PERI CARDIAL ADIPOSE TISSUE OF ALBINO RATS FED LARD PALM KERNEL CAKE AND TREATED WITH HERBAL DECOCTION

BY

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CERTIFICATION

We certify that this project was carried out by **Momah Wendy Chizoba** with Matriculation Number: **BMS1501911** of the Department of Medical Laboratory Science, School of Basic Medical Science, University, Benin City, Edo State, Nigeria and has not been presented in part or full in any diploma or degree awarding institution.

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DEDICATION

This work is dedicated to God Almighty, may I never forget the good things he does for me and his faithfulness towards me and to my parent Barr & Mrs. N.D.F Momah Esq

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ABSTRACT

Pericardial adipose tissues are combined fats from pericardial sac and surrounding external surfaces of the pericardium. Excess deposits often lead to cardiovascular disorders and other heart related diseases. There are claims that an herbal decoction called Aju Mbaise (a combination of medicinal plants wrapped as a combo pack) can be used to treat heart related diseases including obesity which affects many people world-wide. Despite the existing information on Aju Mbaise and its healing claims, there is paucity of scientific data on the effects on pericardial adipose tissues. Therefore, this study was to examine histopathology changes in pericardial adipose tissues of lard-palm kernel cake (L-PKC) diet-fed rats treated with Aju Mbaise herbal decoction. The specific objectives were to investigate the effects of the present herbal decoction on body weight, adiposity indices and lipid profile of experimental rats. Samples of fresh L-PKC were obtained from Uselu market, Benin City while Aju Mbaise was purchased online. Each component was identified and authenticated by an expert taxonomist in the University while voucher numbers were issued for each constituent. Sixteen (16) Sprague-Dawley rats of both sexes, weighing (149-175g) were obtained from a research animal farm in Benin City. Animals acclimatized for 2weeks in Anatomy department University of Benin with ambient temperature (26±3°C), humidity (50% - 60%) and photoperiodicity (12:12hr). They were kept in clean steel gauzed cages and coconut husks used as beddings in a light and humid environment. Rats were fed on standard pellets and water provided adequately. Ethical

approval (V.1034/40) was obtained from Ministry of Agriculture and Natural Resources while rats were used in compliance with laydown policies outlined in the Guide for Care and Use of Laboratory Animals. L-PKC diet was prepared consisting of 90% super feed, 8% pig fat, and 2% PKC mixed appropriately to make up 100%. Herbal decoction was prepared by placing 296g wrap of it in a clean pot while one (1L) litre of water was added and heated for 30minutes with a gas cooker according to producer's recommendation. After cooking, it was cooled and filtered with a white sterile muslin cloth. The dark-brown decoction (filtrate) was refrigerated to avoid decay at 25°C. LD_{50} was conducted to ascertain the lethal dose that will serve as a guide in administering the required dosage. Experimental rats were divided into four (4) groups irrespective of sex, age and weight (n=4). Group A were untreated but received standard rats diet (SRD) and water. Group B was fed with 10% of L-PKC fat diet mixed with 90% SRD and water. Group C was fed 10% L-PKC mixed with 90% SRD and water, and administered 2.5mL/kg body weight of herbal decoction while Group D was fed same way like Group C but treated with 5mL/kg of herbal decoction. Each rat was picked with a hand towel and treated orally for twenty eight (28) days at 2day intervals with a sterile syringe. Average weekly body weights of each rat were recorded with a digital electronic balance. In the end, all animals fasted overnight and were anaesthetized with chloroform. Rats were dissected using scalpel blade while pericardial adipose tissues from the epicardial and paracardial were excised including the myocardium and blotted individually with filter paper prior to grossing while relative tissue weights were recorded. Blood sample was collected from the cardiac region from each animal without anticoagulant with tab gel and centrifuged (BROADBENT, UK) at 3000 rpm for 5 min to obtain the serum content. Sera were stored at -20°C until it was needed for fasting lipid profile using the chemical analyzer. Grossed samples were fixed in 10% neutral buffered formol saline and processed histologically (dehydrated with alcohol, cleared in xylene and impregnated with molten paraffin wax and embedded with the automatic embedding machine. Tissues were de-blocked and sectioned with a microtome at 3-5µ. Sections were stained using H&E method and viewed with the microscope at x10 and x40 magnifications. Data were statistically analyzed using ANOVA and Tukey's post-hoc test for multiple comparisons while $p<0.05$ was considered significant. The effect of the graded treatment (2.5mL/kg and 5mL/kg AJMD) on rats revealed a progressive increase in Group (C and D). All rats experienced increases in weights compared with Day 0 and there was no significant difference in weights within the study period. Total pericardial fat weight was increased in all groups but higher in group B (Lard-PKC). The same condition was seen in total pericardial index with no significant differences ($p<0.05$) in all pericardial weights. Organ weights in L-PKC (Group B) were higher when compared with those in Group A. Those in Group C (L-PKC + 2.5mL/kg AJMD) decreased compared with Group B while Group D (L-PKC +5mL/kg AJMD) increased with no significant difference in the groups. Lipid profile analysis showed that there is a significant decrease in mean TC (mg/dL) $p<0.001$ between Group B and A, and between Group C and B. There was no significant difference in HDL and LDL while VLDL showed a significant difference ($p<0.000$) between Group A and D and between Group B and C. Pericardial adiposity from Group A showed that adipocytes remained intact without distortions in histological architecture. Adipocytes sizes in Group B though appeared normal but bigger in sizes compared with control animals. Pericardial adiposity in Group C and D were normal but slightly reduced in Group D

compared with other groups. Myocardium (Group A) showed normal histological architecture evident with an intact branching muscle fibre. Group B showed evidence of ballooning of branching muscle fibres and nucleus. Group C and D were normal with an intact fibre striation and nucleus. Therefore, Aju Mbaise decoction maintained histological integrity of the myocardium, lipid profile, including reduction in adipose tissue weights and adipocyte sizes of rats in this study. Further investigation from molecular perspectives should be considered to support the current claims.

CHAPTER ONE
INTRODUCTION

1.1 Background of the Study

Pericardial adipose tissues refers to the combination of fat enclosed by the pericardial sac and fat surrounding the external surface of the pericardium which is collectively referred to as pericardial adipose tissues (Britton and Fox, 2011). Adipose tissues composed of adipocytes, which are fat storage cells for triacylglycerol according to Ayala *et al.* (2014). Recently, it's not only considered as a storage organ but seen as tissues with dynamic metabolic ability

because they play an endocrine role capable of synthesizing a number of biological active compounds that regulate metabolic homeostasis (Marisa, 2014). Adipocytes ar e not the only component of adipose tissues, other types called stroma-vascular fraction comprising of blood cells, endothelial cells, pericytes and adipose precursor cells among others exist. Adipose tissues are not uniform and adipocytes are of two types, namely; white adipocytes which are fat-storing cells that have special compartment for such work and brown adipocytes which function to convert chemical energy to heat (Rana, 2015; Zhu *et al.*, 2015). The increasing cases of obesity in some countries like United State and Nigeria have conferred significant risks to human leading to emergence of different chronic diseases and conditions including but not limited to cancer, diabetes and heart diseases (Rana, 2015). Obesity is caused when there is a high intake of fat diet within the range of 30-78% of total energy taken. This high fat condition is created either by adding a particular fat to animal diet or using an assortment of fat and sugar-rich supermarket foods to enrich the diet of interest (Kayihan, 2014). Note that there is a direct relationship between fat intakes and its action on adipose tissues which has been long established (Zhu *et al.*, 2015).

1.2 Statement of the problem

In Nigeria folklore medicine, there are claims that a cocktail herbal mixture commonly known as Aju Mbaise can be used to treat different forms of diseases including obesity. Like other plants, Aju Mbaise is one of the herbal mixtures in use by 80% of the world population that lives particularly in the developing countries as a means to meet their primary health care needs (Ezejindu *et al.,* 2017). Adekule and Adekule, (2009) documented the use of herbal cocktail

mixture for the treatment of some infectious diseases in the respiratory system, urinary tract, gastrointestinal tract, biliary system and even weight management. The secondary plant metabolites in the herbal mixture include alkaloids, flavonoids, terpenoids, saponins, polysaccharides, wax and fatty acids, even simple phenolic and tannins which are thought to be responsible for the actions exerted on aliments to restore normalcy (Kumar *et al.,* 2005). In addition, previous studies have documented its laxative and lipolytic effects (Nnadiukwu *et al*, 2021). Other studies also reported the polyherbal formulations possess, various pharmacological activities including antimalarial, hepatoprotective, wound healing anti-diarrhea, antioxidant and anti-inflammatory effects of this mixture (Nnadiukwu *et al,* 2019). On the other hand, Nnadiukwu *et al.*, (2021) documented the hypolipidemic, analgesic and anti-inflammatory effect of Aju Mbaise herbal mix on rats fed a normal diet. In all of these, since the herbal cocktail (Aju Mbaise) is claimed to have body weight reducing properties, experimental procedures to authenticate this claim is therefore pertinent which is the gap created by existing reports.

1.3 Justification of the study

The importance of nutritional evaluations and its composition become widely recognized on account of dysmetabolic diseases such as obesity, diabetes and cardiovascular complications. Obesity is considered a serious disease affecting a large population of the world, and then excess dietary energy intake from fat has direct relationship with bodyweight gain as well as weight loss due to low intake of fat (Niloofar *et al.,* 2010). Despite the existing information on Aju

Mbaise and its healing claims, there is paucity of scientific data on its effects on pericardial adipose tissues.

1.4 Significance of the study

Obesity and other health complications could be managed with herbal mixture like Aju Mbaise if administered in the right dosage and it is achieved by reduction in cravenness for food, burning of calories fast, possession of diuretic and cholerectic effects as well as laxative effect and increasing lipolysis in the system, which end users of the present herbal cocktail stand to benefit. However, it is believed that at the end of the present research, better understanding of the effects of consuming herbal mixtures indiscriminately without minding the side effects will be established. Data generated will also assist policy makers to make policies in the interest of the populace.

1.5 Aim and Objective

The aim of this study was to examine histopathology changes in pericardial adipose tissues of lard-palm kernel cake (L-PKC) diet-fed rats treated with Aju Mbaise herbal decoction.

The specific objectives were

1. to investigate the effects of the present herbal decoction on body weight.
2. adiposity indices
3. lipid profile of experimental rats.

1.6 Research Questions

- How possible is it to examine histopathologic changes in adipose tissues of lard-palm kernel cake (L-PKC) diet-fed rats treated with Aju Mbaise herbal decoction in the present research?
- Is it possible to determine the effects of the present herbal decoction on body weight, adiposity indices and lipid profile of experimental rats in this study?

1.7 Hypotheses

The null and alternate hypotheses were used in testing the questions arising from this study:

H_0: Examination of histopathologic changes in adipose tissues of lard-palm kernel cake (L-PKC) diet-fed rats treated with Aju Mbaise herbal decoction is achievable in this research.

H_1: Examination of histopathologic changes in adipose tissues of lard-palm kernel cake (L-PKC) diet-fed rats treated with Aju Mbaise herbal decoction is not achievable in this research.

H_0: Effects of Aju Mbaise herbal decoction on body weight, adiposity indices and lipid profile of experimental rats are quite achievable in this research.

H_1: Effects of Aju Mbaise herbal decoction on body weight, adiposity indices and lipid profile of experimental rats are not achievable in this research.

CHAPTER TWO

LITERATURE REVIEWS

2.1 Overview of Aju Mbaise

Aju Mbaise is not a scientific word but rather has its origin from Mbaise of Imo state of Southeastern, Nigeria. It is a mix composed of leaves, roots and trunks of different medicinal plants wrapped together. A wrap of Aju Mbaise has many compositions like, *Cnestis ferruginea, Xylopia aethiopica, Dialium guineense, Uvaria chamae, Palisota hirsuta,* and *Napoleona vogelii* that are called Oko-Aja, Uda,Uziza, Mmiri ohia, Ikpele atutu and Ntum respectively in Igbo language. Others like, *Combretum racemosun, Sphenocentrum jollynum and Heterotis rotundifolia* are also part of the composition of Aju Mbaise. These plants have shown different abilities in fighting microbes as well as reduction of

fat accumulation in the body leading to weight loss most especially in postnatal mothers (Nnadiukwu *et al,* 2019).

From the beginning, plants have been utilized as therapeutic agent in both organized and unorganized forms (Girach *et al.,* 2003). Most prominently, herbal medicine has gained more acceptances in its alternative value as cure to some illness, though this acceptance is not as synthetic antibiotics. From the ancient time, plants have always played important roles is medicine and health, this is because they contain biologically active material and ingredients which have certain effects on the body when taken for both medical and non-medical purposes. World Health Organization (2002) reported that plants that contain one or more substances in its part or organs and even leaves that is capable of having therapeutic effect when taken are said to be called medicinal plants. Such plants with non-nutrient chemical compound or bioactive components called phytochemicals are responsible for protecting the plants against microbial infections or infestation by pests which extends to humans when taken (Nweze *et al.,* 2004). This herbal decoction has many active components that are specific for curing specific ailments when injected or taken orally.

The plants are widely used because of their easy availability and cost effectiveness mostly by rural dwellers (Cordell, 2000). This antimicrobial effect of the decoction maybe due to individual components' contribution of the different plants or plant's part wrapped together as single entity which enables the mixture to be more effective than just a single plant used for the same purpose. Aldehyde and phenolic compounds are main contributors of the antimicrobial activities of this herbal decoction. As the antimicrobial activities

of this herbal concoction is being valued, it is necessary to note that Aju Mbaise has other functions like facilitating weight loss and reduction of fat deposits in the body. The mixture has a bitter alkaline taste with a delicious aroma but the traditional medicinal functions in the body has made it invaluable in the developing countries of the world where 80% of them use traditional plant extracts (medicine) to meet most of their needs in primary health care (WHO,2002). For this reason, it has come to reality that about 3.2 million people around the world use the plants extracts as drugs or fat burning concoction on a regular basis. Based on this, it becomes necessary to know that Aju Mbaise has about four different types of its mixture which includes: fat reduction and weight loss, increase in fertility, treatment of infectious microbes in the body system and management of tumors and other related growth treatment. Of all these therapeutic functions of Aju Mbaise, fat reduction and its relative weight loss is of special interest to this research and how it does that is of great importance. It does this by increasing lack of appetite while burning calories at the expense of its synthesis in the body. While at a continued intake of the concoction, diuretic and choleretic effect of it starts leading to laxation of the gastrointestinal tract. Normally, metabolic lipolysis sets in with a thermogenic effect that promotes increase in body temperature aiding this lipolytic mechanism (Kabir, 2021).

Each part of the plants in the wrap of Aju Mbaise has both nutritional and therapeutic contributions in the decoction against many diseases. The root extract of *S. jollynum* is greatly in use by men as aphrodisiac (Azubuike *et al.,* 2018). The management of mental and inflammatory disorders, pain and

depression by central nervous system (CNS) stimulation was reported too to have been done by *S. jollynum* plant (Bitton *et al.,* 2011). Among numerous therapeutic effect of *S. jollynum* are anti-diabetic (Mbaka *et al.,* 2010), antioxidant (Olorunnisola *et al.,* 2014), anti-inflammatory (Gaya *et al.,*2009), anti-allergic (Hariri *et al.,* 2010), anti-bacterial (Mackey *et al.,* 2013) and anti-malaria (Nia *et al.,* 2004).

Cnestis ferruginea like others also has some of these effects on the body like in vivo hypoglycaemic activity in STZ-induced diabetic rodents. Hypoglycemic and hypocholesterolemic activities of leaves have also been reported (Adisa *et al.,* 2013). This particular therapeutic effect results to loss of fat in the body which invariably leads to weight loss.

Dialium guineense bark is another part of the Aju Mbaise which is used for treatment of both prenatal pains and edema as well as keeping oral hygiene and extendedly to treatment of stomach ache and fever which may be due to some other illness or stress (Nwosu *et al.,* 2000). Even the leaves and stem bark are used for the treatment of diarrhea severe cough, bronchitis, wound, stomach ache, malaria fever, jaundice, anti-ulcer and hemorrhoids (Bero *et al.,* 2009).

Decoction of the fruit or bark of *Xylopia aethiopica* is used in traditional medicine to treat stomach-aches and dysenteric conditions and some respiratory disorder like asthma and bronchitis. It is also researched that the root when powered is useful in dressing of sores, even in treatment of pyorrhea of the gum and cancer locally in Nigeria (Erhirhie *et. al.*, 2014).

Researches have also shown that the pounded root of *Uvaria chamae* is used for the treatment of nose bleeding, some cardiac and pulmonary diseases as well as haematuria (Adams *et al.,* 1999). This plant has high level of antioxidant effect therefore they can be used to treat several diseases in which there is an increase in free radical production. Just like *U. chamae, H. rotundifolia* has antioxidant as well as anti-ulcer and antibacterial potential (Yeboah *et al.*, 2017). This is due to high level of retinol, cholecalciferol, tocopherol, thiamine and low in pyridoxine, niacin, riboflavin, ascorbic acid, phylloqinone and cobalamin in the leaves of the plant. Combined effects of the components make the herbal cocktail to have high levels of therapeutic effects in the body.

Combretum racemosum is traditionally used especially the leaves in Nigeria as antiparasitic, antibacterial and antifungal infections. It is also believed that *C. racemosum* has an effective action against stomach pains, gastric ulcers, dysentery, abdominal disorder and fever. Hutchings *et al.*, (1996) reported that some species of Combretum could be used unscientifically for the treatment of pneumonia, colds, syphilis and mumps The leaves decoction of *N. imperialis* has shown to possess antibacterial and wound healing properties in an experimental rats (Chah *et al.,* 2006). The leaf extracts of *P. hirsuta* possess both anxiolytic and antidepressant effect (Woode *et al.,* 2009). It has been reported according to Boakye *et al.,* (2011) that *P. hirsuta* has anti-inflammatory, antipyretic, and anti-oxidant effect as orthodox medicine.

2.2 Phytochemicals in Aju Mbaise Decoction

Every decoction has bioactive constituents which makes it highly efficacious both in modern and traditional medicine. Medicinal properties of these plants

are dependent on the plant's secondary metabolites which is contained in them and these metabolites are not always found in every plant (Heinrich *et al.,* 2004) Flavonoids, terpenoids, tannin, Hydrogen cyanide, waxes and fatty acids, alkaloids, phenols, saponins and glycosides and their derivative are the main phytochemicals found in most medicinal plants which Aju Mbaise happens to be among and it has higher percentage of them because of its multi plant compositions. These secondary metabolites have both their bioactivity and therapeutic effects with unique characteristic features for identification as well, they are also found to have cholesterol lowering ability (Manta *et al.,* 2013).

2.3 Adipose tissue

Adipose tissue is an organ for storage of triacylglycerol molecules which is released when there is under nutrition or stored during over nutrition. This is done to maintain balance between excess or deficit of lipids in the body. However, adipose tissue is more of a caloric reservoir; it is also a key component in maintaining metabolic homeostasis and is involved in a wide array of biological processes throughout the body (Henrik *et al.,* 2015). Adipose tissue is composed of a loose collection of specialized cells, called adipocytes, embedded in a mesh of collagen fibers. Research over the past two decades has revealed adipose tissue as one of the largest endocrine organs in the body as well as an active tissue for cellular reactions with a key role in energy homeostasis secreting and expressing a wide range of biologically active molecules, which are known as adipokines (Fain *et al.,* 2004). There are three

types of adipose tissue which are white, brown and beige or “brite” (brown in white).This classification is based on differences in their origin, morphology, location, and function (Frühbeck *et al.*, 2009). It is scientifically established that white adipose tissue functions in a wide range that is not limited to but includes immune and inflammatory regulation, glucose and food intake control or metabolism by secreting a great number of adipokines (Catalán *et al.,* 2009). White adipose tissue major role is lipid homeostasis whereby the storage of triglycerides and its release as free fatty acid is well balanced and maintained respectively as when in fed or fasting state (Nnadiukwu *et al,* 2019). Meanwhile brown adipose tissue is the main site of thermogenesis and energy expenditure in mammals (Feng *et al.,* 2013). In recent time, the third type of adipose tissue was discovered which was as a result of activation induced by prolonged cold exposure that led to the “browning” of white adipose. The emergence of brown adipocyte-like cells in white adipose depots led to the formation of beige adipose tissue (Wu *et al.*, 2012). Adipose tissue has adipocytes as the main cells as well as it is responsible for energy homeostasis control and adipokine release. It has also connective tissue formed by other different cellular types including preadipocytes, immune cells, nerve cells, and vascular cells that interplay among themselves contributing to the main functions of each adipose tissue depots (Lynes *et al.,* 2017).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Location and Duration of Study

This study was conducted in the Department of Medical Laboratory Science, Histopathology Sub-Departmental Laboratory, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City. Collection and extraction of plant material, animal acclimatization, determination of $L.D_{50}$, test administration, animal sacrifice, gross examination, fixation, processing, microtomy, staining, microscopic examination of histological sections and photomicrography lasted for 3 months (November 2021 to February 2022).

3.1.1 Collection of Aju Mbaise Combo Pack

Samples of fresh L-PKC were obtained from Uselu market here in Benin City while Aju Mbaise was purchased online from the main producer at Imo State, which was sent via Peace Motors and delivered within 3 days.

3.1.2 Identification and Authentication

Samples of the component of the wrap of Aju Mbaise were removed and sent to an expert taxonomist in the Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin. The wrap consisted of the following: *C. ferruginea, X. aethiopica, D. guineense, U. chamae, P. hirsuta, N. vogelii, C. racemosun, S. jollynum and H. rotundifolia*. They were identified, authenticated and assigned voucher numbers: UBH-$_{C}$346; UBH-$_{X}$347; UBH-$_{D}$348; UBH-$_{U}$349; UBH-$_{P}$350; UBH-$_{N}$351; UBH-$_{C}$352; UBH-$_{S}$353; and UBH-$_{H}$354 while each sample was deposited at the herbarium section.

3.1.3 Animal handling and Ethics declaration

Sixteen (16) Sprague-Dawley rats of varied sexes weighing between 149-175g were obtained from a research animal farm at Uselu axis, Benin City. Animals were left to acclimatize for 14 days before the experiment commenced. The animals were kept at an animal research house in the department of Anatomy University of Benin at ambient temperature of (26±3°C), relative humidity (50%-60%) and photoperiodicity (12:12hr). They were kept in clean steel gauzed cages and coconut husks used as beddings in light and humid environment. Cages were cleaned by replacing bedding husks both morning and evenings. Rats were fed on standardized pellet super starter feeds and water provided adequately. Ethical approval (V.1034/40) was obtained from the

ministry of agriculture and natural resources while animals were used in compliance with policies outlined in the Guide for Care and Use of Laboratory Animals, published by US National Institute of Health (NIH Publication No. 85-23, revised 1996).

3.2 Lard-Palm Kernel Cake Fat Diet Preparation

10kg of super starter finisher feed was weighed out and 10% removed from the 10kg. Thereafter, 8% of pig fat and 2% of palm kernel cake (PKC) were weighed and mixed with the remaining 90% of the feed. The entire components consisted of 90% super feed, 8% pig fat, and 2% PKC mixed appropriately to make up 100% (10kg).

3.2.1 Preparation of Aju Mbaise Herbal Decoction

A pot was properly rinsed and a wrap of Aju Mbaise weighing 296g was placed in it, while one litre of water was added followed by intensive heating on a gas cooker for 30 minutes (Ayala, 2014). This preparation was according to the producer's instruction. It was cooled and filtered with a white sterile muslin cloth into a sterile plastic container. The dark-brown decoction (filtrate) was refrigerated to avoid decay at 25°C all through the study period.

3.2.2 Lethal Dose Determinations (LD_{50})

Acute toxicity study (LD_{50}) was conducted Aju Mbaise decoction using an adaptation to Lorke's method. This was to ascertain the lethal dose that will serve as a guide in administering the required dosage.

3.2.3 Experimental Design

Experimental rats were divided into four (4) groups containing four (4) rats in each cage (n=4). Group A served as the untreated control and received standard rat diet (SRD) and tap water only. Group B was fed with 10% of L-PKC fat diet mixed with 90% SRD and tap water. Group C was fed with 10% L-PKC mixed with 90% SRD and water, before oral administration of 2.5mL/kg body weight of Aju Mbaise herbal decoction (AMHD) treatment. Group D was fed with 10% L-PKC mixed with 90% SRD and water, and was treated with higher dose concentration (5mL/kg) of AMHD. The administration lasted for twenty eight (28) days at 2day intervals in Groups (C and D) using a sterile syringe and a hand towel to pick each rat.

3.2.4 Body and Organ Weight Measurement

Average weekly body weights of each rat were recorded on Day 0 and at seven (7), fourteen (14), Twenty one (21) and day Twenty eight (28) which marked the end of the study. Body weight differences for each group was calculated, and at termination, the heart and other vital organs were weighed using digital electronic balance.

3.2.5 Animal Sacrifice and Organ Harvesting

All animals fasted overnight till the 29^{th} day and were anaesthetized with chloroform. Rats were dissected using scalpel blade while pericardial adipose tissues from the epicardial and paracardial were excised. This refers to the combination of fat enclosed by the pericardial sac and fat surrounding the external surfaces of the pericardium. Cardiac muscle (myocardium) was also

excised and blotted individually with filter paper prior to gross examination for changes like colour, texture and structural integrity.

3.2.6 Relative Pericardial Adipose Tissue Weights

The wet pericardial adipose tissues were grossed immediately after excision while information on the individual weights was documented. Relative tissue weight (RTW) was estimated as ratio of tissue to animal's body weight at the end of experiment x100 for each rat.

Table 3.1: Treatment Protocol for Experimental Animals

Groups	Number of rats	Average Weight	Dosage in mL/kg body weight	Treatment plan	Duration
A	4	161.60	0mL/kg	Untreated (control) received standard rat diet (SRD) and normal tap water alone	28days
B	4	161.55	0mL/kg	fed with 10% of L-PKC fat diet mixed with 90% SRD and normal tap water only	28days
C	4	149.05	2.5mL/kg body weight of AMHD treatment	fed with 10% L-PKC mixed with 90% SRD and normal tap water	28days
D	4	174.45	5mL/kg body weight of AMHD treatment	fed with 10% L-PKC mixed with 90% SRD and normal tap water	28days

Each animal was picked one at a time with a hand towel and appropriate volumes of treatment were administered in group C and D alone.

3.2.7 Biochemical Analysis

Blood sample was collected from the cardiac region from each animal without anticoagulant with tab gel and centrifuged (BROADBENT, UK) at 3000 rpm

for 5 min to obtain the serum content. Sera were stored at -20°C until it was needed for fasting lipid profile.

3.2.8 Lipid Profile

Serum from fasting blood was obtained by centrifuging the clotted blood as described earlier. Chemical analyzer (Erba Chem 5X Analyzer) based on spectrophotometric principle was used for lipid analysis: serum triglycerides (ST), total cholesterol (TC), high density lipoproteins cholesterol (HDL) and low density lipoproteins cholesterol (LDL). Enzymatic photometric test was used for analysis using a wavelength of 546 nm and an optical path of 1cm.

3.2.9 Cut-Up and Tissue Processing

Cardiac muscle (myocardium) and pericardial adipose tissues (fat deposits around the heart) were excised from each group from the heart and cardiac region. Samples were grossed and fixed in 10% neutral buffered formol saline histological processing. Tissues were cut into bits of between 1.5 to 2mm thicknesses during grossing. Histological processing was done using paraffin technique. They were dehydrated by passing them through ascending grades (70%, 90% and 100%) of alcohol. The tissues were cleared in xylene and impregnated by passing tissue through 3 changes of molten paraffin wax for internal support. They were embedded for external support with molten paraffin wax held in tissue cassettes and left to solidify.

3.2.10 Sectioning and Staining

Blocking out was done while tissue blocks were sectioned with a microtome to obtain serial ribbons of about 3-5µ. Sections were placed on a slide (resting on a

flat surface) containing 20% methanol and then floated onto a water bath to flatten up. Sections were picked up with clean grease-free slides and excess water allowed to drain-off and heat-fixed on a hot plate. Sections were then stained using H&E method in which they were dewaxed in xylene and hydrated by passing them through descending grades (100%, 90% and 70%) of alcohol and then to water. Thereafter, sections were stained with Cole's haematoxylin for 10 minutes and then rinsed in water. It was differentiated briefly in 1% acid-alcohol and again rinsed off. Sections were blued in Scot's tap water and counter-stained with eosin for 3 minutes, and rinsed off yet again. The sections were dehydrated by passing them through ascending grades (70%, 90% and 100%) of alcohol, cleared in xylene and mounted in DPX. Slides were later viewed using the microscope at x10 and x40 magnifications.

3.2.11 Microscopy and Photomicrography

Slides were examined using x10 and x40 objective lenses of Binocular microscope. Photomicrography was conducted on the slides using a digital microscope camera attached to the eyepiece of the microscope.

3.2.12 Statistical Analysis

Data obtained from the study were analyzed as mean ± standard error of mean (SEM). The statistical package for social science (SPSS) software version 20 was used for the statistical analysis. Data were statistically analyzed using one way analysis of variation (ANOVA) to determine main effect on treatment

groups while Tukey's post-hoc test was used for multiple comparisons. $P<0.05$ was considered significant.

CHAPTER FOUR
RESULTS

4.1 Body Weight Measurements

The effect of the graded treatment with AJMD on the rats revealed a progressive increase in Group (C and D). All rats in the groups experienced increase in weights compared with Day 0 when they first enrolled in the experiment. On treatment with 2.5mL/kg and 5mL/kg, there was no significant difference in the weights of rats throughout the study period (Table 4.1).

4.2 Relative Pericardial Adiposity Weights

Total pericardial fat weight was increased in all groups but higher in group B (Lard-PKC). The same condition was seen in total pericardial index with no significant differences ($p<0.05$) in all pericardial weights (Table 4.2).

4.3 Organ Weights Measurement

The effect of L-PKC and AJMD treatment was compared to the normal control (Group A).The organ weights of rats in the L-PKC (Group B) were higher when compared with the organ weights of rats in Group A. Those in Group C (L-PKC + 2.5mL/kg AJMD) decreased when compared to Group B while those in Group D (L-PKC +5mL/kg AJMD) increased with no significant differences with other groups (Table 4.3).

4.4 Lipid Biochemistry Analysis

The results of lipid biochemistry analysis showed that there was significant decrease in the mean TC (mg/dL) $p<0.001$ between L-PKC control (Group B) and the main control (Group A).There was also significant increase in mean TC (mg/dL) between 2.5mL/kg AJMD (Group C) and L-PKC control (Group B) .In mean TG, there was significant difference between main control (Group A) and 2.5mL/kg AJMD (Group C) and between L-PKC control and 5mL/kg AJMD (Group D).There was no significant difference in HDL and LDL. However for VLDL analysis, there was a significant difference ($p<0.000$) between the main control (Group A) and 5mL/kg AJMD (Group D) and between L-PKC control and 2.5mL/kg AJMD (Group C) (Table 4.4).

4.5 Histopathology of Pericardial Tissues

Pericardial adiposity from untreated animals (Group A) revealed that the adipocytes remained intact without distortions in histological architecture (Plate 4.1). Adipocytes of Group B though appeared normal but bigger in sizes (Plate 4.2) compared with those of the control (Plate 4.1). The pericardial adiposity from (Group C) on 2.5mL/kg and 5mL/kg (Group D) treatment plans showed normal sizes of adipocytes (Plate 4.3 - 4.4) but slightly reduced in animals on 5mL/kg (Group D) treatment regime (Plate 4.4) compared with other groups.

4.6 Histopathology of the Cardiac Muscles

Myocardium from main control rats (Group A) showed normal histological architecture evident with an intact branching muscle fibre (Plate 4.5). Animals in Group B showed evidence of ballooning of branching muscle fibres and nucleus (Plate 4.6), when compared with the former (Plate 4.5). LPKCD-fed rats on 2.5mL/kg (Group C) and 5mL/kg (Group D) treatment plans showed normal histological architecture evident with the appearance of an intact fibre striation .and nucleus (Plate 4.7).

4.1 Effect Aju Mbaise Decoction Treatment on Body Weights Measurement for 28 Days

		Body weight analysis				
Group	**Treatment**	Day 0	Day 7	Day 14	Day 21	Day 28
Group A	Normal Control	161.60± 13.97	192.25±17.06	220.38± 15.73	242.15± 17.05	248.650± 16.51
Group B	L-PKC control	161.55± 31.27	192.30± 23.43	195.25± 29.62	228.00± 21.81	262.62± 22.17
Group C	2.5mL/kg AJMD + L-PKC	149.05± 35.42	181.17± 26.67	205.70±23.13	212.90± 22.16	233.15± 25.20
Group D	5mL/kg AJMD + L-PKC	174.45 ± 32.71	215.40± 24.62	209.55± 27.64	214.41± 24.37	236.27± 28.60
	F Ratio	0.123	0.433	0.178	0.401	0.325
	Sig	0.945	0.734	0.909	0.755	0.807

Values are presented as Mean ± Standard Error (SE)

* P<0.05 when compared with Group A (Main Control)

# P <0.05 when compared with Group B (LPKC control)

Table 4.2 Effect of Aju Mbaise herbal decoction on Pericardial Weights of Rats

		PERICARDIAL WEIGHT ANALYSIS				
Group	**Treatment**	**Epicardial Fat (g)**	**Paracardial Fat (g)**	**Pericardial fat (g)**	**Pericardial fat index (g)**	**Total pericardial fat index(g)**
Group A	Main Control	0.85±0.14	0.98±0.25	1.73±0.39	0.39±0.87	0.74±0.14
Group B	L-PKC control	0.83±0.14	0.83±0.11	1.68±0.25	0.33±0.07	0.66±0.14
Group C	2.5 mL/kg AJMC + LPKC	0.90±0.13	0.90±0.15	1.85±0.27	0.40±0.08	0.76±0.13
Group D	5mL/kg AJMC + LPKC	0.56±0.05	0.56±0.03	1.01±0.10	0.26±0.36	0.49±0.63
	F Ratio	2.130	1.380	1.896	0.903	1.001
	Sig	0.150	0.296	0.184	0.468	0.426

* P<0.05 when compared with Group A (Main control)

# P <0.05 when compared with Group B (LPKC control)

Note: Epicardial adipose tissue + paracardial adipose tissue makes up the pericardial adipose tissue

Table 4.3: Organ Weights of Treated and Control Animals With A Focus On the Heart

		ORGAN WEIGHT ANALYSIS						
Groups	Treatment	Liver	L (kidney)	R (Kidney)	Spleen	**HEART**	Stomach	Lungs
Group A	Main control	8.00±0.38	0.75±0.029	0.75±0.029	0.85±0.14	0.85±0.029	1.35±0.029	1.75±0.065
Group B	L-PKC control	9.45±0.26	0.88±0.085	0.95±0.65	1.00±0.17	0.98±0.13	1.58±0.085	1.85±0.16
Group C	2.5/kg AJMC+LPKC	8.42±0.54	0.83±0.11	0.78±0.063	0.93±0.12	0.75±0.096	1.55±0.087	1.48±0.063
Group D	5mL/kg AJMC+ LPKC	8.17±0.43	0.63±0.06	0.70±0.041#	0.85±0.065	0.80±0.041	1.30±0.17	1.68±0.13
	F Ratio	1.232	1.940	4.451	0.307	1.366	2.680	2.034
	Sig	0.341	0.177	0.025	0.820	0.300	0.094	0.163

Values are presented as Mean ± Standard Error (SE)

* P<0.05 when compared with Group A (Main control)

# P <0.05 when compared with Group B (LPKC control)

Table 4.4: Relative Organ Weights of Rats Fed L-PKC and Treated with herbal decoction while focusing on the Heart

RELATIVE ORGAN WEIGHT ANALYSIS

Group	Treatment	Relative Liver	Relative Left kidney	Relative Right kidney	Relative Spleen	**RELATIVE HEART**	Relative Stomach	Relative Lung
Group A	Main control	3.29±0.37	0.30±0.01	0.30±0.01	0.36±0.08	0.35±0.03	0.55±0.02	0.72±0.07
Group B	L-PKC control	3.61±0.20	0.33±0.02	0.36±0.01	0.38±0.05	0.37±0.03	0.65±0.04	0.71±0.05
Group C	2.5mL/kg AJMC+LPKC	3.66±0.15	0.35±0.27	0.34±0.04	0.42±0.07	0.32±0.02	0.67±0.03	0.65±0.06
Group D	5mL/kg AJMC+ LPKC	2.76±0.79	0.35±0.85	0.50±0.20	1.20±0.81	0.32±0.03	0.50±0.07	0.63±0.11
	F Ratio	0.829	0.265	0.710	0.977	0.642	2.968	0.337
	Sig	0.504	0.849	0.565	0.436	0.603	0.075	0.799

* P<0.05 when compared with Group A (Main Control)

# P <0.05 when compared with Group B (LPKC control)

Table 4.5: Effect of Aju Mbaise Decoction on Lipid Biochemistry Indices (mg/dL) In Experimental Rats

Group	Treatment	TC (mg/dL)	TG (mg/dL)	HDL(mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Group A	Main control	101.51±0.51#	121.41±5.50	48.98±1.83	33.04±0.28	22.84±0.32
Group B	L-PKC control	80.79±3.67*	89.38±11.97	37.49±4.80	25.76±3.97	17.56±2.64
Group C	2.5mL/kg AJMD + L-PKC	113.04±6.40#	228.22±20.99#	40.82±5.83	25.68±3.76	49.07±5.70#
Group D	5mL/kg AJMD + L-PKC	88.08±4.22	126.29±24.12	34.27±1.50	25.67±1.83	26.19±4.53
	F Ratio	11.300	12.120	2.556	1.612	12.849
	Sig	0.001	0.001	0.104	0.234	0.000

Values are presented as Mean ± Standard Error (SE)

* P<0.05 when compared with Group A (Normal Control)
# P <0.05 when compared with Group B (L-PKC control)
LPKC=Lard-Palm Kernel Cake
AJMC=Aju Mbaise Decoction
TC= Total Cholesterol, TG = triglycerides
HDL=High Density Lipoprotein.
LDL=Low Density Lipoprotein
VLDL= Very Low
Density - Lipoprotein

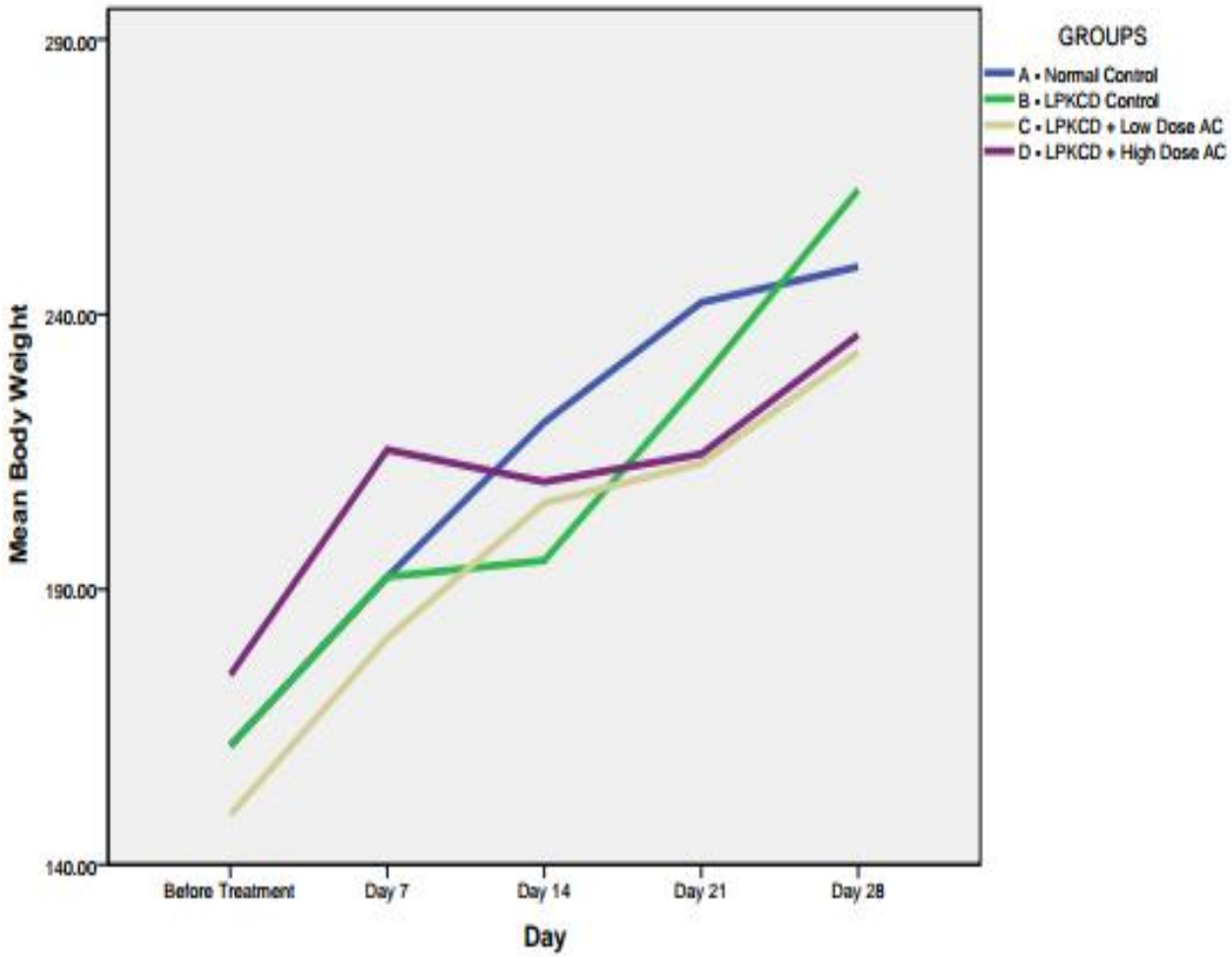

Figure 4.1: Graph of mean body weight

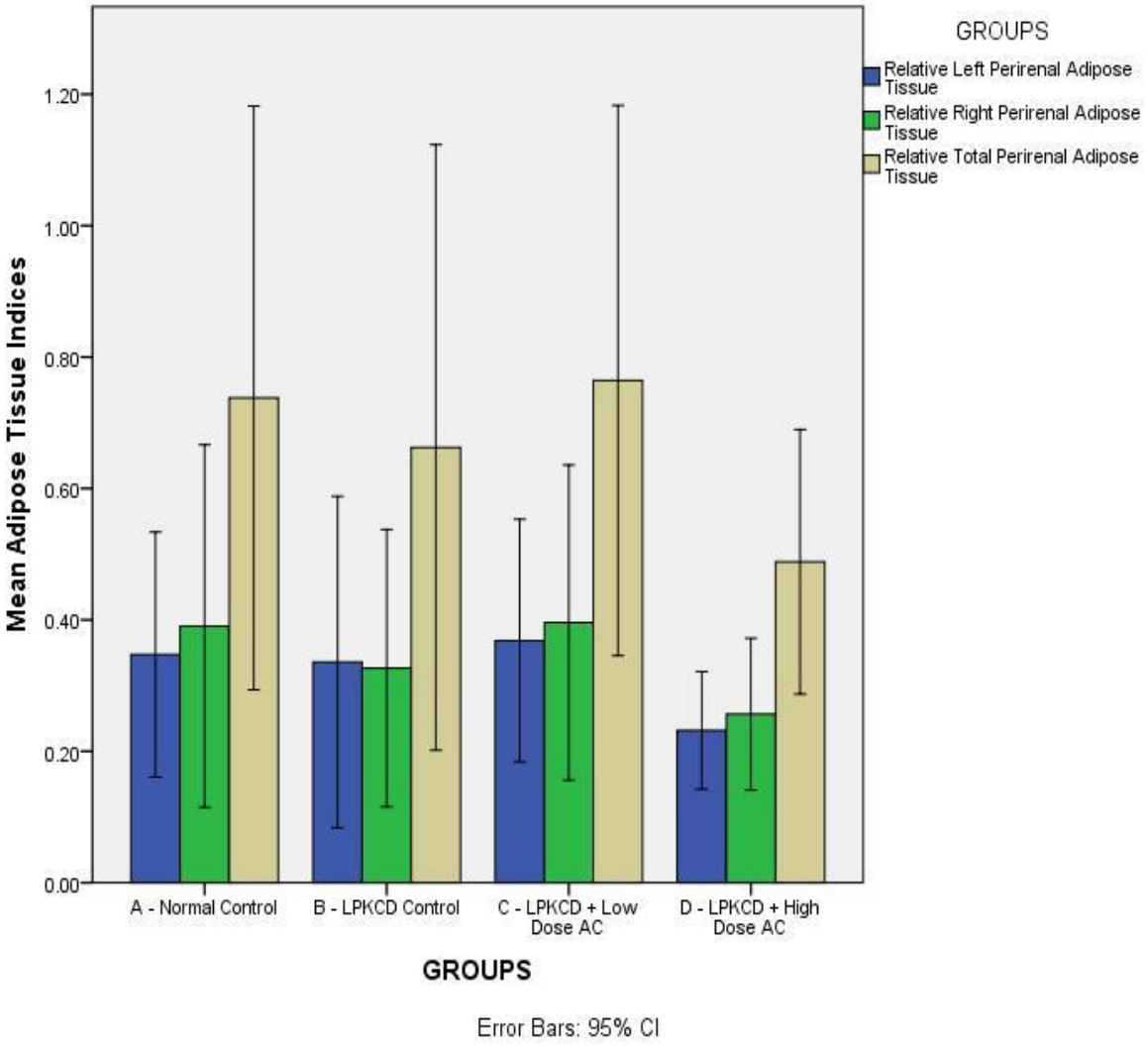

Figure 4.2: Graph of Mean Cardiac Adiposity in Experimental Rats

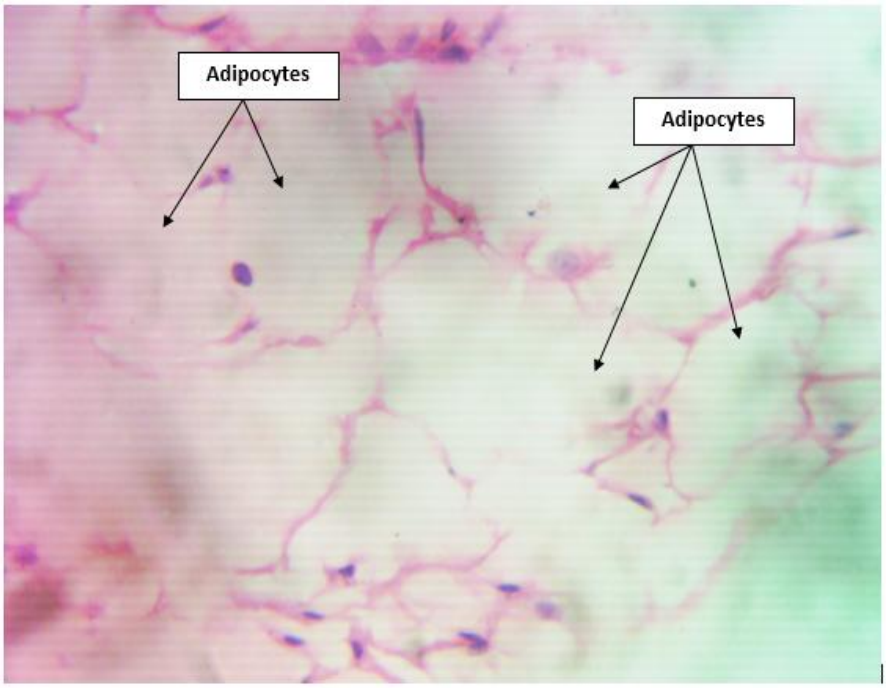

Plate 4.1: Light photomicrograph of Pericardial white adiposity from the main control rats (Group A) showed normal histological architecture. The adipocytes appeared normal (Stain: H&E / Mag: x400).

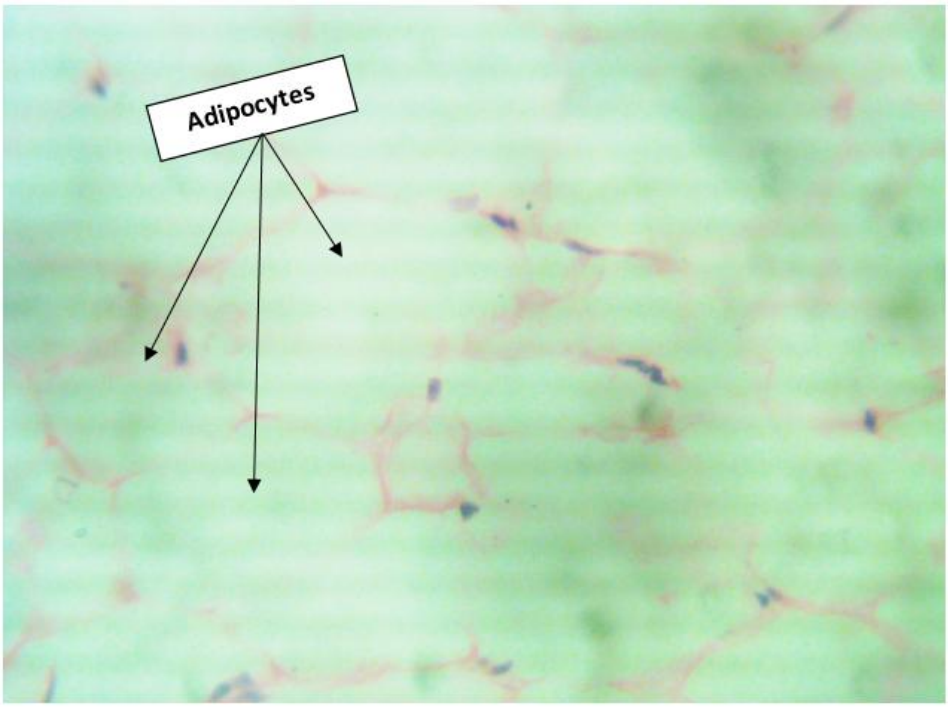

Plate 4.2: Light photomicrograph of Pericardial white adiposity from Lard-Palm Kernel Cake Diet (LPKCD) fed rat (Group B). Adipocytes sizes appeared normal but bigger in sizes compared to those of the untreated animals (control) fed with a standard diet (Stain: H&E/Mag: x400).

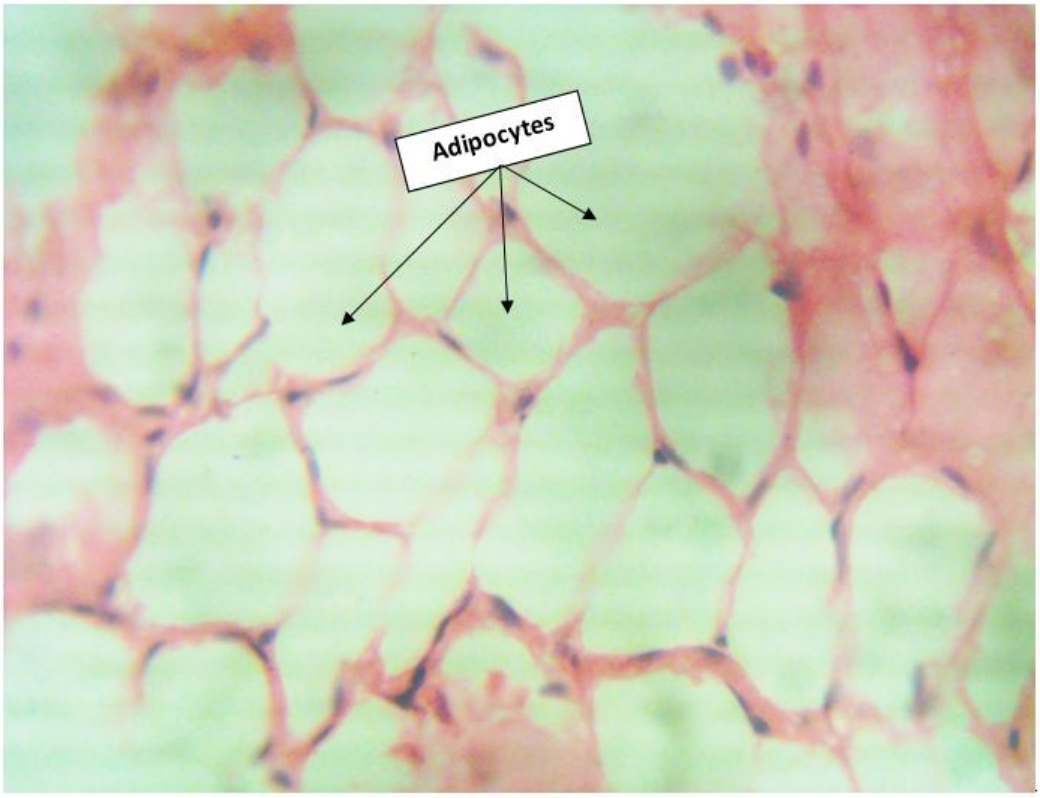

Plate 4.3: Light photomicrograph of pericardial white adiposity from LPKCD-fed rat treated with 2.5mL/kg (Group C) showed normal adipocytes (Stain: H&E/Mag: x400).

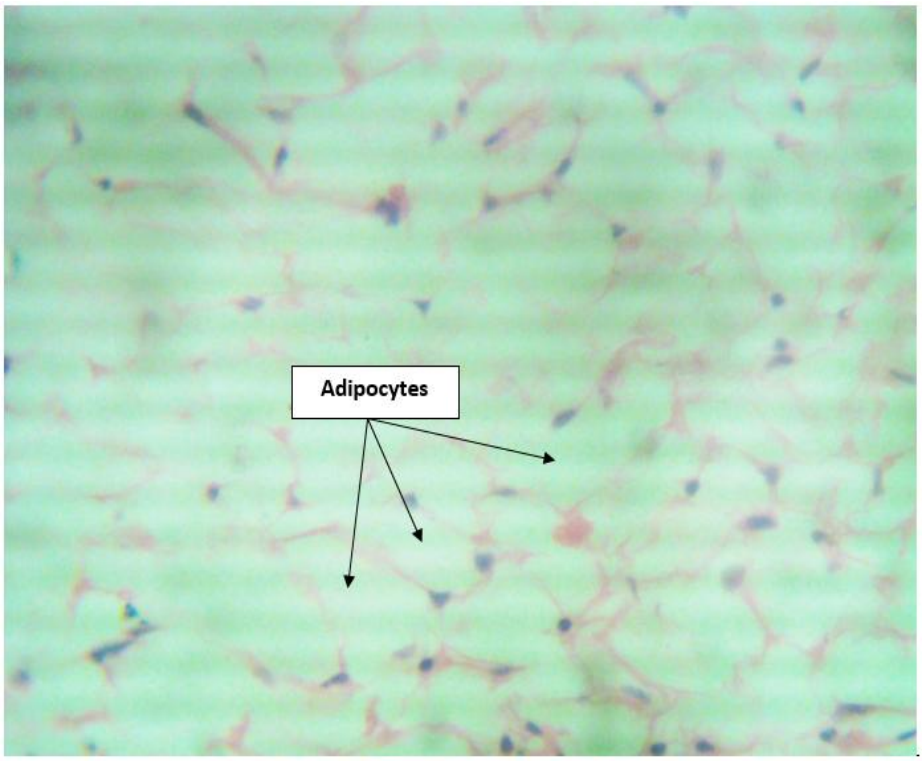

Plate 4.4: Light photomicrograph of pericardial white adiposity from LPKCD-fed rat treated with 5mL/kg AJMC (Group D). Sizes of adipocyte appeared slightly reduced (Stain: H&E/Mag: x400).

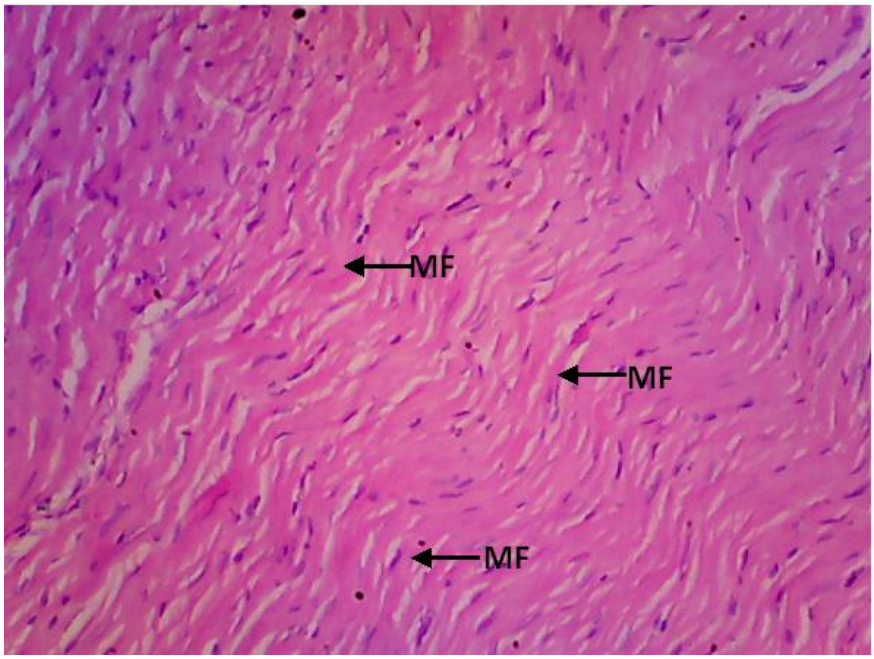

Plate 4.5: Light photomicrograph of cardiac muscle from main control rats (Group A) showed normal histological architecture evident with an intact branching muscle fibre (Stain: H&E / Mag: x400).

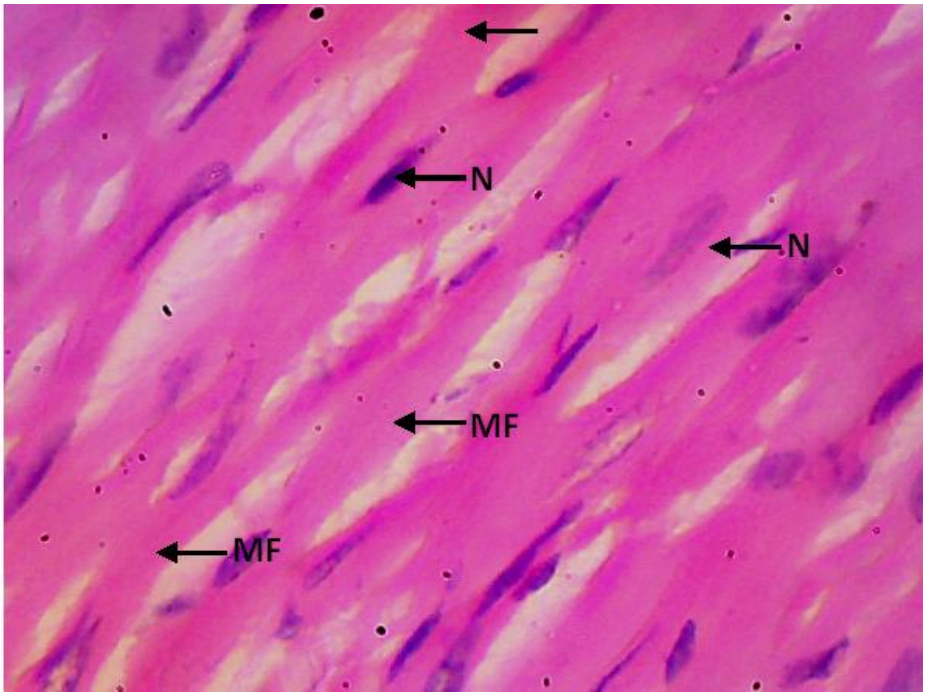

Plate 4.6: Light photomicrograph of cardiac muscle from Lard-Palm Kernel Cake Diet (LPKCD) fed rat (Group B), otherwise served as the LPKCD control. Histopathology appeared normal but with evidence of ballooning of branching muscle fibres and nucleus (Stain: H&E/Mag: x400).

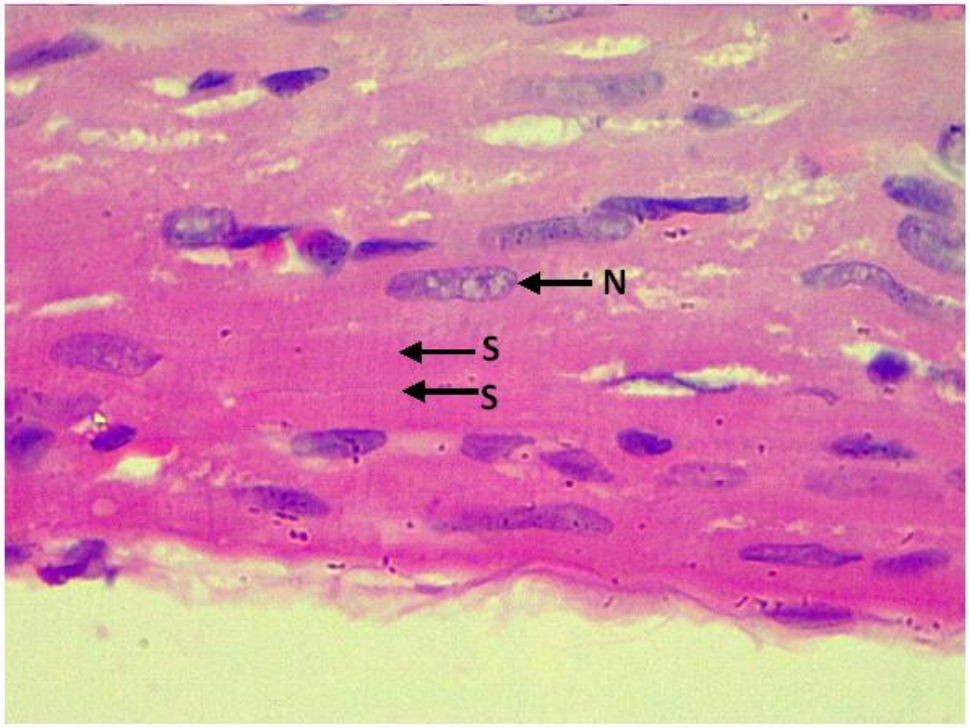

Plate 4.7: Light photomicrograph of cardiac muscles from LPKCD-fed rat treated with 5mL/kg AJMC (Group D). Histology appeared normal and comparable to those of the main control in Group A (Stain: H&E/Mag: x400). Note: Photomicrograph of animals in Group C showed normal histology, therefore there is no need for repetitions.

KEYS:

MF= Branching Muscle fibres

N= Nucles

S=Striation

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

Since prehistoric times, humans have used natural products to treat illnesses. It has been recorded in fossil studies that human use of plants as medicine is dated back to 60,000 years ago (shi *et al*., 2010). The herbal product used in this experiment is Aju Mbaise, which is a wrap of different plants. This product exerted progressive increases in body weight of treated rats similar to those observed in the control groups with a non-significant effect. This suggests that both L-PKC diet feeding and AJMD treatment did not alter the body weight of rats because under normal conditions, middle aged animals should experience an increase in body weight while feeding (Lyratzopoulos *et al..,* 2005). This finding contradicted the folkloric claims on herbal cocktail as being a potential body weight reducing agent (Smith *et al.,* 2012). However, the authentication of this claim maybe further explored in animals placed on a normal rats chow or different animal species for comparison with findings in this study. Diets containing high fats like Lard are well known to induce profound weight gain which may even lead to obesity (Fermin *et al* 2012,). The non-significant differences in body weight of rats maybe due to counter effects from palm kernel cake (PKC) mixed with lard. PKC is a bye product of palm kernel oil extraction, rich in high-fibre, and medium-grade energy source (Manshop, 2011). Palm kernel cake is a protein feed that is best suited for ruminants. Among other similar fodders, palm kernel cake is ranked a little higher than copra cake and cocoa pod husk, but lower than fish meal and groundnut cake, especially in its protein value. It contains 15-21% crude protein with a low level

of lysine, methionine and tryptophan, which can be poorly used as feed for ruminants (Manshop, 2011). On the other hand, lard is a semi-solid white fat product obtained from fatty tissue of a pig (Fermin *et al* 2012,). Many cuisines use lard as a cooking fat while some scientists use it to induce high-fat-fed condition in experimental animals. In this study, PKC may have countered the impacts of lard. Previous studies have shown that PKC is capable of inhibiting body weight gain (Garcia *et al.*, 1999). Another report suggests that PKC-induced weight loss was attributed to poor palatability and lowered acceptability of diets that contained PKC in it leading to decreased feeds intake and subsequently impaired weight gain in animals (Duran *et al.*, 1990). In addition, Boeteng *et al.,* (2008) observed that feeding PKC ration depresses body weight gain but the effect depends on the percentage of PKC used. Therefore, an increase in PKC content of a diet leads to a corresponding decrease in body weight. The spike in rats treated with L-PKC diet alone in the present study particularly on day 28 when compared to other groups maybe due to acclimatization of the animals to an unusual diet. Perhaps a more profound increase in body weight maybe observed in prolonged feeding beyond 28 days.

Organ weight measurements are used in experimental studies to ascertain changes due to diet feeding, drug treatments and toxic agent's exposure (Nisha *et al* 2009, Azubuike *et al* 2014). Organ indices of treated rats in this study did not show any significant changes. Since rat weights were improved in the course of this study, obvious changes in organ weights may not be detected because relative organ weight is an index determined in relationship with the body weight of an animal (Angervall and Carlstrom, 1963). In some cases,

absolute organ weights are usually preferred as it may lead to better interpretation of effects induced by treatments.

Analysis of variance in serum lipid biochemistry in this study showed no changes in HDL and LDL but decreased levels of TC, TG and VLDL upon feeding with L-PKC only. There was significant decreases in TC when compared with animals in Group A, which may connote the anti-hyperlipidemic activities of L-PKC feeding. This effect may be attributed to PKC as it is well known to be rich in fibre and energy leading to lipolyitc effects (Ruiz et al,, 2005). Reduced levels of these parameters observed in high dose treatments with AJMD could be attributed to phytochemical constituents such as saponins, tannins and phenols in the herbal decoction (Ghule *et al.*, 2006) and has been reported that plants rich in these phytochemicals possess hypolipidemic activities (Han *et al.*, 2005).

Pericardial adipose tissues refers to the combination of fat enclosed by the pericardial sac and fat surrounding the external surface of the pericardium which is collectively referred to as pericardial adipose tissues and often resulting from the epicardial and paracardial adipose tissues (Britton and Fox, 2011). Adipose tissues are dynamic organs that play a role in energy balance in the body, thus, changes in their sizes are due to metabolic processes (Lafontan and Langin, 2009). Decreased total pericardial wet adipose tissues and consequently adipocytes sizes observed with the high dose treatment with AJMD in this study suggest either a direct or indirect effect of the decoction on fat pads. Pericardial adiposity from LPKCD-fed rats on 2.5mL/kg and 5mL/kg

treatment plans showed normal adipocytes but slightly reduced in animals on 5mL/kg treatment regime compared to other groups is strongly suggestive of the impact of a high concentration of Aju Mbaise decoction (AJMD) on treated rats. On a second thought, phenol occurs in abundance in AJMD has been implicated in a previous study to be the cause of hypolipidemic effects, dysregulated lipid profiles, and inhibition of adipocytes differentiation (Rice, 1996). Agents or substances which inhibit the growth of adipocytes and fat accumulations are considered as having anti-obesity effects (Kim *et al,,* 2011; Roh *et al,,* 2012). The mechanism or reduction effect of AJMD on fat pads but non-significant effects on body weight of animals in the present study cannot be readily explained. Perhaps, the progressive body weight gain maybe due to muscle mass and not necessarily fat accumulation. Further studies to better understand the responsible mechanism for observed effects may be conducted at a latter research. However, in the current study, the effects seen in photomicrograph of LPKCD-fed rats alone suggest that there is a pathologic onset which was resolved upon introduction of the present decoction and is evident in the histology layout of cardiac muscle of rats. This analysis is strongly supported by Smith *et al.,* (2012) in which they claimed that traditional medicine now composed of natural products used for treatment or management of different ailments and those earlier used, attempted to provide good health to end-users.

5.2 Conclusion

Data obtained in this study suggest that treatment with Aju Mbaise decoction at a dose of 5mL/kg body weight maintained histological integrity of the myocardium, lipid profile, including reduction in adipose tissue weights and adipocyte sizes of rats fed with lard-palm kernel cake diet.

5.3 Recommendation

Further studies on the exact mechanisms of action of Aju Mbaise decoction on the subject matter will be necessary. Investigation from molecular perspectives should also be considered.

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