPx (mgGSH/min/mgprotein)	Between Groups	8.995	4	2.249	29.404	.000
	Within Groups	1.147	15	.076		
	Total	10.142	19			
Heart GST (µmol GSH-CDNB /min/mgprotein)x10^-6	Between Groups	1.714	4	.429	9.555	.000
	Within Groups	.673	15	.045		
	Total	2.387	19			
Serum GST (µmol GSH-	Between Groups	.176	4	.044	2.798	.064

CDNB /min/mgprotein)x10^-6	Within Groups	.236	15	.016		
	Total	.413	19			
Heart CATALASE (µM H2O2/min/mgProtein)x10^-6	Between Groups	11.530	4	2.882	11.216	.000
	Within Groups	3.855	15	.257		
	Total	15.385	19			
Serum CATALASE (um H2O2/min/mgProtein)x10^-6	Between Groups	46.175	4	11.544	16.279	.000
	Within Groups	10.637	15	.709		
	Total	56.811	19			

Post Hoc Tests

Multiple Comparisons

Dependent Variable		(I) OxidativeStress Assay	(J) OxidativeStress Assay	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Serum LDH (U/L)	Tukey HSD	NC	PC	-9.55750*	1.31546	.000	-13.6195	-5.4955
			LDE 250mg/Kgbwt	-5.09500*	1.31546	.011	-9.1570	-1.0330
			HDE 500 mg/Kgbwt	-4.46000*	1.31546	.028	-8.5220	-.3980
			SAD 5mg/Kgbwt	-.00250	1.31546	1.000	-4.0645	4.0595
		PC	NC	9.55750*	1.31546	.000	5.4955	13.6195
			LDE 250mg/Kgbwt	4.46250*	1.31546	.028	.4005	8.5245
			HDE 500 mg/Kgbwt	5.09750*	1.31546	.011	1.0355	9.1595
			SAD 5mg/Kgbwt	9.55500*	1.31546	.000	5.4930	13.6170
		LDE 250mg/Kgbwt	NC	5.09500*	1.31546	.011	1.0330	9.1570
			PC	-4.46250*	1.31546	.028	-8.5245	-.4005
			HDE 500 mg/Kgbwt	.63500	1.31546	.988	-3.4270	4.6970

			SAD 5mg/Kgbwt	5.09250*	1.31546	.011	1.0305	9.1545
			NC	4.46000*	1.31546	.028	.3980	8.5220
			PC	-5.09750*	1.31546	.011	-9.1595	-1.0355
		HDE 500 mg/Kgbwt	LDE 250mg/Kgbwt	-.63500	1.31546	.988	-4.6970	3.4270
			SAD 5mg/Kgbwt	4.45750*	1.31546	.028	.3955	8.5195
			NC	.00250	1.31546	1.000	-4.0595	4.0645
			PC	-9.55500*	1.31546	.000	-13.6170	-5.4930
		SAD 5mg/Kgbwt	LDE 250mg/Kgbwt	-5.09250*	1.31546	.011	-9.1545	-1.0305
			HDE 500 mg/Kgbwt	-4.45750*	1.31546	.028	-8.5195	-.3955
			PC	16.56750*	5.26464	.045	.3107	32.8243
			LDE 250mg/Kgbwt	17.20500*	5.26464	.036	.9482	33.4618
		NC	HDE 500 mg/Kgbwt	2.55000	5.26464	.988	-13.7068	18.8068
			SAD 5mg/Kgbwt	-7.64500	5.26464	.606	-23.9018	8.6118
			NC	-16.56750*	5.26464	.045	-32.8243	-.3107
Heart LDH (U/L)	Tukey HSD		LDE 250mg/Kgbwt	.63750	5.26464	1.000	-15.6193	16.8943
		PC	HDE 500 mg/Kgbwt	-14.01750	5.26464	.108	-30.2743	2.2393
			SAD 5mg/Kgbwt	-24.21250*	5.26464	.003	-40.4693	-7.9557
		LDE 250mg/Kgbwt	NC	-17.20500*	5.26464	.036	-33.4618	-.9482

			PC	-.63750	5.26464	1.000	-16.8943	15.6193
			HDE 500 mg/Kgbwt	-14.65500	5.26464	.087	-30.9118	1.6018
			SAD 5mg/Kgbwt	-24.85000*	5.26464	.002	-41.1068	-8.5932
			NC	-2.55000	5.26464	.988	-18.8068	13.7068
		HDE 500 mg/Kgbwt	PC	14.01750	5.26464	.108	-2.2393	30.2743
			LDE 250mg/Kgbwt	14.65500	5.26464	.087	-1.6018	30.9118
			SAD 5mg/Kgbwt	-10.19500	5.26464	.341	-26.4518	6.0618
			NC	7.64500	5.26464	.606	-8.6118	23.9018
		SAD 5mg/Kgbwt	PC	24.21250*	5.26464	.003	7.9557	40.4693
			LDE 250mg/Kgbwt	24.85000*	5.26464	.002	8.5932	41.1068
			HDE 500 mg/Kgbwt	10.19500	5.26464	.341	-6.0618	26.4518
			PC	6.91500*	1.83017	.013	1.2636	12.5664
			LDE 250mg/Kgbwt	-3.41750	1.83017	.375	-9.0689	2.2339
		NC	HDE 500 mg/Kgbwt	-.41250	1.83017	.999	-6.0639	5.2389
			SAD 5mg/Kgbwt	1.66750	1.83017	.888	-3.9839	7.3189
Heart SOD (IU/mg Protein)x10^-6	Tukey HSD		NC	-6.91500*	1.83017	.013	-12.5664	-1.2636
			LDE 250mg/Kgbwt	-10.33250*	1.83017	.000	-15.9839	-4.6811
		PC	HDE 500 mg/Kgbwt	-7.32750*	1.83017	.009	-12.9789	-1.6761
			SAD 5mg/Kgbwt	-5.24750	1.83017	.075	-10.8989	.4039

			NC	3.41750	1.83017	.375	-2.2339	9.0689
			PC	10.33250*	1.83017	.000	4.6811	15.9839
		LDE 250mg/Kgbwt	HDE 500 mg/Kgbwt	3.00500	1.83017	.495	-2.6464	8.6564
			SAD 5mg/Kgbwt	5.08500	1.83017	.088	-.5664	10.7364
			NC	.41250	1.83017	.999	-5.2389	6.0639
			PC	7.32750*	1.83017	.009	1.6761	12.9789
		HDE 500 mg/Kgbwt	LDE 250mg/Kgbwt	-3.00500	1.83017	.495	-8.6564	2.6464
			SAD 5mg/Kgbwt	2.08000	1.83017	.785	-3.5714	7.7314
			NC	-1.66750	1.83017	.888	-7.3189	3.9839
			PC	5.24750	1.83017	.075	-.4039	10.8989
		SAD 5mg/Kgbwt	LDE 250mg/Kgbwt	-5.08500	1.83017	.088	-10.7364	.5664
			HDE 500 mg/Kgbwt	-2.08000	1.83017	.785	-7.7314	3.5714
			PC	7.97250*	.94608	.000	5.0511	10.8939
			LDE 250mg/Kgbwt	2.75000	.94608	.070	-.1714	5.6714
		NC	HDE 500 mg/Kgbwt	-.10250	.94608	1.000	-3.0239	2.8189
			SAD 5mg/Kgbwt	3.53750*	.94608	.014	.6161	6.4589
SOD (IU/mg Protein)x10^-6	Tukey HSD		NC	-7.97250*	.94608	.000	-10.8939	-5.0511
			LDE 250mg/Kgbwt	-5.22250*	.94608	.000	-8.1439	-2.3011
		PC	HDE 500 mg/Kgbwt	-8.07500*	.94608	.000	-10.9964	-5.1536
			SAD 5mg/Kgbwt	-4.43500*	.94608	.002	-7.3564	-1.5136

		LDE 250mg/Kgbwt	NC	-2.75000	.94608	.070	-5.6714	.1714
			PC	5.22250*	.94608	.000	2.3011	8.1439
			HDE 500 mg/Kgbwt	-2.85250	.94608	.057	-5.7739	.0689
			SAD 5mg/Kgbwt	.78750	.94608	.916	-2.1339	3.7089
		HDE 500 mg/Kgbwt	NC	.10250	.94608	1.000	-2.8189	3.0239
			PC	8.07500*	.94608	.000	5.1536	10.9964
			LDE 250mg/Kgbwt	2.85250	.94608	.057	-.0689	5.7739
			SAD 5mg/Kgbwt	3.64000*	.94608	.012	.7186	6.5614
		SAD 5mg/Kgbwt	NC	-3.53750*	.94608	.014	-6.4589	-.6161
			PC	4.43500*	.94608	.002	1.5136	7.3564
			LDE 250mg/Kgbwt	-.78750	.94608	.916	-3.7089	2.1339
			HDE 500 mg/Kgbwt	-3.64000*	.94608	.012	-6.5614	-.7186
		NC	PC	33.32750*	3.15745	.000	23.5775	43.0775
			LDE 250mg/Kgbwt	10.14500*	3.15745	.040	.3950	19.8950
			HDE 500 mg/Kgbwt	1.21000	3.15745	.995	-8.5400	10.9600
			SAD 5mg/Kgbwt	-2.17250	3.15745	.956	-11.9225	7.5775
Heart Protein (g/L)	Tukey HSD	PC	NC	-33.32750*	3.15745	.000	-43.0775	-23.5775
			LDE 250mg/Kgbwt	-23.18250*	3.15745	.000	-32.9325	-13.4325
			HDE 500 mg/Kgbwt	-32.11750*	3.15745	.000	-41.8675	-22.3675

			SAD 5mg/Kgbwt	-35.50000*	3.15745	.000	-45.2500	-25.7500
			NC	-10.14500*	3.15745	.040	-19.8950	-.3950
		LDE 250mg/Kgbwt	PC	23.18250*	3.15745	.000	13.4325	32.9325
			HDE 500 mg/Kgbwt	-8.93500	3.15745	.080	-18.6850	.8150
			SAD 5mg/Kgbwt	-12.31750*	3.15745	.011	-22.0675	-2.5675
			NC	-1.21000	3.15745	.995	-10.9600	8.5400
		HDE 500 mg/Kgbwt	PC	32.11750*	3.15745	.000	22.3675	41.8675
			LDE 250mg/Kgbwt	8.93500	3.15745	.080	-.8150	18.6850
			SAD 5mg/Kgbwt	-3.38250	3.15745	.818	-13.1325	6.3675
			NC	2.17250	3.15745	.956	-7.5775	11.9225
		SAD 5mg/Kgbwt	PC	35.50000*	3.15745	.000	25.7500	45.2500
			LDE 250mg/Kgbwt	12.31750*	3.15745	.011	2.5675	22.0675
			HDE 500 mg/Kgbwt	3.38250	3.15745	.818	-6.3675	13.1325
			PC	-.39500*	.02527	.000	-.4730	-.3170
		NC	LDE 250mg/Kgbwt	-.11750*	.02527	.002	-.1955	-.0395
			HDE 500 mg/Kgbwt	-.06500	.02527	.126	-.1430	.0130
Heart MDA (nmol/mgprotein)	Tukey HSD		SAD 5mg/Kgbwt	-.03250	.02527	.703	-.1105	.0455
			NC	.39500*	.02527	.000	.3170	.4730
		PC	LDE 250mg/Kgbwt	.27750*	.02527	.000	.1995	.3555

			HDE 500 mg/Kgbwt	.33000*	.02527	.000	.2520	.4080
			SAD 5mg/Kgbwt	.36250*	.02527	.000	.2845	.4405
			NC	.11750*	.02527	.002	.0395	.1955
			PC	-.27750*	.02527	.000	-.3555	-.1995
		LDE 250mg/Kgbwt	HDE 500 mg/Kgbwt	.05250	.02527	.279	-.0255	.1305
			SAD 5mg/Kgbwt	.08500*	.02527	.030	.0070	.1630
			NC	.06500	.02527	.126	-.0130	.1430
			PC	-.33000*	.02527	.000	-.4080	-.2520
		HDE 500 mg/Kgbwt	LDE 250mg/Kgbwt	-.05250	.02527	.279	-.1305	.0255
			SAD 5mg/Kgbwt	.03250	.02527	.703	-.0455	.1105
			NC	.03250	.02527	.703	-.0455	.1105
			PC	-.36250*	.02527	.000	-.4405	-.2845
		SAD 5mg/Kgbwt	LDE 250mg/Kgbwt	-.08500*	.02527	.030	-.1630	-.0070
			HDE 500 mg/Kgbwt	-.03250	.02527	.703	-.1105	.0455
			PC	-.22250*	.01242	.000	-.2608	-.1842
			LDE 250mg/Kgbwt	-.07000*	.01242	.000	-.1083	-.0317
		NC	HDE 500 mg/Kgbwt	-.07750*	.01242	.000	-.1158	-.0392
Serum MDA (nmol/mgprotein)	Tukey HSD		SAD 5mg/Kgbwt	-.04750*	.01242	.012	-.0858	-.0092
			NC	.22250*	.01242	.000	.1842	.2608
		PC	LDE 250mg/Kgbwt	.15250*	.01242	.000	.1142	.1908

			HDE 500 mg/Kgbwt	.14500*	.01242	.000	.1067	.1833
			SAD 5mg/Kgbwt	.17500*	.01242	.000	.1367	.2133
			NC	.07000*	.01242	.000	.0317	.1083
			PC	-.15250*	.01242	.000	-.1908	-.1142
		LDE 250mg/Kgbwt	HDE 500 mg/Kgbwt	-.00750	.01242	.972	-.0458	.0308
			SAD 5mg/Kgbwt	.02250	.01242	.403	-.0158	.0608
			NC	.07750*	.01242	.000	.0392	.1158
			PC	-.14500*	.01242	.000	-.1833	-.1067
		HDE 500 mg/Kgbwt	LDE 250mg/Kgbwt	.00750	.01242	.972	-.0308	.0458
			SAD 5mg/Kgbwt	.03000	.01242	.164	-.0083	.0683
			NC	.04750*	.01242	.012	.0092	.0858
			PC	-.17500*	.01242	.000	-.2133	-.1367
		SAD 5mg/Kgbwt	LDE 250mg/Kgbwt	-.02250	.01242	.403	-.0608	.0158
			HDE 500 mg/Kgbwt	-.03000	.01242	.164	-.0683	.0083
			PC	1.56000*	.14813	.000	1.1026	2.0174
			LDE 250mg/Kgbwt	.31250	.14813	.266	-.1449	.7699
		NC	HDE 500 mg/Kgbwt	.58750*	.14813	.009	.1301	1.0449
Heart GPx (mgGSH/min/mg protein)	Tukey HSD		SAD 5mg/Kgbwt	.97250*	.14813	.000	.5151	1.4299
			NC	-1.56000*	.14813	.000	-2.0174	-1.1026
		PC	LDE 250mg/Kgbwt	-1.24750*	.14813	.000	-1.7049	-.7901

			HDE 500 mg/Kgbwt	-.97250*	.14813	.000	-1.4299	-.5151
			SAD 5mg/Kgbwt	-.58750*	.14813	.009	-1.0449	-.1301
			NC	-.31250	.14813	.266	-.7699	.1449
			PC	1.24750*	.14813	.000	.7901	1.7049
		LDE 250mg/Kgbwt	HDE 500 mg/Kgbwt	.27500	.14813	.380	-.1824	.7324
			SAD 5mg/Kgbwt	.66000*	.14813	.004	.2026	1.1174
			NC	-.58750*	.14813	.009	-1.0449	-.1301
			PC	.97250*	.14813	.000	.5151	1.4299
		HDE 500 mg/Kgbwt	LDE 250mg/Kgbwt	-.27500	.14813	.380	-.7324	.1824
			SAD 5mg/Kgbwt	.38500	.14813	.121	-.0724	.8424
			NC	-.97250*	.14813	.000	-1.4299	-.5151
			PC	.58750*	.14813	.009	.1301	1.0449
		SAD 5mg/Kgbwt	LDE 250mg/Kgbwt	-.66000*	.14813	.004	-1.1174	-.2026
			HDE 500 mg/Kgbwt	-.38500	.14813	.121	-.8424	.0724
			PC	2.02000*	.19555	.000	1.4162	2.6238
			LDE 250mg/Kgbwt	.89500*	.19555	.003	.2912	1.4988
		NC	HDE 500 mg/Kgbwt	1.01250*	.19555	.001	.4087	1.6163
Serum GPx (mgGSH/min/mg protein)	Tukey HSD		SAD 5mg/Kgbwt	1.48000*	.19555	.000	.8762	2.0838
			NC	-2.02000*	.19555	.000	-2.6238	-1.4162
		PC	LDE 250mg/Kgbwt	-1.12500*	.19555	.000	-1.7288	-.5212

			HDE 500 mg/Kgbwt	-1.00750*	.19555	.001	-1.6113	-.4037
			SAD 5mg/Kgbwt	-.54000	.19555	.091	-1.1438	.0638
			NC	-.89500*	.19555	.003	-1.4988	-.2912
			PC	1.12500*	.19555	.000	.5212	1.7288
		LDE 250mg/Kgbwt	HDE 500 mg/Kgbwt	.11750	.19555	.973	-.4863	.7213
			SAD 5mg/Kgbwt	.58500	.19555	.060	-.0188	1.1888
			NC	-1.01250*	.19555	.001	-1.6163	-.4087
			PC	1.00750*	.19555	.001	.4037	1.6113
		HDE 500 mg/Kgbwt	LDE 250mg/Kgbwt	-.11750	.19555	.973	-.7213	.4863
			SAD 5mg/Kgbwt	.46750	.19555	.171	-.1363	1.0713
			NC	-1.48000*	.19555	.000	-2.0838	-.8762
			PC	.54000	.19555	.091	-.0638	1.1438
		SAD 5mg/Kgbwt	LDE 250mg/Kgbwt	-.58500	.19555	.060	-1.1888	.0188
			HDE 500 mg/Kgbwt	-.46750	.19555	.171	-1.0713	.1363
			PC	-.84750*	.14975	.000	-1.3099	-.3851
			LDE 250mg/Kgbwt	-.61000*	.14975	.008	-1.0724	-.1476
Heart GST (µmol GSH-CDNB /min/mgprotein)x 10^-6	Tukey HSD	NC	HDE 500 mg/Kgbwt	-.25250	.14975	.470	-.7149	.2099
			SAD 5mg/Kgbwt	-.34500	.14975	.197	-.8074	.1174
			NC	.84750*	.14975	.000	.3851	1.3099
		PC	LDE 250mg/Kgbwt	.23750	.14975	.527	-.2249	.6999

			HDE 500 mg/Kgbwt	.59500*	.14975	.009	.1326	1.0574
			SAD 5mg/Kgbwt	.50250*	.14975	.030	.0401	.9649
			NC	.61000*	.14975	.008	.1476	1.0724
			PC	-.23750	.14975	.527	-.6999	.2249
		LDE 250mg/Kgbwt	HDE 500 mg/Kgbwt	.35750	.14975	.172	-.1049	.8199
			SAD 5mg/Kgbwt	.26500	.14975	.425	-.1974	.7274
			NC	.25250	.14975	.470	-.2099	.7149
			PC	-.59500*	.14975	.009	-1.0574	-.1326
		HDE 500 mg/Kgbwt	LDE 250mg/Kgbwt	-.35750	.14975	.172	-.8199	.1049
			SAD 5mg/Kgbwt	-.09250	.14975	.970	-.5549	.3699
			NC	.34500	.14975	.197	-.1174	.8074
			PC	-.50250*	.14975	.030	-.9649	-.0401
		SAD 5mg/Kgbwt	LDE 250mg/Kgbwt	-.26500	.14975	.425	-.7274	.1974
			HDE 500 mg/Kgbwt	.09250	.14975	.970	-.3699	.5549
			PC	-.23250	.08878	.117	-.5066	.0416
			LDE 250mg/Kgbwt	.01000	.08878	1.000	-.2641	.2841
Serum GST (µmol GSH-CDNB /min/mgprotein)x 10^-6	Tukey HSD	NC	HDE 500 mg/Kgbwt	.01750	.08878	1.000	-.2566	.2916
			SAD 5mg/Kgbwt	-.03750	.08878	.993	-.3116	.2366
			NC	.23250	.08878	.117	-.0416	.5066
		PC	LDE 250mg/Kgbwt	.24250	.08878	.096	-.0316	.5166

			HDE 500 mg/Kgbwt	.25000	.08878	.082	-.0241	.5241
			SAD 5mg/Kgbwt	.19500	.08878	.233	-.0791	.4691
			NC	-.01000	.08878	1.000	-.2841	.2641
			PC	-.24250	.08878	.096	-.5166	.0316
		LDE 250mg/Kgbwt	HDE 500 mg/Kgbwt	.00750	.08878	1.000	-.2666	.2816
			SAD 5mg/Kgbwt	-.04750	.08878	.982	-.3216	.2266
			NC	-.01750	.08878	1.000	-.2916	.2566
			PC	-.25000	.08878	.082	-.5241	.0241
		HDE 500 mg/Kgbwt	LDE 250mg/Kgbwt	-.00750	.08878	1.000	-.2816	.2666
			SAD 5mg/Kgbwt	-.05500	.08878	.970	-.3291	.2191
			NC	.03750	.08878	.993	-.2366	.3116
			PC	-.19500	.08878	.233	-.4691	.0791
		SAD 5mg/Kgbwt	LDE 250mg/Kgbwt	.04750	.08878	.982	-.2266	.3216
			HDE 500 mg/Kgbwt	.05500	.08878	.970	-.2191	.3291
			PC	1.05250	.35847	.066	-.0544	2.1594
			LDE 250mg/Kgbwt	.24750	.35847	.956	-.8594	1.3544
		NC	HDE 500 mg/Kgbwt	-.75500	.35847	.268	-1.8619	.3519
Heart CATALASE (µM H2O2/min/mgProtein)x10^-6	Tukey HSD		SAD 5mg/Kgbwt	-1.09750	.35847	.053	-2.2044	.0094
			NC	-1.05250	.35847	.066	-2.1594	.0544
		PC	LDE 250mg/Kgbwt	-.80500	.35847	.216	-1.9119	.3019

			HDE 500 mg/Kgbwt	-1.80750*	.35847	.001	-2.9144	-.7006
			SAD 5mg/Kgbwt	-2.15000*	.35847	.000	-3.2569	-1.0431
			NC	-.24750	.35847	.956	-1.3544	.8594
			PC	.80500	.35847	.216	-.3019	1.9119
		LDE 250mg/Kgbwt	HDE 500 mg/Kgbwt	-1.00250	.35847	.085	-2.1094	.1044
			SAD 5mg/Kgbwt	-1.34500*	.35847	.014	-2.4519	-.2381
			NC	.75500	.35847	.268	-.3519	1.8619
			PC	1.80750*	.35847	.001	.7006	2.9144
		HDE 500 mg/Kgbwt	LDE 250mg/Kgbwt	1.00250	.35847	.085	-.1044	2.1094
			SAD 5mg/Kgbwt	-.34250	.35847	.870	-1.4494	.7644
			NC	1.09750	.35847	.053	-.0094	2.2044
			PC	2.15000*	.35847	.000	1.0431	3.2569
		SAD 5mg/Kgbwt	LDE 250mg/Kgbwt	1.34500*	.35847	.014	.2381	2.4519
			HDE 500 mg/Kgbwt	.34250	.35847	.870	-.7644	1.4494
			PC	3.83000*	.59545	.000	1.9913	5.6687
			LDE 250mg/Kgbwt	1.90250*	.59545	.041	.0638	3.7412
Serum CATALASE (um H2O2/min/mgProtein)x10^-6	Tukey HSD	NC	HDE 500 mg/Kgbwt	.60000	.59545	.848	-1.2387	2.4387
			SAD 5mg/Kgbwt	-.32750	.59545	.980	-2.1662	1.5112
			NC	-3.83000*	.59545	.000	-5.6687	-1.9913
		PC	LDE 250mg/Kgbwt	-1.92750*	.59545	.038	-3.7662	-.0888

		HDE 500 mg/Kgbwt	-3.23000*	.59545	.001	-5.0687	-1.3913
		SAD 5mg/Kgbwt	-4.15750*	.59545	.000	-5.9962	-2.3188
		NC	-1.90250*	.59545	.041	-3.7412	-.0638
		PC	1.92750*	.59545	.038	.0888	3.7662
	LDE 250mg/Kgbwt	HDE 500 mg/Kgbwt	-1.30250	.59545	.237	-3.1412	.5362
		SAD 5mg/Kgbwt	-2.23000*	.59545	.014	-4.0687	-.3913
		NC	-.60000	.59545	.848	-2.4387	1.2387
		PC	3.23000*	.59545	.001	1.3913	5.0687
	HDE 500 mg/Kgbwt	LDE 250mg/Kgbwt	1.30250	.59545	.237	-.5362	3.1412
		SAD 5mg/Kgbwt	-.92750	.59545	.544	-2.7662	.9112
		NC	.32750	.59545	.980	-1.5112	2.1662
		PC	4.15750*	.59545	.000	2.3188	5.9962
	SAD 5mg/Kgbwt	LDE 250mg/Kgbwt	2.23000*	.59545	.014	.3913	4.0687
		HDE 500 mg/Kgbwt	.92750	.59545	.544	-.9112	2.7662

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

Homogeneous Subsets

Serum LDH (U/L)

	OxidativeStressAssay	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	NC	4	13.3825		
	SAD 5mg/Kgbwt	4	13.3850		
	HDE 500 mg/Kgbwt	4		17.8425	
	LDE 250mg/Kgbwt	4		18.4775	
	PC	4			22.9400

	Sig.		1.000	.988	1.000
Duncan[a]	NC	4	13.3825		
	SAD 5mg/Kgbwt	4	13.3850		
	HDE 500 mg/Kgbwt	4		17.8425	
	LDE 250mg/Kgbwt	4		18.4775	
	PC	4			22.9400
	Sig.		.999	.636	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Heart LDH (U/L)

	OxidativeStressAssay	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	LDE 250mg/Kgbwt	4	72.6475	
	PC	4	73.2850	
	HDE 500 mg/Kgbwt	4	87.3025	87.3025
	NC	4		89.8525
	SAD 5mg/Kgbwt	4		97.4975
	Sig.		.087	.341
Duncan[a]	LDE 250mg/Kgbwt	4	72.6475	
	PC	4	73.2850	
	HDE 500 mg/Kgbwt	4		87.3025
	NC	4		89.8525
	SAD 5mg/Kgbwt	4		97.4975
	Sig.		.905	.085

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Heart SOD (IU/mg Protein)x10^-6

	OxidativeStressAssay	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	PC	4	19.3725		
	SAD 5mg/Kgbwt	4	24.6200	24.6200	
	NC	4		26.2875	
	HDE 500 mg/Kgbwt	4		26.7000	

	LDE 250mg/Kgbwt	4		29.7050	
	Sig.		.075	.088	
	PC	4	19.3725		
	SAD 5mg/Kgbwt	4		24.6200	
	NC	4		26.2875	26.2875
Duncan[a]	HDE 500 mg/Kgbwt	4		26.7000	26.7000
	LDE 250mg/Kgbwt	4			29.7050
	Sig.		1.000	.298	.096

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

SOD (IU/mg Protein)x10^-6

	OxidativeStressAssay	N	Subset for alpha = 0.05		
			1	2	3
	PC	4	20.5125		
	SAD 5mg/Kgbwt	4		24.9475	
	LDE 250mg/Kgbwt	4		25.7350	25.7350
Tukey HSD[a]	NC	4			28.4850
	HDE 500 mg/Kgbwt	4			28.5875
	Sig.		1.000	.916	.057
	PC	4	20.5125		
	SAD 5mg/Kgbwt	4		24.9475	
	LDE 250mg/Kgbwt	4		25.7350	
Duncan[a]	NC	4			28.4850
	HDE 500 mg/Kgbwt	4			28.5875
	Sig.		1.000	.418	.915

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Heart Protein (g/L)

	OxidativeStressAssay	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	PC	4	29.9450		

	LDE 250mg/Kgbwt	4		53.1275	
	HDE 500 mg/Kgbwt	4		62.0625	62.0625
	NC	4			63.2725
	SAD 5mg/Kgbwt	4			65.4450
	Sig.		1.000	.080	.818
Duncan[a]	PC	4	29.9450		
	LDE 250mg/Kgbwt	4		53.1275	
	HDE 500 mg/Kgbwt	4			62.0625
	NC	4			63.2725
	SAD 5mg/Kgbwt	4			65.4450
	Sig.		1.000	1.000	.326

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Heart MDA (nmol/mgprotein)

	OxidativeStressAssay	N	Subset for alpha = 0.05			
			1	2	3	4
	NC	4	.2500			
	SAD 5mg/Kgbwt	4	.2825			
	HDE 500 mg/Kgbwt	4	.3150	.3150		
Tukey HSD[a]	LDE 250mg/Kgbwt	4		.3675		
	PC	4			.6450	
	Sig.		.126	.279	1.000	
	NC	4	.2500			
	SAD 5mg/Kgbwt	4	.2825	.2825		
	HDE 500 mg/Kgbwt	4		.3150	.3150	
Duncan[a]	LDE 250mg/Kgbwt	4			.3675	
	PC	4				.6450
	Sig.		.218	.218	.055	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Serum MDA (nmol/mgprotein)

	OxidativeStressAssay	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	NC	4	.1550			
	SAD 5mg/Kgbwt	4		.2025		
	LDE 250mg/Kgbwt	4		.2250		
	HDE 500 mg/Kgbwt	4		.2325		
	PC	4			.3775	
	Sig.		1.000	.164	1.000	
Duncan[a]	NC	4	.1550			
	SAD 5mg/Kgbwt	4		.2025		
	LDE 250mg/Kgbwt	4		.2250	.2250	
	HDE 500 mg/Kgbwt	4			.2325	
	PC	4				.3775
	Sig.		1.000	.090	.555	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Heart GPx (mgGSH/min/mgprotein)

	OxidativeStressAssay	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	PC	4	2.2925			
	SAD 5mg/Kgbwt	4		2.8800		
	HDE 500 mg/Kgbwt	4		3.2650	3.2650	
	LDE 250mg/Kgbwt	4			3.5400	3.5400
	NC	4				3.8525
	Sig.		1.000	.121	.380	.266
Duncan[a]	PC	4	2.2925			
	SAD 5mg/Kgbwt	4		2.8800		
	HDE 500 mg/Kgbwt	4			3.2650	
	LDE 250mg/Kgbwt	4			3.5400	3.5400
	NC	4				3.8525
	Sig.		1.000	1.000	.083	.052

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Serum GPx (mgGSH/min/mgprotein)

	OxidativeStressAssay	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	PC	4	1.5200			
	SAD 5mg/Kgbwt	4	2.0600	2.0600		
	HDE 500 mg/Kgbwt	4		2.5275		
	LDE 250mg/Kgbwt	4		2.6450		
	NC	4			3.5400	
	Sig.		.091	.060	1.000	
Duncan[a]	PC	4	1.5200			
	SAD 5mg/Kgbwt	4		2.0600		
	HDE 500 mg/Kgbwt	4			2.5275	
	LDE 250mg/Kgbwt	4			2.6450	
	NC	4				3.5400
	Sig.		1.000	1.000	.557	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Heart GST (µmol GSH-CDNB /min/mgprotein)x10^-6

	OxidativeStressAssay	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	NC	4	.8150			
	HDE 500 mg/Kgbwt	4	1.0675	1.0675		
	SAD 5mg/Kgbwt	4	1.1600	1.1600		
	LDE 250mg/Kgbwt	4		1.4250	1.4250	
	PC	4			1.6625	
	Sig.		.197	.172	.527	
Duncan[a]	NC	4	.8150			
	HDE 500 mg/Kgbwt	4	1.0675	1.0675		
	SAD 5mg/Kgbwt	4		1.1600	1.1600	
	LDE 250mg/Kgbwt	4			1.4250	1.4250
	PC	4				1.6625
	Sig.		.112	.546	.097	.134

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Serum GST (µmol GSH-CDNB /min/mgprotein)x10^-6

	OxidativeStressAssay	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	HDE 500 mg/Kgbwt	4	.7800	
	LDE 250mg/Kgbwt	4	.7875	
	NC	4	.7975	
	SAD 5mg/Kgbwt	4	.8350	
	PC	4	1.0300	
	Sig.		.082	
Duncan[a]	HDE 500 mg/Kgbwt	4	.7800	
	LDE 250mg/Kgbwt	4	.7875	
	NC	4	.7975	
	SAD 5mg/Kgbwt	4	.8350	
	PC	4		1.0300
	Sig.		.577	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Heart CATALASE (µM H2O2/min/mgProtein)x10^-6

	OxidativeStressAssay	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	PC	4	5.0300			
	LDE 250mg/Kgbwt	4	5.8350	5.8350		
	NC	4	6.0825	6.0825	6.0825	
	HDE 500 mg/Kgbwt	4		6.8375	6.8375	
	SAD 5mg/Kgbwt	4			7.1800	
	Sig.		.066	.085	.053	
Duncan[a]	PC	4	5.0300			
	LDE 250mg/Kgbwt	4		5.8350		
	NC	4		6.0825	6.0825	

	HDE 500 mg/Kgbwt	4			6.8375	6.8375
	SAD 5mg/Kgbwt	4				7.1800
	Sig.		1.000	.500	.052	.354

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Serum CATALASE (um H2O2/min/mgProtein)x10^-6

	OxidativeStressAssay	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	PC	4	12.1950		
	LDE 250mg/Kgbwt	4		14.1225	
	HDE 500 mg/Kgbwt	4		15.4250	15.4250
	NC	4			16.0250
	SAD 5mg/Kgbwt	4			16.3525
	Sig.		1.000	.237	.544
Duncan[a]	PC	4	12.1950		
	LDE 250mg/Kgbwt	4		14.1225	
	HDE 500 mg/Kgbwt	4			15.4250
	NC	4			16.0250
	SAD 5mg/Kgbwt	4			16.3525
	Sig.		1.000	1.000	.160

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

APPENDIX III

Lipid profiles

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Total Cholesterol (mg/dl)	Between Groups	24179.205	4	6044.801	119.984	.000
	Within Groups	755.703	15	50.380		
	Total	24934.907	19			

Triglycerides (mg/dl)	Between Groups	31469.249	4	7867.312	488.927	.000
	Within Groups	241.365	15	16.091		
	Total	31710.614	19			
VLDL (mg/dl)	Between Groups	1258.761	4	314.690	487.912	.000
	Within Groups	9.675	15	.645		
	Total	1268.436	19			
HDL (mg/dl)	Between Groups	1163.331	4	290.833	27.220	.000
	Within Groups	160.268	15	10.685		
	Total	1323.599	19			
LDL (mg/dl)	Between Groups	915.097	4	228.774	59.089	.000
	Within Groups	58.075	15	3.872		
	Total	973.172	19			
CRI-I	Between Groups	1.394	4	.348	70.979	.000
	Within Groups	.074	15	.005		
	Total	1.467	19			
CRI-II	Between Groups	35.060	4	8.765	105.611	.000
	Within Groups	1.245	15	.083		
	Total	36.305	19			
AC (TC-HDLC)/HDLC	Between Groups	35.060	4	8.765	105.611	.000
	Within Groups	1.245	15	.083		
	Total	36.305	19			
AIP(Log TG/HDLC)	Between Groups	1.261	4	.315	276.964	.000
	Within Groups	.017	15	.001		
	Total	1.278	19			

Post Hoc Tests

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) LipidProfileAssay	(J) LipidProfileAssay	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound

		PC	-86.22500*	5.01897	.000	-101.7232	-70.7268
		LDE 250mg/Kgbwt	-19.75750*	5.01897	.010	-35.2557	-4.2593
	NC	HDE 500mg/Kgbwt	-1.79500	5.01897	.996	-17.2932	13.7032
		SAD 5mg/Kgbwt	10.48000	5.01897	.275	-5.0182	25.9782
		NC	86.22500*	5.01897	.000	70.7268	101.7232
		LDE 250mg/Kgbwt	66.46750*	5.01897	.000	50.9693	81.9657
	PC	HDE 500mg/Kgbwt	84.43000*	5.01897	.000	68.9318	99.9282
		SAD 5mg/Kgbwt	96.70500*	5.01897	.000	81.2068	112.2032
		NC	19.75750*	5.01897	.010	4.2593	35.2557
Total Cholesterol (mg/dl)		PC	-66.46750*	5.01897	.000	-81.9657	-50.9693
	LDE 250mg/Kgbwt	HDE 500mg/Kgbwt	17.96250*	5.01897	.020	2.4643	33.4607
		SAD 5mg/Kgbwt	30.23750*	5.01897	.000	14.7393	45.7357
		NC	1.79500	5.01897	.996	-13.7032	17.2932
		PC	-84.43000*	5.01897	.000	-99.9282	-68.9318
	HDE 500mg/Kgbwt	LDE 250mg/Kgbwt	-17.96250*	5.01897	.020	-33.4607	-2.4643
		SAD 5mg/Kgbwt	12.27500	5.01897	.156	-3.2232	27.7732
		NC	-10.48000	5.01897	.275	-25.9782	5.0182
		PC	-96.70500*	5.01897	.000	-112.2032	-81.2068
	SAD 5mg/Kgbwt	LDE 250mg/Kgbwt	-30.23750*	5.01897	.000	-45.7357	-14.7393

		HDE 500mg/Kgbwt	-12.27500	5.01897	.156	-27.7732	3.2232
		PC	-83.33500*	2.83646	.000	-92.0938	-74.5762
		LDE 250mg/Kgbwt	2.30750	2.83646	.922	-6.4513	11.0663
	NC	HDE 500mg/Kgbwt	14.87000*	2.83646	.001	6.1112	23.6288
		SAD 5mg/Kgbwt	30.76750*	2.83646	.000	22.0087	39.5263
		NC	83.33500*	2.83646	.000	74.5762	92.0938
		LDE 250mg/Kgbwt	85.64250*	2.83646	.000	76.8837	94.4013
	PC	HDE 500mg/Kgbwt	98.20500*	2.83646	.000	89.4462	106.9638
		SAD 5mg/Kgbwt	114.10250*	2.83646	.000	105.3437	122.8613
		NC	-2.30750	2.83646	.922	-11.0663	6.4513
		PC	-85.64250*	2.83646	.000	-94.4013	-76.8837
Triglycerides (mg/dl)	LDE 250mg/Kgbwt	HDE 500mg/Kgbwt	12.56250*	2.83646	.004	3.8037	21.3213
		SAD 5mg/Kgbwt	28.46000*	2.83646	.000	19.7012	37.2188
		NC	-14.87000*	2.83646	.001	-23.6288	-6.1112
		PC	-98.20500*	2.83646	.000	-106.9638	-89.4462
	HDE 500mg/Kgbwt	LDE 250mg/Kgbwt	-12.56250*	2.83646	.004	-21.3213	-3.8037
		SAD 5mg/Kgbwt	15.89750*	2.83646	.000	7.1387	24.6563
		NC	-30.76750*	2.83646	.000	-39.5263	-22.0087
		PC	-114.10250*	2.83646	.000	-122.8613	-105.3437
	SAD 5mg/Kgbwt	LDE 250mg/Kgbwt	-28.46000*	2.83646	.000	-37.2188	-19.7012

		HDE 500mg/Kgbwt	-15.89750*	2.83646	.000	-24.6563	-7.1387
		PC	-16.67000*	.56788	.000	-18.4236	-14.9164
		LDE 250mg/Kgbwt	.46000	.56788	.923	-1.2936	2.2136
	NC	HDE 500mg/Kgbwt	2.97000*	.56788	.001	1.2164	4.7236
		SAD 5mg/Kgbwt	6.15000*	.56788	.000	4.3964	7.9036
		NC	16.67000*	.56788	.000	14.9164	18.4236
		LDE 250mg/Kgbwt	17.13000*	.56788	.000	15.3764	18.8836
	PC	HDE 500mg/Kgbwt	19.64000*	.56788	.000	17.8864	21.3936
		SAD 5mg/Kgbwt	22.82000*	.56788	.000	21.0664	24.5736
		NC	-.46000	.56788	.923	-2.2136	1.2936
		PC	-17.13000*	.56788	.000	-18.8836	-15.3764
VLDL (mg/dl)	LDE 250mg/Kgbwt	HDE 500mg/Kgbwt	2.51000*	.56788	.004	.7564	4.2636
		SAD 5mg/Kgbwt	5.69000*	.56788	.000	3.9364	7.4436
		NC	-2.97000*	.56788	.001	-4.7236	-1.2164
		PC	-19.64000*	.56788	.000	-21.3936	-17.8864
	HDE 500mg/Kgbwt	LDE 250mg/Kgbwt	-2.51000*	.56788	.004	-4.2636	-.7564
		SAD 5mg/Kgbwt	3.18000*	.56788	.000	1.4264	4.9336
		NC	-6.15000*	.56788	.000	-7.9036	-4.3964
		PC	-22.82000*	.56788	.000	-24.5736	-21.0664
	SAD 5mg/Kgbwt	LDE 250mg/Kgbwt	-5.69000*	.56788	.000	-7.4436	-3.9364
		HDE 500mg/Kgbwt	-3.18000*	.56788	.000	-4.9336	-1.4264
HDL (mg/dl)	NC	PC	15.08000*	2.31133	.000	7.9428	22.2172

		LDE 250mg/Kgbwt	6.98250	2.31133	.057	-.1547	14.1197
		HDE 500mg/Kgbwt	.00000	2.31133	1.000	-7.1372	7.1372
		SAD 5mg/Kgbwt	-7.54000*	2.31133	.036	-14.6772	-.4028
		NC	-15.08000*	2.31133	.000	-22.2172	-7.9428
	PC	LDE 250mg/Kgbwt	-8.09750*	2.31133	.023	-15.2347	-.9603
		HDE 500mg/Kgbwt	-15.08000*	2.31133	.000	-22.2172	-7.9428
		SAD 5mg/Kgbwt	-22.62000*	2.31133	.000	-29.7572	-15.4828
		NC	-6.98250	2.31133	.057	-14.1197	.1547
	LDE 250mg/Kgbwt	PC	8.09750*	2.31133	.023	.9603	15.2347
		HDE 500mg/Kgbwt	-6.98250	2.31133	.057	-14.1197	.1547
		SAD 5mg/Kgbwt	-14.52250*	2.31133	.000	-21.6597	-7.3853
		NC	.00000	2.31133	1.000	-7.1372	7.1372
	HDE 500mg/Kgbwt	PC	15.08000*	2.31133	.000	7.9428	22.2172
		LDE 250mg/Kgbwt	6.98250	2.31133	.057	-.1547	14.1197
		SAD 5mg/Kgbwt	-7.54000*	2.31133	.036	-14.6772	-.4028
		NC	7.54000*	2.31133	.036	.4028	14.6772
	SAD 5mg/Kgbwt	PC	22.62000*	2.31133	.000	15.4828	29.7572
		LDE 250mg/Kgbwt	14.52250*	2.31133	.000	7.3853	21.6597
		HDE 500mg/Kgbwt	7.54000*	2.31133	.036	.4028	14.6772
LDL (mg/dl)	NC	PC	-18.67750*	1.39135	.000	-22.9739	-14.3811

		LDE 250mg/Kgbwt	-4.66500*	1.39135	.030	-8.9614	-.3686
		HDE 500mg/Kgbwt	-4.08500	1.39135	.066	-8.3814	.2114
		SAD 5mg/Kgbwt	-.78000	1.39135	.979	-5.0764	3.5164
		NC	18.67750*	1.39135	.000	14.3811	22.9739
	PC	LDE 250mg/Kgbwt	14.01250*	1.39135	.000	9.7161	18.3089
		HDE 500mg/Kgbwt	14.59250*	1.39135	.000	10.2961	18.8889
		SAD 5mg/Kgbwt	17.89750*	1.39135	.000	13.6011	22.1939
		NC	4.66500*	1.39135	.030	.3686	8.9614
	LDE 250mg/Kgbwt	PC	-14.01250*	1.39135	.000	-18.3089	-9.7161
		HDE 500mg/Kgbwt	.58000	1.39135	.993	-3.7164	4.8764
		SAD 5mg/Kgbwt	3.88500	1.39135	.086	-.4114	8.1814
		NC	4.08500	1.39135	.066	-.2114	8.3814
	HDE 500mg/Kgbwt	PC	-14.59250*	1.39135	.000	-18.8889	-10.2961
		LDE 250mg/Kgbwt	-.58000	1.39135	.993	-4.8764	3.7164
		SAD 5mg/Kgbwt	3.30500	1.39135	.176	-.9914	7.6014
		NC	.78000	1.39135	.979	-3.5164	5.0764
	SAD 5mg/Kgbwt	PC	-17.89750*	1.39135	.000	-22.1939	-13.6011
		LDE 250mg/Kgbwt	-3.88500	1.39135	.086	-8.1814	.4114
		HDE 500mg/Kgbwt	-3.30500	1.39135	.176	-7.6014	.9914
		PC	-.69000*	.04954	.000	-.8430	-.5370
CRI-I	NC	LDE 250mg/Kgbwt	-.16500*	.04954	.032	-.3180	-.0120

		HDE 500mg/Kgbwt	-.07750	.04954	.540	-.2305	.0755
		SAD 5mg/Kgbwt	.03250	.04954	.963	-.1205	.1855
		NC	.69000*	.04954	.000	.5370	.8430
		LDE 250mg/Kgbwt	.52500*	.04954	.000	.3720	.6780
	PC	HDE 500mg/Kgbwt	.61250*	.04954	.000	.4595	.7655
		SAD 5mg/Kgbwt	.72250*	.04954	.000	.5695	.8755
		NC	.16500*	.04954	.032	.0120	.3180
		PC	-.52500*	.04954	.000	-.6780	-.3720
	LDE 250mg/Kgbwt	HDE 500mg/Kgbwt	.08750	.04954	.427	-.0655	.2405
		SAD 5mg/Kgbwt	.19750*	.04954	.009	.0445	.3505
		NC	.07750	.04954	.540	-.0755	.2305
		PC	-.61250*	.04954	.000	-.7655	-.4595
	HDE 500mg/Kgbwt	LDE 250mg/Kgbwt	-.08750	.04954	.427	-.2405	.0655
		SAD 5mg/Kgbwt	.11000	.04954	.225	-.0430	.2630
		NC	-.03250	.04954	.963	-.1855	.1205
		PC	-.72250*	.04954	.000	-.8755	-.5695
	SAD 5mg/Kgbwt	LDE 250mg/Kgbwt	-.19750*	.04954	.009	-.3505	-.0445
		HDE 500mg/Kgbwt	-.11000	.04954	.225	-.2630	.0430
		PC	-3.26500*	.20371	.000	-3.8940	-2.6360
		LDE 250mg/Kgbwt	-.75000*	.20371	.016	-1.3790	-.1210
	NC	HDE 500mg/Kgbwt	-.03500	.20371	1.000	-.6640	.5940
		SAD 5mg/Kgbwt	.42000	.20371	.286	-.2090	1.0490
		NC	3.26500*	.20371	.000	2.6360	3.8940
		LDE 250mg/Kgbwt	2.51500*	.20371	.000	1.8860	3.1440
	PC	HDE 500mg/Kgbwt	3.23000*	.20371	.000	2.6010	3.8590

CRI-II

		SAD 5mg/Kgbwt	3.68500*	.20371	.000	3.0560	4.3140
		NC	.75000*	.20371	.016	.1210	1.3790
		PC	-2.51500*	.20371	.000	-3.1440	-1.8860
	LDE 250mg/Kgbwt	HDE 500mg/Kgbwt	.71500*	.20371	.022	.0860	1.3440
		SAD 5mg/Kgbwt	1.17000*	.20371	.000	.5410	1.7990
		NC	.03500	.20371	1.000	-.5940	.6640
		PC	-3.23000*	.20371	.000	-3.8590	-2.6010
	HDE 500mg/Kgbwt	LDE 250mg/Kgbwt	-.71500*	.20371	.022	-1.3440	-.0860
		SAD 5mg/Kgbwt	.45500	.20371	.220	-.1740	1.0840
		NC	-.42000	.20371	.286	-1.0490	.2090
		PC	-3.68500*	.20371	.000	-4.3140	-3.0560
	SAD 5mg/Kgbwt	LDE 250mg/Kgbwt	-1.17000*	.20371	.000	-1.7990	-.5410
		HDE 500mg/Kgbwt	-.45500	.20371	.220	-1.0840	.1740
		PC	-3.26500*	.20371	.000	-3.8940	-2.6360
	NC	LDE 250mg/Kgbwt	-.75000*	.20371	.016	-1.3790	-.1210
		HDE 500mg/Kgbwt	-.03500	.20371	1.000	-.6640	.5940
		SAD 5mg/Kgbwt	.42000	.20371	.286	-.2090	1.0490
		NC	3.26500*	.20371	.000	2.6360	3.8940
		LDE 250mg/Kgbwt	2.51500*	.20371	.000	1.8860	3.1440
AC (TC-HDLC)/HDLC	PC	HDE 500mg/Kgbwt	3.23000*	.20371	.000	2.6010	3.8590
		SAD 5mg/Kgbwt	3.68500*	.20371	.000	3.0560	4.3140
		NC	.75000*	.20371	.016	.1210	1.3790
		PC	-2.51500*	.20371	.000	-3.1440	-1.8860
	LDE 250mg/Kgbwt	HDE 500mg/Kgbwt	.71500*	.20371	.022	.0860	1.3440
		SAD 5mg/Kgbwt	1.17000*	.20371	.000	.5410	1.7990
	HDE	NC	.03500	.20371	1.000	-.5940	.6640

	500mg/Kgbwt	PC	-3.23000*	.20371	.000	-3.8590	-2.6010
		LDE	-.71500*	.20371	.022	-1.3440	-.0860
		250mg/Kgbwt					
		SAD	.45500	.20371	.220	-.1740	1.0840
		5mg/Kgbwt					
		NC	-.42000	.20371	.286	-1.0490	.2090
		PC	-3.68500*	.20371	.000	-4.3140	-3.0560
	SAD	LDE	-1.17000*	.20371	.000	-1.7990	-.5410
	5mg/Kgbwt	250mg/Kgbwt					
		HDE	-.45500	.20371	.220	-1.0840	.1740
		500mg/Kgbwt					
		PC	-.47693*	.02385	.000	-.5506	-.4033
		LDE	-.05197	.02385	.240	-.1256	.0217
		250mg/Kgbwt					
	NC	HDE	.09484*	.02385	.009	.0212	.1685
		500mg/Kgbwt					
		SAD	.28382*	.02385	.000	.2102	.3575
		5mg/Kgbwt					
		NC	.47693*	.02385	.000	.4033	.5506
		LDE	.42496*	.02385	.000	.3513	.4986
		250mg/Kgbwt					
	PC	HDE	.57177*	.02385	.000	.4981	.6454
		500mg/Kgbwt					
		SAD	.76074*	.02385	.000	.6871	.8344
AIP(Log		5mg/Kgbwt					
TG/HDLC)		NC	.05197	.02385	.240	-.0217	.1256
		PC	-.42496*	.02385	.000	-.4986	-.3513
	LDE	HDE	.14681*	.02385	.000	.0732	.2205
	250mg/Kgbwt	500mg/Kgbwt					
		SAD	.33578*	.02385	.000	.2621	.4094
		5mg/Kgbwt					
		NC	-.09484*	.02385	.009	-.1685	-.0212
		PC	-.57177*	.02385	.000	-.6454	-.4981
	HDE	LDE	-.14681*	.02385	.000	-.2205	-.0732
	500mg/Kgbwt	250mg/Kgbwt					
		SAD	.18897*	.02385	.000	.1153	.2626
		5mg/Kgbwt					
	SAD	NC	-.28382*	.02385	.000	-.3575	-.2102
	5mg/Kgbwt	PC	-.76074*	.02385	.000	-.8344	-.6871

	LDE 250mg/Kgbwt	-.33578*	.02385	.000	-.4094	-.2621
	HDE 500mg/Kgbwt	-.18897*	.02385	.000	-.2626	-.1153

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

Homogeneous Subsets

Total Cholesterol (mg/dl)

Tukey HSD[a]

LipidProfileAssay	N	Subset for alpha = 0.05		
		1	2	3
SAD 5mg/Kgbwt	4	80.5400		
NC	4	91.0200		
HDE 500mg/Kgbwt	4	92.8150		
LDE 250mg/Kgbwt	4		110.7775	
PC	4			177.2450
Sig.		.156	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Triglycerides (mg/dl)

Tukey HSD[a]

LipidProfileAssay	N	Subset for alpha = 0.05			
		1	2	3	4
SAD 5mg/Kgbwt	4	45.6425			
HDE 500mg/Kgbwt	4		61.5400		
LDE 250mg/Kgbwt	4			74.1025	
NC	4			76.4100	
PC	4				159.7450
Sig.		1.000	1.000	.922	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

VLDL (mg/dl)

Tukey HSD[a]

LipidProfileAssay	N	Subset for alpha = 0.05

		1	2	3	4
SAD 5mg/Kgbwt	4	9.1300			
HDE 500mg/Kgbwt	4		12.3100		
LDE 250mg/Kgbwt	4			14.8200	
NC	4			15.2800	
PC	4				31.9500
Sig.		1.000	1.000	.923	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

HDL (mg/dl)

Tukey HSD[a]

LipidProfileAssay	N	Subset for alpha = 0.05		
		1	2	3
PC	4	35.1850		
LDE 250mg/Kgbwt	4		43.2825	
NC	4		50.2650	
HDE 500mg/Kgbwt	4		50.2650	
SAD 5mg/Kgbwt	4			57.8050
Sig.		1.000	.057	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

LDL (mg/dl)

Tukey HSD[a]

LipidProfileAssay	N	Subset for alpha = 0.05		
		1	2	3
NC	4	18.0950		
SAD 5mg/Kgbwt	4	18.8750	18.8750	
HDE 500mg/Kgbwt	4	22.1800	22.1800	
LDE 250mg/Kgbwt	4		22.7600	
PC	4			36.7725
Sig.		.066	.086	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

CRI-I

Tukey HSD[a]

LipidProfileAssay	N	Subset for alpha = 0.05		
		1	2	3
SAD 5mg/Kgbwt	4	.3300		
NC	4	.3625		
HDE 500mg/Kgbwt	4	.4400	.4400	
LDE 250mg/Kgbwt	4		.5275	
PC	4			1.0525
Sig.		.225	.427	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

CRI-II

Tukey HSD[a]

LipidProfileAssay	N	Subset for alpha = 0.05		
		1	2	3
SAD 5mg/Kgbwt	4	1.3950		
NC	4	1.8150		
HDE 500mg/Kgbwt	4	1.8500		
LDE 250mg/Kgbwt	4		2.5650	
PC	4			5.0800
Sig.		.220	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

AC (TC-HDLC)/HDLC

Tukey HSD[a]

LipidProfileAssay	N	Subset for alpha = 0.05		
		1	2	3
SAD 5mg/Kgbwt	4	.3950		
NC	4	.8150		
HDE 500mg/Kgbwt	4	.8500		
LDE 250mg/Kgbwt	4		1.5650	
PC	4			4.0800
Sig.		.220	1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

AIP(Log TG/HDLC)

Tukey HSD[a]

LipidProfileAssay	N	Subset for alpha = 0.05			
		1	2	3	4
SAD 5mg/Kgbwt	4	-.1020			
HDE 500mg/Kgbwt	4		.0870		
NC	4			.1819	
LDE 250mg/Kgbwt	4			.2338	
PC	4				.6588
Sig.		1.000	1.000	.240	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

APPENDIX IV

Liver Enzymes

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Albumin (g/L)	Between Groups	236.471	4	59.118	13.277	.000
	Within Groups	66.788	15	4.453		
	Total	303.259	19			
Total Protein (g/L)	Between Groups	475.343	4	118.836	11.760	.000
	Within Groups	151.577	15	10.105		
	Total	626.920	19			
Serum Globulin (g/l)	Between Groups	140.445	4	35.111	3.054	.050
	Within Groups	172.468	15	11.498		
	Total	312.913	19			
ALT (U/L)	Between Groups	280.317	4	70.079	11.807	.000
	Within Groups	89.031	15	5.935		
	Total	369.348	19			
AST (U/L)	Between Groups	398.304	4	99.576	5.953	.004
	Within Groups	250.889	15	16.726		
	Total	649.193	19			
ALP (U/L)	Between Groups	51354.474	4	12838.618	8.045	.001
	Within Groups	23937.885	15	1595.859		
	Total	75292.358	19			

	Between Groups	.010	4	.003	6.143	.004
Tbilirubin (mg/dl)	Within Groups	.006	15	.000		
	Total	.017	19			

Post Hoc Tests

Multiple Comparisons

Dependent Variable		(I) LiverFunctionAssay	(J) LiverFunctionAssay	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Albumin (g/L)	Tukey HSD	NC	PC	6.44500*	1.49207	.005	1.8376	11.0524
			LDE 250 mg/Kgbwt	3.87750	1.49207	.121	-.7299	8.4849
			HDE 500 mg/Kgbwt	-.22500	1.49207	1.000	-4.8324	4.3824
			SAD 5mg/Kgbwt	-3.39000	1.49207	.207	-7.9974	1.2174
		PC	NC	-6.44500*	1.49207	.005	-11.0524	-1.8376
			LDE 250 mg/Kgbwt	-2.56750	1.49207	.451	-7.1749	2.0399
			HDE 500 mg/Kgbwt	-6.67000*	1.49207	.004	-11.2774	-2.0626
			SAD 5mg/Kgbwt	-9.83500*	1.49207	.000	-14.4424	-5.2276
		LDE 250 mg/Kgbwt	NC	-3.87750	1.49207	.121	-8.4849	.7299
			PC	2.56750	1.49207	.451	-2.0399	7.1749
			HDE 500 mg/Kgbwt	-4.10250	1.49207	.093	-8.7099	.5049
			SAD 5mg/Kgbwt	-7.26750*	1.49207	.002	-11.8749	-2.6601

		HDE 500 mg/Kgbwt	NC	.22500	1.49207	1.000	-4.3824	4.8324
			PC	6.67000*	1.49207	.004	2.0626	11.2774
			LDE 250 mg/Kgbwt	4.10250	1.49207	.093	-.5049	8.7099
			SAD 5mg/Kgbwt	-3.16500	1.49207	.262	-7.7724	1.4424
		SAD 5mg/Kgbwt	NC	3.39000	1.49207	.207	-1.2174	7.9974
			PC	9.83500*	1.49207	.000	5.2276	14.4424
			LDE 250 mg/Kgbwt	7.26750*	1.49207	.002	2.6601	11.8749
			HDE 500 mg/Kgbwt	3.16500	1.49207	.262	-1.4424	7.7724
			PC	12.71000*	2.24779	.000	5.7690	19.6510
		NC	LDE 250 mg/Kgbwt	2.92250	2.24779	.695	-4.0185	9.8635
			HDE 500 mg/Kgbwt	2.48500	2.24779	.801	-4.4560	9.4260
			SAD 5mg/Kgbwt	-1.02000	2.24779	.990	-7.9610	5.9210
			NC	-12.71000*	2.24779	.000	-19.6510	-5.7690
			LDE 250 mg/Kgbwt	-9.78750*	2.24779	.004	-16.7285	-2.8465
Total Protein (g/L)	Tukey HSD	PC	HDE 500 mg/Kgbwt	-10.22500*	2.24779	.003	-17.1660	-3.2840
			SAD 5mg/Kgbwt	-13.73000*	2.24779	.000	-20.6710	-6.7890
			NC	-2.92250	2.24779	.695	-9.8635	4.0185
			PC	9.78750*	2.24779	.004	2.8465	16.7285
		LDE 250 mg/Kgbwt	HDE 500 mg/Kgbwt	-.43750	2.24779	1.000	-7.3785	6.5035
			SAD 5mg/Kgbwt	-3.94250	2.24779	.433	-10.8835	2.9985

		HDE 500 mg/Kgbwt	NC	-2.48500	2.24779	.801	-9.4260	4.4560
			PC	10.22500*	2.24779	.003	3.2840	17.1660
			LDE 250 mg/Kgbwt	.43750	2.24779	1.000	-6.5035	7.3785
			SAD 5mg/Kgbwt	-3.50500	2.24779	.543	-10.4460	3.4360
		SAD 5mg/Kgbwt	NC	1.02000	2.24779	.990	-5.9210	7.9610
			PC	13.73000*	2.24779	.000	6.7890	20.6710
			LDE 250 mg/Kgbwt	3.94250	2.24779	.433	-2.9985	10.8835
			HDE 500 mg/Kgbwt	3.50500	2.24779	.543	-3.4360	10.4460
		NC	PC	7.02250	2.39769	.067	-.3814	14.4264
			LDE 250 mg/Kgbwt	-.19250	2.39769	1.000	-7.5964	7.2114
			HDE 500 mg/Kgbwt	3.46750	2.39769	.610	-3.9364	10.8714
			SAD 5mg/Kgbwt	3.12750	2.39769	.693	-4.2764	10.5314
		PC	NC	-7.02250	2.39769	.067	-14.4264	.3814
			LDE 250 mg/Kgbwt	-7.21500	2.39769	.058	-14.6189	.1889
Serum Globulin (g/l)	Tukey HSD		HDE 500 mg/Kgbwt	-3.55500	2.39769	.588	-10.9589	3.8489
			SAD 5mg/Kgbwt	-3.89500	2.39769	.505	-11.2989	3.5089
		LDE 250 mg/Kgbwt	NC	.19250	2.39769	1.000	-7.2114	7.5964
			PC	7.21500	2.39769	.058	-.1889	14.6189
			HDE 500 mg/Kgbwt	3.66000	2.39769	.562	-3.7439	11.0639
			SAD 5mg/Kgbwt	3.32000	2.39769	.646	-4.0839	10.7239

			NC	-3.46750	2.39769	.610	-10.8714	3.9364
			PC	3.55500	2.39769	.588	-3.8489	10.9589
		HDE 500 mg/Kgbwt	LDE 250 mg/Kgbwt	-3.66000	2.39769	.562	-11.0639	3.7439
			SAD 5mg/Kgbwt	-.34000	2.39769	1.000	-7.7439	7.0639
			NC	-3.12750	2.39769	.693	-10.5314	4.2764
			PC	3.89500	2.39769	.505	-3.5089	11.2989
		SAD 5mg/Kgbwt	LDE 250 mg/Kgbwt	-3.32000	2.39769	.646	-10.7239	4.0839
			HDE 500 mg/Kgbwt	.34000	2.39769	1.000	-7.0639	7.7439
			PC	-10.52000*	1.72270	.000	-15.8396	-5.2004
			LDE 250 mg/Kgbwt	-9.80000*	1.72270	.000	-15.1196	-4.4804
		NC	HDE 500 mg/Kgbwt	-6.52000*	1.72270	.013	-11.8396	-1.2004
			SAD 5mg/Kgbwt	-5.61750*	1.72270	.036	-10.9371	-.2979
			NC	10.52000*	1.72270	.000	5.2004	15.8396
			LDE 250 mg/Kgbwt	.72000	1.72270	.993	-4.5996	6.0396
ALT (U/L)	Tukey HSD	PC	HDE 500 mg/Kgbwt	4.00000	1.72270	.192	-1.3196	9.3196
			SAD 5mg/Kgbwt	4.90250	1.72270	.078	-.4171	10.2221
			NC	9.80000*	1.72270	.000	4.4804	15.1196
			PC	-.72000	1.72270	.993	-6.0396	4.5996
		LDE 250 mg/Kgbwt	HDE 500 mg/Kgbwt	3.28000	1.72270	.357	-2.0396	8.5996
			SAD 5mg/Kgbwt	4.18250	1.72270	.161	-1.1371	9.5021

			NC	6.52000*	1.72270	.013	1.2004	11.8396
			PC	-4.00000	1.72270	.192	-9.3196	1.3196
		HDE 500 mg/Kgbwt	LDE 250 mg/Kgbwt	-3.28000	1.72270	.357	-8.5996	2.0396
			SAD 5mg/Kgbwt	.90250	1.72270	.983	-4.4171	6.2221
			NC	5.61750*	1.72270	.036	.2979	10.9371
			PC	-4.90250	1.72270	.078	-10.2221	.4171
		SAD 5mg/Kgbwt	LDE 250 mg/Kgbwt	-4.18250	1.72270	.161	-9.5021	1.1371
			HDE 500 mg/Kgbwt	-.90250	1.72270	.983	-6.2221	4.4171
			PC	-11.11500*	2.89188	.012	-20.0449	-2.1851
			LDE 250 mg/Kgbwt	-11.60250*	2.89188	.009	-20.5324	-2.6726
		NC	HDE 500 mg/Kgbwt	-10.43250*	2.89188	.019	-19.3624	-1.5026
			SAD 5mg/Kgbwt	-5.26500	2.89188	.398	-14.1949	3.6649
			NC	11.11500*	2.89188	.012	2.1851	20.0449
			LDE 250 mg/Kgbwt	-.48750	2.89188	1.000	-9.4174	8.4424
AST (U/L)	Tukey HSD	PC	HDE 500 mg/Kgbwt	.68250	2.89188	.999	-8.2474	9.6124
			SAD 5mg/Kgbwt	5.85000	2.89188	.302	-3.0799	14.7799
			NC	11.60250*	2.89188	.009	2.6726	20.5324
			PC	.48750	2.89188	1.000	-8.4424	9.4174
		LDE 250 mg/Kgbwt	HDE 500 mg/Kgbwt	1.17000	2.89188	.994	-7.7599	10.0999
			SAD 5mg/Kgbwt	6.33750	2.89188	.235	-2.5924	15.2674

			NC	10.43250*	2.89188	.019	1.5026	19.3624
			PC	-.68250	2.89188	.999	-9.6124	8.2474
		HDE 500 mg/Kgbwt	LDE 250 mg/Kgbwt	-1.17000	2.89188	.994	-10.0999	7.7599
			SAD 5mg/Kgbwt	5.16750	2.89188	.416	-3.7624	14.0974
			NC	5.26500	2.89188	.398	-3.6649	14.1949
			PC	-5.85000	2.89188	.302	-14.7799	3.0799
		SAD 5mg/Kgbwt	LDE 250 mg/Kgbwt	-6.33750	2.89188	.235	-15.2674	2.5924
			HDE 500 mg/Kgbwt	-5.16750	2.89188	.416	-14.0974	3.7624
			PC	-143.52000*	28.24765	.001	-230.7466	-56.2934
			LDE 250 mg/Kgbwt	-98.90000*	28.24765	.023	-186.1266	-11.6734
		NC	HDE 500 mg/Kgbwt	-46.92000	28.24765	.485	-134.1466	40.3066
			SAD 5mg/Kgbwt	-33.81000	28.24765	.753	-121.0366	53.4166
			NC	143.52000*	28.24765	.001	56.2934	230.7466
			LDE 250 mg/Kgbwt	44.62000	28.24765	.531	-42.6066	131.8466
ALP (U/L)	Tukey HSD	PC	HDE 500 mg/Kgbwt	96.60000*	28.24765	.027	9.3734	183.8266
			SAD 5mg/Kgbwt	109.71000*	28.24765	.011	22.4834	196.9366
			NC	98.90000*	28.24765	.023	11.6734	186.1266
			PC	-44.62000	28.24765	.531	-131.8466	42.6066
		LDE 250 mg/Kgbwt	HDE 500 mg/Kgbwt	51.98000	28.24765	.388	-35.2466	139.2066
			SAD 5mg/Kgbwt	65.09000	28.24765	.197	-22.1366	152.3166

		HDE 500 mg/Kgbwt	NC	46.92000	28.24765	.485	-40.3066	134.1466
			PC	-96.60000*	28.24765	.027	-183.8266	-9.3734
			LDE 250 mg/Kgbwt	-51.98000	28.24765	.388	-139.2066	35.2466
			SAD 5mg/Kgbwt	13.11000	28.24765	.989	-74.1166	100.3366
		SAD 5mg/Kgbwt	NC	33.81000	28.24765	.753	-53.4166	121.0366
			PC	-109.71000*	28.24765	.011	-196.9366	-22.4834
			LDE 250 mg/Kgbwt	-65.09000	28.24765	.197	-152.3166	22.1366
			HDE 500 mg/Kgbwt	-13.11000	28.24765	.989	-100.3366	74.1166
		NC	PC	-.07000*	.01449	.002	-.1147	-.0253
			LDE 250 mg/Kgbwt	-.04500*	.01449	.048	-.0897	-.0003
			HDE 500 mg/Kgbwt	-.04000	.01449	.091	-.0847	.0047
			SAD 5mg/Kgbwt	-.03000	.01449	.282	-.0747	.0147
		PC	NC	.07000*	.01449	.002	.0253	.1147
			LDE 250 mg/Kgbwt	.02500	.01449	.449	-.0197	.0697
			HDE 500 mg/Kgbwt	.03000	.01449	.282	-.0147	.0747
Tbilirubin (mg/dl)	Tukey HSD		SAD 5mg/Kgbwt	.04000	.01449	.091	-.0047	.0847
			NC	.04500*	.01449	.048	.0003	.0897
		LDE 250 mg/Kgbwt	PC	-.02500	.01449	.449	-.0697	.0197
			HDE 500 mg/Kgbwt	.00500	.01449	.997	-.0397	.0497
			SAD 5mg/Kgbwt	.01500	.01449	.835	-.0297	.0597
		HDE 500 mg/Kgbwt	NC	.04000	.01449	.091	-.0047	.0847
			PC	-.03000	.01449	.282	-.0747	.0147
			LDE 250 mg/Kgbwt	-.00500	.01449	.997	-.0497	.0397
			SAD 5mg/Kgbwt	.01000	.01449	.956	-.0347	.0547

		NC	.03000	.01449	.282	-.0147	.0747
		PC	-.04000	.01449	.091	-.0847	.0047
	SAD 5mg/Kgbwt	LDE 250 mg/Kgbwt	-.01500	.01449	.835	-.0597	.0297
		HDE 500 mg/Kgbwt	-.01000	.01449	.956	-.0547	.0347

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

Homogeneous Subsets

Albumin (g/L)

	LiverFunctionAssay	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	PC	4	23.6600		
	LDE 250 mg/Kgbwt	4	26.2275	26.2275	
	NC	4		30.1050	30.1050
	HDE 500 mg/Kgbwt	4		30.3300	30.3300
	SAD 5mg/Kgbwt	4			33.4950
	Sig.		.451	.093	.207
Duncan[a]	PC	4	23.6600		
	LDE 250 mg/Kgbwt	4	26.2275		
	NC	4		30.1050	
	HDE 500 mg/Kgbwt	4		30.3300	30.3300
	SAD 5mg/Kgbwt	4			33.4950
	Sig.		.106	.882	.051

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Total Protein (g/L)

	LiverFunctionAssay	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	PC	4	54.0475	
	LDE 250 mg/Kgbwt	4		63.8350
	HDE 500 mg/Kgbwt	4		64.2725
	NC	4		66.7575
	SAD 5mg/Kgbwt	4		67.7775
	Sig.		1.000	.433
Duncan[a]	PC	4	54.0475	
	LDE 250 mg/Kgbwt	4		63.8350
	HDE 500 mg/Kgbwt	4		64.2725
	NC	4		66.7575
	SAD 5mg/Kgbwt	4		67.7775
	Sig.		1.000	.126

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Serum Globulin (g/l)

	LiverFunctionAssay	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	PC	4	30.3875	
	HDE 500 mg/Kgbwt	4	33.9425	
	SAD 5mg/Kgbwt	4	34.2825	
	NC	4	37.4100	
	LDE 250 mg/Kgbwt	4	37.6025	
	Sig.		.058	
Duncan[a]	PC	4	30.3875	
	HDE 500 mg/Kgbwt	4	33.9425	33.9425

	SAD 5mg/Kgbwt	4	34.2825	34.2825
	NC	4		37.4100
	LDE 250 mg/Kgbwt	4		37.6025
	Sig.		.143	.179

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

ALT (U/L)

	LiverFunctionAssay	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey HSD[a]	NC	4	22.3550			
	SAD 5mg/Kgbwt	4		27.9725		
	HDE 500 mg/Kgbwt	4		28.8750		
	LDE 250 mg/Kgbwt	4		32.1550		
	PC	4		32.8750		
	Sig.		1.000	.078		
Duncan[a]	NC	4	22.3550			
	SAD 5mg/Kgbwt	4		27.9725		
	HDE 500 mg/Kgbwt	4		28.8750	28.8750	
	LDE 250 mg/Kgbwt	4			32.1550	32.1550
	PC	4				32.8750
	Sig.		1.000	.608	.076	.682

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

AST (U/L)

	LiverFunctionAssay	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	NC	4	34.3100	
	SAD 5mg/Kgbwt	4	39.5750	39.5750
	HDE 500 mg/Kgbwt	4		44.7425
	PC	4		45.4250
	LDE 250 mg/Kgbwt	4		45.9125
	Sig.		.398	.235
Duncan[a]	NC	4	34.3100	

	SAD 5mg/Kgbwt	4	39.5750	39.5750
	HDE 500 mg/Kgbwt	4		44.7425
	PC	4		45.4250
	LDE 250 mg/Kgbwt	4		45.9125
	Sig.		.089	.060

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

ALP (U/L)

	LiverFunctionAssay	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	NC	4	207.6900		
	SAD 5mg/Kgbwt	4	241.5000	241.5000	
	HDE 500 mg/Kgbwt	4	254.6100	254.6100	
	LDE 250 mg/Kgbwt	4		306.5900	306.5900
	PC	4			351.2100
	Sig.		.485	.197	.531
Duncan[a]	NC	4	207.6900		
	SAD 5mg/Kgbwt	4	241.5000		
	HDE 500 mg/Kgbwt	4	254.6100	254.6100	
	LDE 250 mg/Kgbwt	4		306.5900	306.5900
	PC	4			351.2100
	Sig.		.135	.086	.135

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

Tbilirubin (mg/dl)

	LiverFunctionAssay	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	NC	4	.2700		
	SAD 5mg/Kgbwt	4	.3000	.3000	
	HDE 500 mg/Kgbwt	4	.3100	.3100	
	LDE 250 mg/Kgbwt	4		.3150	

Duncan[a]	PC	4		.3400	
	Sig.		.091	.091	
	NC	4	.2700		
	SAD 5mg/Kgbwt	4	.3000	.3000	
	HDE 500 mg/Kgbwt	4		.3100	.3100
	LDE 250 mg/Kgbwt	4		.3150	.3150
	PC	4			.3400
	Sig.		.056	.342	.067

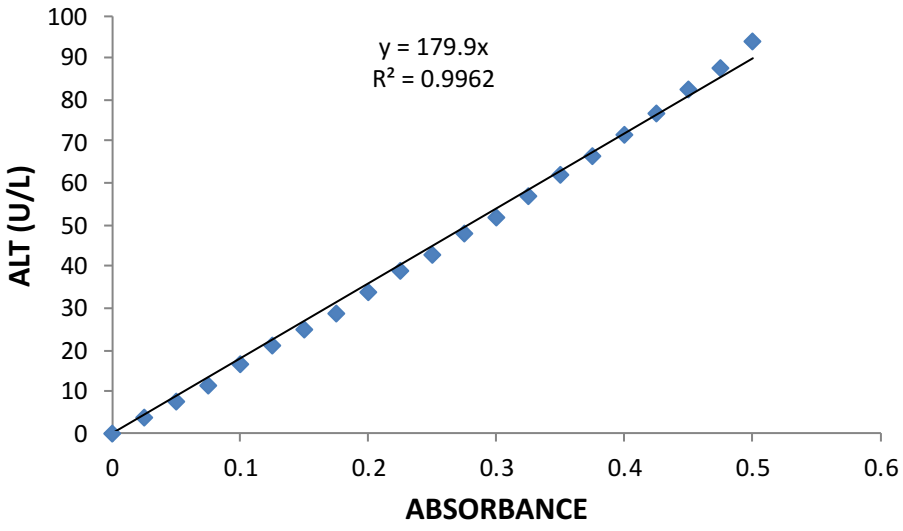
Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 4.000.

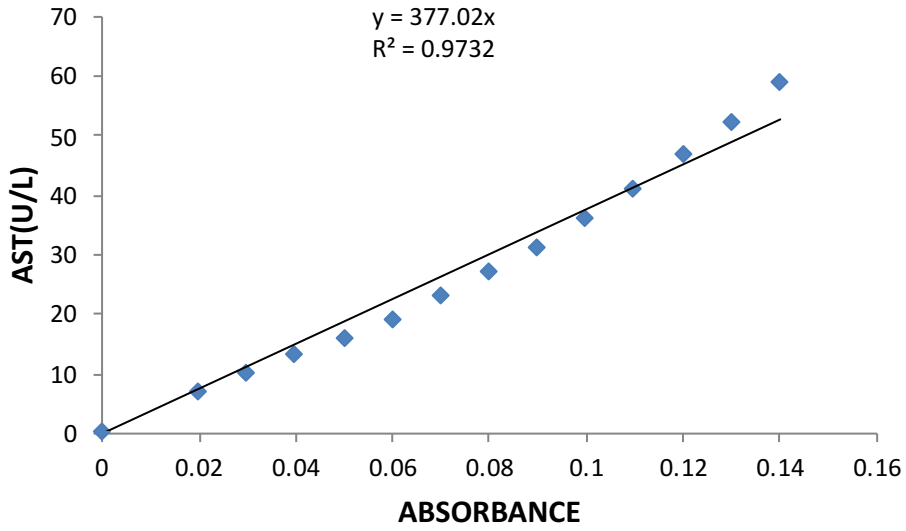
b.

APPENDIX V

Calibration curve for Alanine aminotransferase (ALT)



Calibration curve for Aspartate aminotransferase (AST)



APPENDIX VI

Liver function tests

Data for Serum Albumin, Serum Protein and Serum Globulin levels, Alanine transaminase, Aspartate transaminase, Total Bilirubin, Serum Lactate dehydrogenase and Heart Lactate dehydrogenase

	Groups	Serum Albumin (g/L)	Serum Protein (g/l)	Serum Globulin (g/l)	ALT (U/L)	AST (U/L)	ALP (U/L)	T. Bilirubin (mg/dl)	Serum LDH (U/L)	Heart LDH (U/L)
	Gp1-1	28.47	66.32	37.85	24.65	39.38	181.24	0.29	15.29	84.12
NC	Gp1-2	33.83	65.73	35.12	18.71	35.48	250.24	0.26	15.29	96.86
	Gp1-3	29.36	70.12	40.58	23.57	27.68	145.36	0.27	10.20	91.76
	Gp1-4	28.76	64.86	36.09	22.49	34.70	253.92	0.26	12.75	86.67
	Gp2-1	24.14	51.71	27.57	32.38	49.13	333.96	0.32	20.39	79.02
PC	Gp2-2	23.40	52.00	28.60	31.66	47.57	390.08	0.31	22.94	79.02
	Gp2-3	23.55	57.26	33.71	34.72	46.01	357.88	0.37	25.49	63.73
	Gp2-4	23.55	55.22	31.67	32.74	38.99	322.92	0.36	22.94	71.37
	Gp3-1	25.63	62.52	36.88	34.90	46.79	262.20	0.31	17.84	84.12
LDE	Gp3-2	24.14	66.32	42.17	30.76	48.74	299.92	0.32	18.84	71.37
	Gp3-3	29.06	63.69	34.63	30.58	44.06	364.32	0.33	17.29	63.73

	Gp3-4	26.08	62.81	36.73	32.38	44.06	299.92	0.30	19.94	71.37
	Gp4-1	29.51	66.61	37.10	28.78	46.40	247.48	0.29	19.94	94.31
HDE	Gp4-2	29.66	59.89	30.23	25.55	44.45	277.84	0.31	15.75	94.31
	Gp4-3	29.21	66.03	36.81	34.18	39.38	227.24	0.35	17.84	76.47
	Gp4-4	32.94	64.56	31.63	26.99	48.74	265.88	0.29	17.84	84.12
	Gp5-1	33.53	65.73	32.20	26.09	42.89	200.56	0.29	12.20	107.06
SAD	Gp5-2	29.36	64.86	35.50	27.34	43.67	303.60	0.30	12.75	91.76
	Gp5-3	35.32	75.37	40.05	28.24	36.65	239.20	0.31	15.84	96.86
	Gp5-4	35.77	65.15	29.38	30.22	35.09	222.64	0.30	12.75	94.31

APPENDIX VII

Oxidative stress markers

Data for Superoxide dismutase, Glutathione peroxidase, Glutathione S-transferase and Catalase activities in both heart and serum

	Groups	Heart SOD	Serum SOD	Heart GPx	Serum GPx	Heart GST	Serum GST	Heart CAT	Serum CAT
	Gp1-1	25.40	27.96	4.05	3.89	0.76	0.80	5.77	14.68
NC	Gp1-2	25.07	29.28	3.89	3.42	0.81	0.91	5.97	16.54
	Gp1-3	28.70	27.26	3.89	3.27	0.81	0.76	6.54	16.41
	Gp1-4	25.98	29.44	3.58	3.58	0.88	0.72	6.05	16.47
	Gp2-1	22.84	21.22	2.49	1.25	1.68	1.21	5.19	12.84
PC	Gp2-2	14.84	21.65	2.33	1.40	1.35	0.90	4.91	13.01
	Gp2-3	18.89	19.19	2.33	1.56	1.74	0.93	5.38	11.37
	Gp2-4	20.92	19.99	2.02	1.87	1.88	1.08	4.64	11.56
	Gp3-1	25.84	26.69	3.58	2.96	1.22	0.75	5.04	14.26

LDE	Gp3-2	29.79	25.13	3.58	2.33	1.24	0.71	5.71	13.25
	Gp3-3	30.92	24.91	3.42	2.80	1.71	0.74	6.30	14.49
	Gp3-4	32.27	26.21	3.58	2.49	1.53	0.95	6.29	14.49
	Gp4-1	24.88	27.79	2.80	2.96	0.85	0.71	6.35	15.47
HDE	Gp4-2	25.12	31.21	3.42	2.49	0.91	0.73	6.45	16.99
	Gp4-3	30.41	26.89	3.42	2.33	1.45	0.71	7.60	14.46
	Gp4-4	26.39	28.46	3.42	2.33	1.06	0.97	6.95	14.78
	Gp5-1	22.26	24.69	2.96	2.18	1.02	0.72	6.81	16.25
SAD	Gp5-2	23.30	26.37	2.65	1.71	0.96	0.77	6.54	16.96
	Gp5-3	26.04	22.89	3.11	2.02	1.40	0.79	7.45	15.51
	Gp5-4	26.88	25.84	2.80	2.33	1.26	1.06	7.92	16.69

Data for Lipid Peroxidation expressed as Malondialdehyde (MDA) activity

	Groups	Heart MDA	Serum MDA
	Gp1-1	0.24	0.17
NC	Gp1-2	0.25	0.15
	Gp1-3	0.27	0.15
	Gp1-4	0.24	0.15
	Gp2-1	0.70	0.39
PC	Gp2-2	0.57	0.39
	Gp2-3	0.69	0.35
	Gp2-4	0.62	0.38
	Gp3-1	0.32	0.25

LDE	Gp3-2	0.40	0.23
	Gp3-3	0.37	0.21
	Gp3-4	0.38	0.21
	Gp4-1	0.30	0.24
HDE	Gp4-2	0.30	0.25
	Gp4-3	0.35	0.22
	Gp4-4	0.31	0.22
	Gp5-1	0.26	0.23
SAD	Gp5-2	0.26	0.18
	Gp5-3	0.30	0.19
	Gp5-4	0.31	0.21

APPENDIX VIII

Lipid profiles

Data for Total cholesterol, Triglyceride, High density lipoprotein cholesterol, Low density lipoprotein cholesterol and Very low density lipoprotein cholesterol

	Groups	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
	Gp1-1	101.796	77.948	15.590	48.032	19.455
NC	Gp1-2	85.030	80	16	51.383	14.008
	Gp1-3	85.030	72.821	14.564	49.149	20.233
	Gp1-4	92.216	74.872	14.974	52.5	18.677
	Gp2-1	174.85	165.128	33.026	31.277	35.020
PC	Gp2-2	180.838	161.026	32.205	35.745	36.580

	Gp2-3	178.443	158.974	31.795	33.511	38.911
	Gp2-4	174.85	153.846	30.769	40.213	36.576
	Gp3-1	114.97	70.769	14.154	43.564	24.125
LDE	Gp3-2	102.994	71.795	14.359	40.213	24.125
	Gp3-3	110.18	75.897	15.179	46.915	21.012
	Gp3-4	114.97	77.949	15.590	42.447	21.790
	Gp4-1	83.832	69.743	13.949	53.617	21.012
HDE	Gp4-2	86.228	57.436	11.487	46.915	19.455
	Gp4-3	105.389	57.436	11.487	49.149	24.903
	Gp4-4	95.808	61.539	12.308	51.383	23.346
	Gp5-1	91.018	48.205	9.641	62.553	19.455
SAD	Gp5-2	75.449	45.128	9.026	53.617	18.677
	Gp5-3	79.042	44.102	8.821	54.734	19.455
	Gp5-4	76.647	45.128	9.026	60.319	17.899

Data for Atherogenic ratios and indices (Castelli's risk index I (CRI-I), Castelli's risk index II (CRI-II), Atherogenic Coefficient (AC) and Atherogenic index of plasma (AIP)

	Groups	**CRI-I**	**CRI-II**	**AC**	**AIP**
	Gp1-1	0.405	2.119	1.119340864	0.2102
NC	Gp1-2	0.273	1.655	0.654826211	0.1922

	Gp1-3	0.412	1.730	0.730045584	0.171
	Gp1-4	0.356	1.756	0.756487619	0.154
	Gp2-1	1.120	5.590	4.590442177	0.723
PC	Gp2-2	1.023	5.059	4.059158333	0.654
	Gp2-3	1.161	5.325	4.32497	0.676
	Gp2-4	0.910	4.348	3.348121693	0.582
	Gp3-1	0.554	2.639	1.639115995	0.211
LDE	Gp3-2	0.5999	2.561	1.561226455	0.252
	Gp3-3	0.448	2.3485	1.348507937	0.209
	Gp3-4	0.513	2.709	1.708566416	0.264
	Gp4-1	0.392	1.5635	0.563538929	0.114
HDE	Gp4-2	0.415	1.8379	0.837955782	0.088
	Gp4-3	0.507	2.1442	1.144278355	0.068
	Gp4-4	0.454	1.8645	0.86459412	0.078
	Gp5-1	0.311	1.4550	0.45504966	-0.113
SAD	Gp5-2	0.348	1.4072	0.407185595	-0.074
	Gp5-3	0.355	1.4441	0.444108571	-0.094
	Gp5-4	0.297	1.2707	0.270686032	-0.126

APPENDIX IX

Weekly weight of rats in all experimental groups

Rat weight (g)

Groups	1st week	2nd week	3rd week	4th week	5th week	6th week

NC	90.23 ± 5.50	120.43 ± 4.06	138 ± 16.67	159.23 ± 15.97	171.25 ± 9.08	171.95 ± 20.98
PC	92.28 ± 7.79	122.6 ± 7.50	141.50 ± 5.43	170.9 ± 7.0	178.6 ± 28.6	185.6 ± 22.93
LDE	42.48 ± 2.86	89.21 ± 8.74	110.48 ± 9.55	116.35 ± 9.58	138.25 ± 12.67	151.16 ± 7.11
HDE	50.77 ± 3.36	73.0 ± 10.58	96.17 ± 17.51	114.58 ± 15.77	128.48 ± 12.55	156.16 ± 17.0
SAD	81.02 ± 8.67	118.13 ± 7.80	134.53 ± 8.24	148.18 ± 6.44	159.6 ± 12.12	175.51 ± 16.67