Ameliorative potentials of ethanol extract of Alchornea cordifolia on some biochemical and physiological parameters in high-fat diet-induced obese Wistar rats

Chinedum Martins Ekeleme

martinsekeleme@yahoo.co.uk

Arthur Jarvis University

Diana Ochuole Odey

University of Calabar

Chidinma Emmanuel Ibeneme

University of Calabar

Esien David-Oku

University of Calabar

Eyong Ubana Eyong

University of Calabar

Item Justin Atangwho

University of Calabar

Godwin Eneji Egbung

University of Calabar

Edet Effiong Asanga

Arthur Jarvis University

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Chinedum Martins **Ekeleme**[1,2], Diana Ochuole **Odey**[2,3] Chidinma Emmanuel **Ibeneme**[2], Esien **David-Oku**[2], Eyong Ubana **Eyong**[2], Item Justin **Atangwho**[2], Godwin Eneji **Egbung**[2], Edet Effiong **Asanga**[1]

[1] Department of Biochemistry, Arthur Jarvis University, Calabar, Cross River state, Nigeria
[2] Department of Biochemistry, College of Medical Sciences, University of Calabar, Calabar, Cross River state, Nigeria
[3] Department of Biochemistry, University of Cross River state, Calabar Cross River state.

Corresponding authors: Chinedum Martins Ekeleme,
ORCID ID: 0009-0008-7973-3296
Email: martinsekeleme@yahoo.co.uk
Current address: Department of Biochemistry, Arthur Jarvis University, Calabar, Cross River state, Nigeria

Statements & Declarations

Ethical approval

Ethical approval for the research was obtained from the University of Calabar Ethical Review Committee (with number FAREC/PA/UC/049/245BCM3823), which is in accordance with the ethical standards as reviewed in the Principles of Laboratory Animal Care (NIH publication no. 8523, revised 1985).

Consent to participate

The study did not involve human participants; therefore, no informed consent was necessary.

Consent to publish

The researchers did not involve any human participants in their study. As a result, there was no need to seek consent from individuals before publishing their data in this journal.

Availability of data and materials

All data from study are available and will be released on request

Competing Interests

The authors have no relevant financial or non-financial interests to disclose

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Author Contributions

All authors contributed to the study's conception and design. Chinedum Martins Ekeleme, Item Justin Atangwho, Esien David-Oku: Conceptualization, Methodology, Software Chinedum Martins Ekeleme, Esien David-Oku: Data curation, Writing- Original draft preparation. Chinedum Martins Ekeleme, Diana Ochuole Odey, Chidinma Emmanuel Ibeneme: Visualization, Investigation. Godwin Eneji Egbung, Item Justin Atangwho, Esien David-Oku, Eyong Ubana Eyong*:* Supervision.: Edet Effiong Asanga: Software, Validation.: Chinedum Martins Ekeleme: Writing- Reviewing and Editing.

Availability of data and materials

All data supporting the findings of this study are available within the paper.

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Authors' information

Name:
Chinedum Martins **Ekeleme**
Affiliations:
Department of Biochemistry, Arthur Jarvis University, Calabar, Cross River state, Nigeria,

Department of Biochemistry, College of Medical Sciences, University of Calabar, Calabar, Cross River state, Nigeria
Department of Biochemistry, Delta State University of Science and Technology, Ozoro, Delta State, Nigeria,
Email:
martinsekeleme@yahoo.co.uk
ORCID:
0009-0008-7973-3296

Name:
Diana Ochuole **Odey**
Affiliations:
Department of Biochemistry, University of Cross River state, Calabar Cross River state.
Email:
odianaodey@gmail.com

Name:
Chidinma Emmanuel **Ibeneme**
Affiliations:
Department of Biochemistry, College of Medical Sciences, University of Calabar, Calabar, Cross River state, Nigeria
Email:
chidimma.e.ibeneme@gmail.com

Name:
Esien **David-Oku**
Affiliations:
Department of Biochemistry, College of Medical Sciences, University of Calabar, Calabar, Cross River state, Nigeria
Email:
drdavidoku@gmail.com
ORCID:
0000-0002-8261-3868

Name:
Eyong Ubana **Eyong**
Affiliations:
Department of Biochemistry, College of Medical Sciences, University of Calabar, Calabar, Cross River state, Nigeria
Email:
eyongubana@unical.edu.ng
ORCID:
0000-0003-3857-8732

Name:

Item Justin **Atangwho**
Affiliations:
Department of Biochemistry, College of Medical Sciences, University of Calabar, Calabar, Cross River state, Nigeria
Email:
dratangwho@gmail.com
ORCID:
0000-0002-3757-9760

Name:
Prof. Godwin Eneji **Egbung**
Affiliations:
Department of Biochemistry, College of Medical Sciences, University of Calabar, Calabar, Cross River state, Nigeria
Email:
eneji6@gmail.com
ORCID:
0000-0001-7056-7772

Name:
Dr. Edet Effiong **Asanga**
Affiliations:
Department of Biochemistry, Arthur Jarvis University, Calabar, Cross River state, Nigeria,
Email:
edyasangae1@gmail.com
ORCID:
0000-0001-5997-9557

ABSTRACT

This study investigated the effect of ethanol extract of *A. cordifolia* leaves on high-fat diet (HFD)-induced obesity and its associated metabolic abnormalities in rats. Thirty male rats were randomly divided into five groups (Six rats each)- Normal control, Obese control, Orlistat group, *A.cordifolia* groups (500 and 1000 mg/kg body weight). Dietary intake, anthropometric indices, lipid profile, atherogenic indices, blood glucose, hepatic and cardiac function, HMG CoA reductase activity and antioxidative properties were investigated. Intake of HFD led to significant increases in bodyweight, BMI, Lee's index, waist-circumference, liver, adipose tissue, heart weight and lipids, T.cholesterol, triglycerides, atherogenic index, and LDL-c, while decreasing HDL-c. Additionally, HFD consumption increased fasting and serum blood glucose, insulin, HOMA-IR, amylase, and lipase activity, while SOD, GPx, and GSH levels were reduced, and MDA increased. Moreover, HFD increased ALT, ALP, AST, LDH and creatine kinase levels. Furthermore, HMG-CoA reductase activity decreased, and histological analysis revealed inflammation, fat accumulation, and distortion in adipose tissue architecture in the liver and adipose tissue of obese rats. *A.cordifolia* supplementation effectively reversed HFD-induced alterations, demonstrating weight-reducing, anti-hyperlipidemic, antioxidant, and anti-inflammatory properties. Therefore, *A.cordifolia* possesses anti-obesity potential and may be used as a possible therapeutic alternative for obesity and its associated pathologies.

Keywords

Obesity, *A. cordifolia*, Metabolic abnormalities, High-fat diet, Antioxidant properties, HMG CoA reductase activity

1. Introduction

Globally, obesity is a significant public health issue which impacts men, women and children [1][2]. Consistent intake of high-energy-density foods, like high-fat diets, has emerged as one of the significant factors linked to the occurrence and rise in obesity prevalence over time [3]. Obesity is linked to several health issues, including osteoarthritis, cancer, type-2 diabetes, heart disease, hypertension, liver damage, and dyslipidemia [4]. Over 1.9 billion people worldwide were overweight in 2014, according to the World Health Organisation (WHO), including more than 600 million obese people [5]. According to a study by Mabiama *et al.* [6], around 30% of individuals in Africa have a weight issue and are considered overweight or obese. In Nigeria, a projected 21 million people aged 15 years and above were overweight or obese in 2020, resulting in age-adjusted prevalence rates of 20.3% and 11.6%, respectively [7].

Previous studies have demonstrated that a high-fat diet (HFD) increases the generation of reactive oxygen species (ROS) in adipocytes and many other organs, leading to oxidative stress [8][9]. Additionally, an increase in the peroxidation of lipids and the reduction of both antioxidant defence mechanisms are signs of an unbalanced redox status [10]. Moreover, oxidative stress has been linked to the emergence of obesity and related metabolic issues [11]. These metabolic changes include increased plasma triglyceride levels, total low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) cholesterol, and low high-density lipoprotein (HDL) cholesterol levels [11]. Hepatic, pancreatic, and muscular tissues are examples of non-adipose tissues where excess fatty acids in the blood are stored. Hepatic fatty acid accumulation contributes to the onset of insulin resistance and non-alcoholic fatty liver disease (NAFLD) by raising the release of glucose into the liver [12]. Hepatocellular necrosis, steatosis, and fibrosis can all result from NAFLD [13]. Lipid accumulation in heart muscle cells induces insulin resistance, myocardial hypertrophy, and myocardial infarction and promotes a reduction in overall cardiac functioning [14].

Several pharmaceutical interventions, including orlistat, phentermine/topiramate, lorcaserin, naltrexone/ bupropion, and liraglutide, have been authorized for use over time to treat obesity [15]. Nevertheless, in addition to hepatic and renal dysfunctions, these medications have been known to cause a number of other side effects, including nausea, diarrhoea, dry skin, rhabdomyolysis, cramping in the muscles, myopathy, and memory loss [15][16]. Consequently, scientists are

seeking safer alternatives by using plants and compounds from natural sources that possess anti-obesity and antioxidant properties. Natural remedies are readily available, more easily utilised, have no negative side effects, and are affordable.

The plant *Alchornea cordifolia* (Schumach.) Müll. Arg. is a member of the *Euphorbiaceae* family and is commonly found in African regions as well as extensively utilised in traditional medicine [17][18]. In Nigeria, it is known by various local names like "ububo" (Igbo), "ipaesinyin" (Yoruba), "banbani" (Hausa), "upia" (Igede), "uwonwen" (Benin), "mbom" (Efik), and "ukpaoromi" (Ijaw), among others [19]. Several studies have reported that various parts of *A. cordifolia* possess biological activities such as anti-inflammatory, antidiarrheal, hepatoprotective, antiviral, and antidiabetic properties [20][21][22][23][24]. The antimicrobial screenings of different parts of *A. cordifolia* have also shown that it is effective against a wide range of pathogenic microbes, including gastrointestinal, skin, respiratory, and urinary tract pathogens, thus supporting its traditional use for treating such ailments [25][26][27][28]. *A. cordifolia* leaves are commonly used to treat cuts, wounds, and sores [29]. Furthermore, this plant has been used to treat coughs, headaches, and colds, to control spontaneous abortion, and to manage and relieve asthmatic attacks [30]. The root and stem barks are used to treat jaundice, while the powdered leaves are used to cure diarrhoea and wounds [31]. Other studies have shown that crude leaf extract of *A. cordifolia* exhibits significant antimalarial activity [32]. The plant has also been traditionally used to treat gastrointestinal and urinary disorders, leprosy, and snake venom antidote. The fruit of this plant is used to treat eye and pigmentation problems, while a decoction of leafy twigs is used to treat malaria, fever, and rheumatic pains [33].

Some bioactive compounds such as (5–methyl 4’ -propenoxy anthocyanidines 7-O-β-D – diglucopyranoside) isolated from different parts of *A. cordifolia* have been linked to its significant pharmacological actions [34]. Additionally, fatty acids [35][36], phenolic compounds [34][35], and alkaloids [21] are the main groups of phytochemicals in *A.cordifolia* parts, reported by several studies responsible for their medicinal properties. Despite the ethnobotanical applications, there is limited information on the effect of *A,cordifolia* on serum/tissue lipid profile and anthropogenic indices in a high fat diet-induced obese rat model. We, therefore, evaluated the ameliorative potentials of *A.cordifolia* on dyslipidemia in high-fat diet-induced obese Wistar rats.

2. Materials

2.1 Collection and processing of plant materials

Fresh *Alchornea cordifolia* (Schumach.) Müll.Arg. leaves were obtained from where they grow naturally in Moniya Village, located at latitude 7.5249260 and longitude 3.9151850 in the Akinyele Local Government Area of Ibadan, Oyo State, between November and December 2022. The plant was authenticated by a qualified botanist who assigned the voucher number Bot/Herb/UCC/611. The plant name was checked with https://www.worldfloraonline.org/ and found as assigned Linnaea 34: 170 (1865). The specimen was then deposited at the Herbarium of the Department of Plant and Ecological Studies, University of Calabar, for future reference. The fresh leaves of *A. cordifolia* were first rinsed under flowing water to get rid of sand and other solid impurities before air drying at room temperature and pressure in a shaded space. Afterwards, using an electric mill, dried-up leaves were ground to a fine powder form and stored in an airtight jar at room temperature. The ethanol extract was obtained from powdered *A.cordifolia* leaves using Soxhlet extraction following the addition of 3.0 L of absolute ethanol to 600 g of the powder in a round bottom flask heated at 60 °C, with continuous reflux for 16 h to get a filtrate. A rotary evaporator operating at 40 degrees Celsius concentrated the extract's solvent (filtrate) under reduced pressure, yielding a semi-solid residue. This residue was stored at 2-4 degrees Celsius in a sealed container in the refrigerator until it was needed.

3. Methods

3.1 Analysis of *Alchornea cordifolia* using High-Performance Liquid Chromatography (HPLC)

A UV-Vis DAD-equipped Waters 2695 Alliance HPLC system (Waters Inc., Milford, CT, USA) was used for the HPLC examination of flavonoids and phenolic chemicals. A 250 mm long, 4.6 mm wide, and 5 m thick Waters SunfireTM C18 reverse-phase chromatography column was used for the separation. Using an autoinjector, the already calibrated solutions (standards) and formulations were infused into the system at 200 µl/min. To establish an appropriate separation technique for the benchmarks for comparisons, various liquid chromatographic and gradient mobile phases were assessed at varied flow velocity and column temperature levels.

The gradient procedure was finally selected after a series of previous research and used a combination of acetonitrile (mobile phase A, HPLC grade 99.9%; Honeywell Seelze, from Germany) and phosphoric acid (mobile phase B), which was constituted gradual input of 85 per

cent orthophosphoric acid (Sigma-Aldrich, Merck, Darmstadt, Germany) to HPLC grade water (Carlo Erba) until pH=2 was reached. The method ran for a total of 60 minutes. The composition gradient was changed as follows: initially, five per cent A and ninety-five per cent B, then fifteen minutes of thirty-five per cent A and sixty-five per cent B, then twenty minutes of thirty-five per cent A and sixty-five per cent B, then thirty minutes of forty per cent A and sixty per cent B, then forty minutes of fifty per cent A and fifty per cent B, then fifty-two minutes of seventy per cent A and thirty per cent B, and finally sixty minutes of five per cent A and ninety-five per cent B. A temperature of 5 °C and a flowing pace of 0.5 mL/min were set up. Following a detailed analysis of the UV spectra of each standard, three wavelengths (250, 254, and 272 nm) were selected for investigation in this study using the HPLC-DAD.

3.2 Diet preparation and analysis

3.2.1 Composition and preparation of high-fat diet (HFD)

All components of the HFD were carefully weighed daily (to avoid spoilage) based on the ratio outlined below and mixed gently before administering to the animals. The ingredients of the HFD and their respective quantities were prepared as listed in Table 1.

3.2.2 Proximate and energy composition of the normal feed (NF) and high-fat diet (HFD)

Moisture, fat, ash, crude fibre, and protein content of the HFD and NF were analysed utilising the Association of Official Analytical Chemists-approved methodology AOAC [38] and presented in Table 2. Total energy was determined using the At-Water general factor system by multiplying fat by 37KJ/g, carbohydrate- by 14KJ/g, and protein - by 14KJ/g.

3.3 Animal studies:

3.3.1 Experimental animals

Male Wistar rats (120–170 g) were acquired from the Animals House and housed in plastic cages with stainless steel covers in the Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar (UNICAL), Cross River State. The animals were kept for one week to acclimatize; after that, they were administered a HFD to achieve an obese condition. They were randomly divided into control and experimental groups after obesity had been achieved. Ethical approval for the research was obtained from the University of Calabar Ethical Review Committee (with number FAREC/PA/UC/049/245BCM3823), which is in accordance with the ethical

standards as reviewed in the Principles of Laboratory Animal Care (NIH publication no. 8523, revised 1985).

3.3.2 Dietary induction of obesity

To achieve obesity in the test animals, they were administered consistently for four weeks the formulated HFD as well as received water freely. With few adjustments, the experimental HFD composition (in g/kg of diet) was derived using a similar composition (Basic feed- 67%, egg yolk powder- 10%, lard- 10%, sucrose- 10%, cholesterol- 2%, porcine bile salt- 0.8%, salt- 0.2%) provided by Wang *et al*. [39]. According to Rotimi et al. [40] and Novelli et al. [41], rats with BMIs of 20% higher than controls and Lee's indices of 0.3 and above were classified as obese.

3.3.3 Anthropometric measurements

These formulas were used to determine the Lee's Index and BMI, which were employed as indicators of obesity:

Lee's Index = (Body weights [g]) $^{1/3}$ **/** Nasal – anal length (cm)

BMI = Body weight (g) **/** Nasal – anal length ^{2} (cm^2)

TABLE 1:
Composition of high-fat diet

Ingredients	**Percentage quantity (per100g)**
Normal feed	64
Lard	15
Sucrose	10
Milk	5
Egg yolk powder	5
Sodium chloride	0.2
Porcine bile salt	0.8

TABLE 2:

Proximate and energy composition of the normal-feed (NF) and high-fat diet (HFD)

Proximate parameters	**High-fat diet (HFD)**	**Calorie (KJ/100g)**	**Normal feed (NF)**	**Calorie (KJ/100g)**
% Moisture Content	5.87±0.10		9.63±0.36	
% Fat	20.10±0.83	743.70	7.29±0.40	195.73
% Ash	3.20±0.18		11.18±0.85	
% Crude Fibre	9.19±0.87		12.78±1.00	

% Protein	13.06±0.87	222.02	20.57±1.04	269.73
% Carbohydrate	48.58±1.15	825.86	38.55±1.27	655.3
Total energy		**1791.58**		**1120.76**

3.3.4 Nutritional analysis

Daily food intake was recorded for each cage, and the average weekly consumption of diets was calculated. The animals were given a freshly prepared high-fat diet each day, and their feed intake was determined by weighing the remaining feed after consumption and subtracting it from the initial weight of the diet. The weekly caloric intake was calculated by adding the average weekly caloric intake to the calorie content of the diet.

I. Feed intake (g/day) = Initial weight of the diet - leftover weight of the diet consumed (9 weeks).

II. Energy intake (kJ/day) = Mean diet consumed x dietary metabolisable energy [41].

3.3.5 Animal handling, sacrifice/tissue sample collection, and preservation

The experimentally obese animals were separated into four new groups comprising six Wistar rats and normal rats and given the treatments. The rats were kept in a room with a temperature of 25 °C ± 3 °C and fed rat pellets and tap water as needed. After initiating treatment, the animals' body weights and fasting blood glucose/sugar (FBG/FBS) levels were assessed. These measurements were repeated weekly and biweekly for six (5) weeks. Meanwhile, the fasting blood glucose was measured weekly using one Touch® glucometer, and feed/energy intake and body weights were measured weekly with a digital weighing balance.

3.4 Experimental design

Six rats were assigned to each of the five groups after being acclimatized. The first group, referred to as the normal control (NC), was given a regular pelleted feed (7.29% fat) throughout the study. The second group, serving as the obese control (OC), was fed a high-fat diet (20 kJ% fat per 100g). The third group was given a standard drug, orlistat (30 mg/kg per body weight BW orally daily), along with the high-fat diet. The fourth and fifth groups were both fed the high-fat diet and given *A.cordifolia* orally at 500 and 1000 mg/kg BW daily. Throughout the experiment, the weights of all rats in the different groups were recorded. The reference anti-obesity drug- Orlistat, was made to dissolve in deionized water and administered to the rats at 30 mg/kg dosage. Likewise, the ethanol extract of *A. cordifolia* leaves was diluted in distilled water before being administered to

the rats. This study used two dose regimes of 500 mg/kg and 1000 mg/kg. The choice of these doses stemmed from an LD_{50} study we conducted according to the method of Lodhi *et al.* [42] and Singh *et al.* [43], which showed that ethanol extract of *A.cordifolia* leaves was not toxic since LD50 was greater than 5000 mg/kg body weight. All pharmacological treatments were administered orally. The duration of the administration was 5 weeks, commencing from the start of the 5th week and concluding on the 9th week.

3.5. Sampling and tissue preparation

At week 9, the animals were fasted overnight and given ketamine (2 uL/kg b.w.) anaesthesia after a nine-week period before blood was collected. Blood was obtained via a puncture in the heart and left at a temperature of 37 ◦C for 30 minutes. This was followed by centrifugation at 3000 x g for a duration of 10 minutes to separate the serum. The resulting separated serum was then stored at -4°C until it was ready to be used for laboratory analysis. The liver, adipose (perirenal and epididymal), and heart tissues were collected immediately, weighed, and stored in sample bottles containing normal saline for biochemical assay. The liver tissue that was separated from other tissues was blended with 10 times the amount of ice-cold 0.05 M potassium phosphate buffer (pH 7.4), and then placed in a centrifuge, which was set at 3000 x g for 10 minutes at 4 ◦C. After centrifuging, the resulting supernatant was kept at -80 ◦C and used for biochemical analysis. For the purpose of histological examination, a part of the liver tissues was stored in 10% formalin.

3.6. Serum biochemical assays

3.6.1 Fasting blood glucose (FBG), serum glucose, α-Amylase and insulin levels

Once a week, the animals were fasted and their fasting blood glucose (FBG) was measured for nine consecutive weeks using a glucometer-Accu-chek Performa. (manufactured by Roche in Germany). On the 9^{th} week after the experiment, serum glucose, α-Amylase and insulin levels was assayed from serum of blood collected from the animals. According to the directions on the Randox® kit, the glucose oxidase method [44] was used to measure the amount of glucose in the serum (Randox® Laboratories Ltd., Ardmore, United Kingdom). Furthermore, serum α-Amylase was assayed following the instructions in the MTD diagnostics kit. The insulin level was determined using an enzyme-linked immunoassay (ELISA) kit from Elabscience® USA, Catalog No: E-El-R3034.

Insulin resistance (IR) was calculated by making use of the IR homeostatic evaluation model (HOMA-IR) was calculated according to the following formula

$$\text{HOMA} - \text{IR} = \frac{\text{fasting glucose (mg/dl)} \times \text{fasting insulin } (\mu\text{U/l})}{405}$$

3.6.2 Determination of serum lipid profile and lipase activity

Fasting serum concentrations of total cholesterol (TC) and triacylglycerol (TG) were evaluated by standardised enzymatic colourimetric techniques employing a Fortress Diagnostics Ltd. assay kit (Antrim, UK). By using an enzyme-mediated clearance technique, high-density lipoprotein cholesterol (HDL-c) was assessed (Fortress Diagnostics Ltd. (Antrim, UK) LDL-c and VLDL-c values, on the other hand, were determined using Friedewald's formula, as stated by Friedewald *et al.* [45].

$$\text{LDL} - \text{C (mg/dl)} = \text{Total Cholesterol} - (\text{Triglycerides}/5 + \text{HDL} - \text{C})$$

$$\text{VLDL} - \text{C (mg/dl)} = \text{Triglygeride}/5$$

Lipase activity was determined using Fortress Diagnostics Ltd. Northern Ireland, U.K.

3.6.4. Assessment of cardiac and liver function

Lactate dehydrogenase (LDH) and creatine kinase (CK) were determined to assess heart function using MTD diagnostics kit. Additionally, alanine transaminase (ALT), alkaline phosphatase (ALP), aspartate transaminase (AST), total protein, and bilirubin were measured for assessment of liver function using Randox kit. All instructions and procedures were carried out as outlined by the manufacturer.

3.7. Atherogenic ratios

The Atherogenic ratios were calculated according to the methods of Bhardwaj *et al.* [46], and the formulae are as follows:

Atherogenic Index of Plasma (AIP) = log TG/HDLc,

Cardiac risk ratio (CRR), = TC/HDLc

Castelli's Risk Index (CRI) = LDLc/HDLc

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

3.8. Determination of tissue lipids

Determination of lipids in tissues was preceded by tissue homogenisation and extraction, after which the individual assays were carried out using the tissue lipid extract as samples (except serum). Tissue lipids were extracted and purified using the method of Folch *et al.* [47]. The lipids

were extracted by homogenizing 0.2g of each tissue (liver and adipose) in 1.8 ml of methanol-chloroform (1: 2 volume/volume ratio). The homogenates were transferred into 2 ml Eppendorf tubes and left to stand at a freezing temperature for approximately 10 minutes. Thereafter, the homogenates were centrifuged at 2400 r.p.m for 10 minutes. Furthermore, a biphasic system with a lipid-containing lower layer of 75 per cent chloroform and an upper layer of 25 per cent aqueous methanol containing non-lipid components like cerebrosides and gangliosides (i.e. lipid extract) was observed. The chloroform layer was carefully aspirated into clean and correctly labelled tubes using micropipettes. The extract was further washed by adding 0.2ml 0.05MKCl and centrifuged at 2400 r.p.m for 10 min. The uppermost non-lipid portion was carefully removed using a 20-ml micropipette, and the residual bottom chloroform layer was stored at -20 °C until further investigation.

3.8.1 Estimation of total lipids in tissue homogenates

Using an Ecotherm Heating or Chilling dry bath, 200ul of the lipid extracted from tissues was pipetted into sample tubes that had been previously weighed and dried at 50 °C. Calculations were made to determine the total lipids' weight by dividing the weight of the previously weighed tubes before drying at 50 °C by the weight of the tubes after they had dried, and the final result was represented as milligram/gram of tissue [47].

3.8.2 Estimation of total phospholipids in tissue homogenates

The Connerty et al. [48] method was used to estimate the phospholipids. The lipid extract (100 ml) was evaporated until dry using an Ecotherm Heating or Chilling dry bath at 60 °C. To this, 430ul of distilled water and 50ul of molybdate II reagent (2.5% ammonium-molybdate in 3N H_2SO_4) were added, and the mixture was left to stand at room temperature for 10 minutes. Next, 20ul of amino-napthosulphonic acid (ANSA) reagent (0.5gms of ANSA in 195 ml of 15% sodium bisulphite and 5ml of 20% sodium sulphite) was added, and the mixture was incubated for 20 minutes at room temperature. The quantity of phospholipids was determined by multiplying the absorbance measured at 660 nm by 25, and the results were expressed as mg/100ml.

3.8.3 Triacylglycerol estimation in tissue homogenates

Lipids that had already been extracted were measured into clean autoclaved sample tubes and 50ul of them were evaporated to dryness at 60°C using Ecotherm Heating or Chilling dry bath. Next, 200ul of ethanol was added to the sample tubes, and the mixture was vigorously stirred using a

mechanical stirrer. Then, the Triacylglycerol present in the solution was determined using the Fortress Diagnostics Ltd. assay kits, following the manufacturer's instructions.

3.8.4 Estimation of total cholesterol in tissue homogenates

First, 50ul of the lipid extracted from tissues was transferred into sterile sample tubes, evaporated at 60 °C in the Ecotherm Heating/Chilling dry bath, and dried to a dry consistency. Then, 20 µl of Triton-X100: Chloroform was then added (1:1). This solution was used as the sample for cholesterol determination using the Randox® assay kit.

3.9 Oxidative stress markers

For sample preparation, 0.2g of each organ/tissue (liver and adipose tissue) was homogenized in a homogenizing cup with 1.8 ml of ice-cold homogenizing buffer (pH 7.2) containing 125mM sucrose, 125mM mannitol, 1mM EGTA, and 5mM HEPES using a Teflon pestle homogenizer. The homogenate was then transferred into a 2ml Ependorf microtube and centrifuged at 3000rpm for 10 minutes. The resulting supernatant was stored at -20 °C until it was analyzed for lipid peroxidation and antioxidant indicators.

To evaluate oxidative stress biomarkers, malondialdehyde (MDA) levels were assessed using the protocol described by Buege and Aust [49]. Additionally, the contents of reduced glutathione (GSH) were measured based on Ellman's method [50].

3.10. Antioxidant enzymatic activities

The evaluation of superoxide dismutase (SOD) and catalase (CAT) activities was performed using the techniques outlined in the study conducted by Zou et al. [51]. Additionally, glutathione peroxidase (GPx) activities were determined in accordance with Ellman's method [50], while the estimation of glutathione-S-transferase activity was based on the methods described by Habig et al. [52].

3.11 Evaluation of Hydroxylmethylglutaryl (HMG)-CoA reductase activity in Liver homogenates

The method of Rao and Ramakrishnan [53] was used to extract tissue for the indirect evaluation of HMG-CoA reductase activity.

To assess the variation in the activity of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (NADPH) in liver tissue, the 3-Hydroxy-3-methylglutaryl-CoA and mevalonate concentrations in the tissue homogenate were estimated and the ratio between the two was taken as an index of the enzyme's activity. This enzyme catalyzes the conversion of 3-hydroxy-3-methylglutaryl-CoA to

mevalonate. The ratio increases when the activity of this enzyme in the liver decreases (e.g. fasting, cholesterol feeding, and the effect of statins and related compounds) and decreases when the activity of this enzyme increases (e.g. high energy diets).

HMG-CoA was determined by reacting with hydroxylamine at weakly acidic pH and measuring the resulting hydroxamic acid's colorimetric properties by forming complexes with ferric salts.

Alkaline hydroxylamine was used to estimate specifically HMG-CoA only, as mevalonate interferes in this estimation at acid or neutral pH. Additionally, interference by coenzyme A was minimal when readings were taken at 540 nm.

The procedure involves mixing equal amounts of fresh 10% tissue homogenate and diluted perchloric acid, allowing the mixture to stand at room temperature for 5 minutes, and then centrifuging it at 2000 rpm for 10 minutes. After that, 1.0 ml of the supernatant is combined with 0.5 ml of freshly prepared hydroxylamine reagent (alkaline hydroxylamine reagent in the case of HMG-CoA), and then mixed. After 5 minutes, 1.5 ml of ferric chloride reagent is added to the same tube and vortexed. The readings are taken after 10 minutes at 540 nm versus a similarly treated saline/arsenate blank. Mevalonate is estimated using the same reagents but at a more acidic pH of 4.1 using the hydroxylamine hydrochloride reagent for mevalonate. At this pH, the lactone form of mevalonate reacts with hydroxylamine to form the hydroxamate. The tissue mevalonic acid ratio is calculated using the following formula:

$$\text{Tissue Mevalonic Acid Ratio (TMAR)} = \frac{\text{Absorbance of HMG} - \text{CoA at 540nm}}{\text{Absorbance of mevalonate at 540nm}}$$

3.11.1 Determination of the magnitude of cholesterol synthesis (MCS)

The percentage of mevalonic acid synthesis per gram liver weight was calculated by dividing the fresh weight of the entire animal liver by TMAR and multiplying the result by 100. This calculation allowed for the determination of the magnitude of cholesterol synthesis per gram liver weight of fresh wet tissues.

$$\text{MCS (\%/g liver weight)} = \frac{\text{Fresh weight of liver}}{\text{TMAR}} \times 100$$

Where:

MCS = Magnitude of Cholesterol synthesis (MCS)

TMAR = Tissue Mevalonic Acid Ratio

3.11.2 Percentage reduction in HMG-CoA reductase activity:

This was calculated as 100 - MCS%

Where MCS = Magnitude of cholesterol synthesis

3.12. Histological examination

Histological examinations were conducted to evaluate the liver function and fat accumulation in the white adipose tissue. The liver and adipose tissues were fixed in 10% buffered formaldehyde and then embedded in paraffin. Following that, the embedded tissue samples were sectioned (5 μm) and stained with hematoxylin and eosin to observe the general histological features. The intensity of hepatic tissue damage and deformity of adipose tissues' fat cells was assessed and documented.

4. Results

TABLE 3:
Identity, peak area, height, and the retention time for various alkaloids, flavonoids, and phenolics in *A.cordifolia* leaf samples.

Compound	Peak No.	Peak ID	Ret Time	Height	Area	Conc (μg/100g)
Alkaloids	1	Unidentified	0.12	1934.19	8034.85	0.03
	2	Gallic acid	0.57	465.74	2248.40	0.01
	3	Caffeic acid	1.46	594713.80	9508041.00	41.33
	4	Coumaric acid	1.67	524107.80	3841493.00	16.70
	5	Sinapic acid	1.77	503388.70	9073413.00	39.44
	6	Ellagic acid	3.88	7580.42	572009.90	2.49
Flavonoids	1	Unidentified	0.10	738.33	1574.90	0.01
	2	Chlorogenic Acid	1.39	602104.63	8271610.00	39.63
	3	Benzoic acid	1.58	526960.06	12324179.00	59.04
	4	Quercetin	5.54	3414.01	277279.94	1.33
Phenolics	1	Unidentified	0.03	614.57	521.85	0.00
	2	Salicylic acid	0.11	3356.89	796.85	0.00
	3	Unidentified	1.13	346683.00	1975516.88	3.81
	4	Myricetin	1.30	581049.75	5260227.50	10.14
	5	Luteolin	1.54	915602.75	12300992.00	23.72
	6	Catechin	1.71	955172.50	14203063.00	27.39
	7	Gallic acid	1.97	568230.19	7929978.50	15.29
	8	Apigenin	2.32	325226.00	2239196.75	4.32
	9	Kaempferol	2.45	380470.84	7947065.00	15.32

FIG 1: HPLC chromatogram for alkaloids in *A.cordifolia* leaf samples

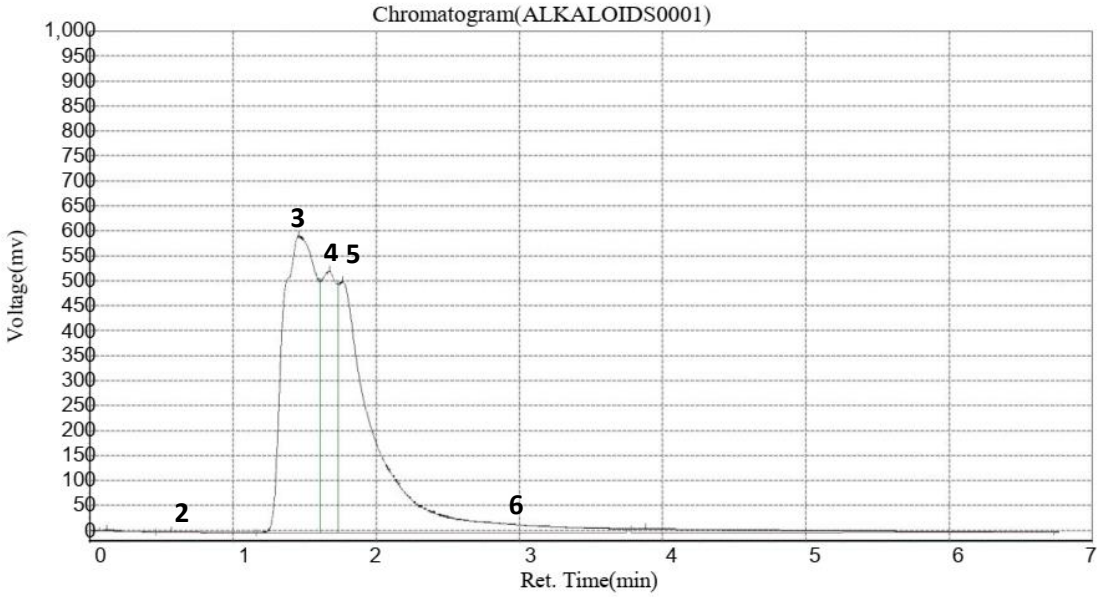


FIG 2: HPLC chromatogram for flavonoids in *A.cordifolia* leaf samples

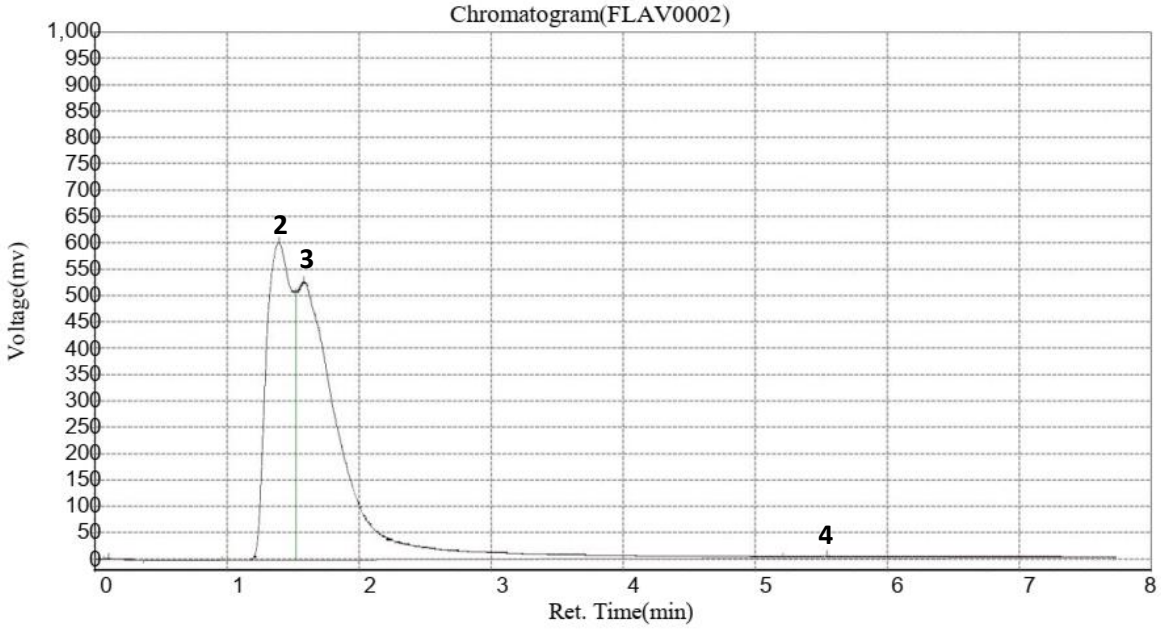
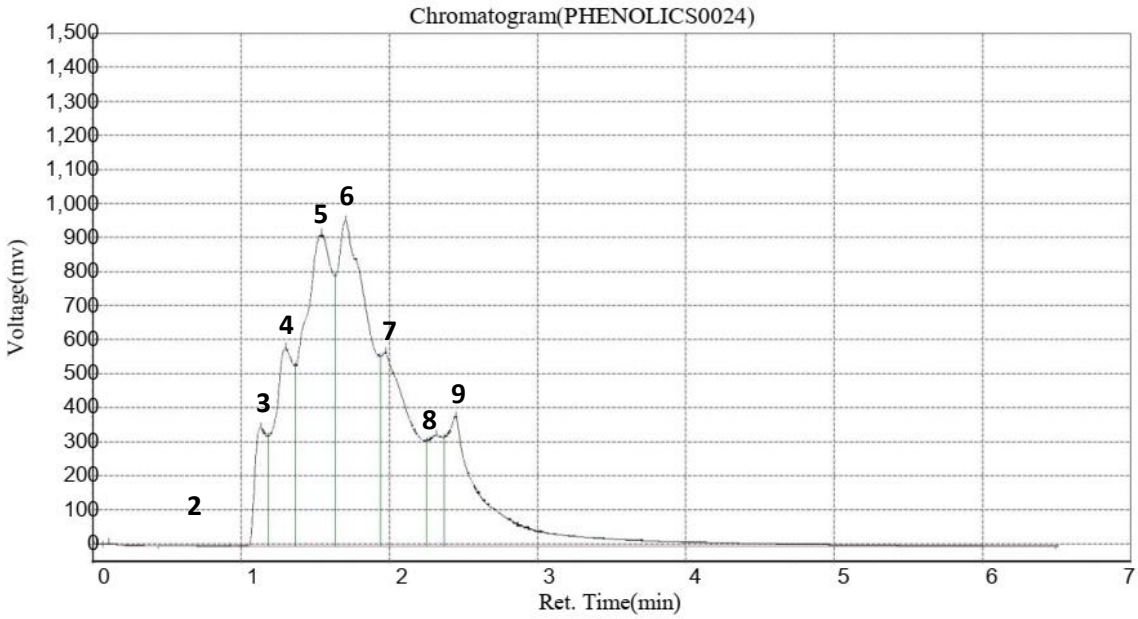


FIG 3: HPLC chromatogram for phenols in *A.cordifolia* leaf samples



4.1 High-performance liquid chromatography (HPLC) analysis

Table 3 summarizes the relative abundance of the specific phytochemicals belonging to the major classes of alkaloids, flavonoids, and phenols. The respective HPLC chromatographs from which the results were computed are presented in Figs. 1-3. The HPLC results for the alkaloids in the *A.cordifolia* leaf ethanol extract indicated six compounds, namely Gallic acid, Caffeic acid, Coumaric acid, Sinapic acid, Ellagic acid and on unidentified compound. It was noticed that Caffeic acid, Sinapic acid, and Coumaric acid had the highest concentration compared with other compounds and was between 17 - 41 µg/100g. For flavonoids, results showed the presence of four compounds of which they identified as Chlorogenic Acid, Benzoic acid, Quercetin, and one unidentified compound. The concentration for these compounds ranged between 40 – 60 µg/100mg. Furthermore, nine phenolic compounds were identified in *A.cordifolia* leaf samples. Salicylic acid, Myricetin, Luteolin, Catechin, Gallic acid, and Kaempferol were the compounds

identified with concentrations between 3.81- 27.39 µg/100g. Luteolin and Catechin had the highest concentration, followed by Kaempferol and Gallic acid.

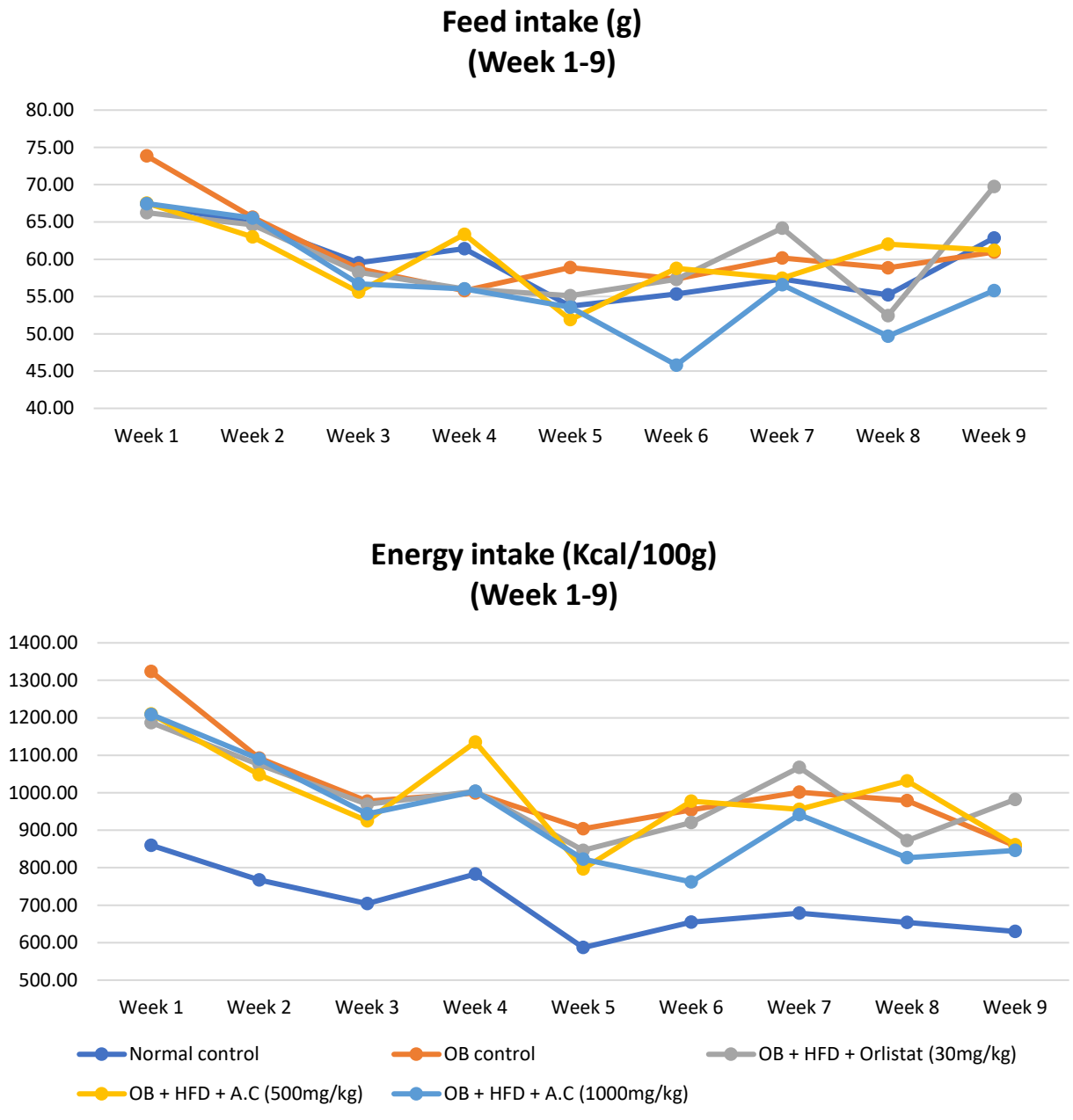


FIG 4: Feed intake and energy consumption (Kcal per day) in rats given a HFD for nine weeks and administered with *A.cordifolia*; (n = 6)**.**

4.2 Nutritional and energy composition analysis of normal feed (NF) and high-fat diet (HFD)

4.2.1 Effects of *A. cordifolia* on feed and energy intake of normal feed (NF) and high-fat diet (HFD) given to Wistar rats

The food intake (g) and energy intake (Kcal/day) of rats fed HFD and *A. cordifolia* treatment for nine weeks are reported in Figure 4. At the beginning of the study, the daily feed intake of all the groups had a similar average. However, as treatment progressed, specifically between weeks 6 and 9, a clearly noticeable reduction in food consumption of obese rats treated with 1000 mg/kg b.w of *A. cordifolia* was observed. Additionally, energy intake for all groups was substantially more than that of the normal control group at week one. However, after treatment at week 9, the energy intake of all treatment groups reduced significantly with the obese group treated with 1000 mg/kg b.w of *A. cordifolia* showing a more significant effect.

4.2.2 Effect of *A. cordifolia* on anthropometric indices of obese rats produced by a high-fat diet consumption.

The mean of body weight, percentage weight gain, body mass index (BMI), Lee's index, and waist measurements prior to and after inducing obesity in the animals, as well as after treatment are presented in Table 4. At Week 0, the body weights were within 150-170 g. However, after induction at Week 4, the normal control group (180.30±10.42 g) had significantly lower body weight compared with the obese (OB) control (negative control group) (231.05±18.17 g), while the percentage weight gain of all treatments compared with the normal control group was between 52.50% - 67.81%. At the end of treatment in the 9th week, there was significant ($p<0.05$) weight reduction detected in all treatment groups when compared with the obese (OB) control group with 1000mg/kg b.w treated animals having the lowest mean body weight. Furthermore, a similar dose-dependent reduction trend was seen in the percentage difference in body weight, Lees's index, BMI,

and waist circumference between weeks 4 and 9 by 500mg/kg and 1000mg/kg of *A.cordifolia* treatment administered to obese rats compared to that of the control group of obese animals.

TABLE 4:

Mean of body weight, percentage changes of body weight, lee's index, body mass index (BMI), and waist circumference of rats between Weeks 0-9.

Groups	Treatments	Week 0	Week 4	Week 9	% Change btw (Week 4 and 9)			
					Body Weight	Lee's Index	BMI	Waist circumference
1	Normal control	152.25±7.27^{c}	180.30±10.42^{b}	205.08±10.54^{c}	12.08	1.00	-1.26	1.20
2	OB control	156.69±4.18^{bc}	231.05±18.17^{a}	295.75±15.46^{a}	21.88	3.83	14.02	13.54
3	OB + HFD + Orlistat (30mg/kg)	168.84±8.58^{a}	219.22±13.26^{a}	243.13±17.98^{b}	4.45	-27.92	-63.16	-18.18
4	OB + HFD + A.C (500mg/kg)	166.46±9.36^{ab}	217.94±11.16^{a}	236.97±13.52^{b}	9.83	-24.94	-50.36	-20.31
5	OB + HFD + A.C (1000mg/kg)	162.73±12.61^{abc}	220.50±13.91^{a}	233.01±13.39^{b}	5.76	-35.33	-83.34	-16.67

Results in table 4 are presented as mean±SEM, n=6. Means with comparable superscript letter are not significantly distinct from one another ($p>0.05$). OB- obese rats; HFD- High-fat diet; A.C- *Alchornea cordifolia*

TABLE 5:

Effect of *A.cordifolia* on relative adipose tissue, liver and heart weights of HFD – induced obese rats

Groups	Treatments	Relative Adipose tissue weight (g)	Relative Liver weight (g)	Relative Heart weight (g)
1	Normal control	0.97±0.18^{a}	2.72±0.01^{a}	0.26±0.07^{a}
2	OB control	2.69±0.59^{c}	2.77±0.04^{a}	0.30±0.01^{a}
3	OB + HFD + Orlistat (30mg/kg)	1.97±0.12^{bc}	2.62±0.05^{a}	0.27±0.01^{a}
4	OB + HFD + A.C (500mg/kg)	1.75±0.20^{ab}	3.06±0.07^{a}	0.29±0.01^{a}
5	OB + HFD + A.C (1000mg/kg)	1.65±0.47^{ab}	2.96±0.51^{a}	0.28±0.02^{a}

Results in Table 5 are presented as mean±SEM, n=6. Means with comparable superscript letter are not significantly distinct from one another ($p<0.05$). OB- obese rats; HFD- High-fat diet; A.C- *Alchornea cordifolia*

4.3 Effect of *Alchornea cordifolia* relative organ weight

The effect of *A.cordifolia* on relative organ weight of High Fat Diet (HFD) – induced obese rats is presented in Table 5. The relative organ weight values of HFD control were significantly ($p < 0.05$) higher for adipose tissue only. However, liver and heart relative weights were non-significantly higher when compared with normal control. *A.cordifolia* treatment significantly ($p < 0.05$) lowered the relative organ weight of adipose tissue. At the same time, there was a dose-dependent, non-significant decrease in relative organ weight of the liver and heart compared to HFD control.

4.4 Effect of *Alchornea cordifolia* on serum lipid profile and ratios of high-fat diet (HFD) - induced obese Wistar rats

Table 6 shows the changes in the serum lipid levels and ratios of obese rats fed with HFD and orally administered ethanol extract of *A. cordifolia*. The rats given HFD for 9 weeks (obese control) demonstrated a significant rise ($p < 0.05$) in serum TC (123.76±6.48 mg/dL), TG (122.97±14.90 mg/dL), and LDL-c (78.25±0.75 mg/dL) and significant decrease ($p < 0.05$) in HDL-c (20.92±2.75 mg/dl) concentration when compared with the normal rats group.

TABLE 6:

Effect of orally administered ethanol extract of ***Alchornea cordifolia*** **(A.C)** on serum lipid levels and ratios in the HFD-induced obese rats.

Groups	Treatments	TC (mg/dl)	HDL-c (mg/dl)	TG (mg/dl)	LDL-c (mg/dl)	Lipid ratios			
						HDL-c/TC %	TC-HDL-c/HDL-c	Log TC/HDL-c	TC/HDL-c
1	Normal control	56.38±1.84^{c}	39.50±1.38^{a}	44.98±4.44^{b}	7.89±0.43^{e}	70.05±0.17^{a}	0.43±0.01^{c}	0.06±0.01^{b}	1.43±0.01^{c}
2	OB control	123.76±6.48^{a}	20.92±2.75^{d}	122.97±14.90^{a}	78.25±0.75^{a}	16.85±0.77^{d}	4.96±0.27^{a}	0.77±0.01^{a}	5.96±0.27^{a}
3	OB + HFD + Orlistat (30mg/kg)	58.51±0.61^{c}	25.78±0.25^{c}	28.92±2.60^{c}	26.95±0.16^{c}	44.06±0.02^{c}	1.27±0.01^{b}	0.05±0.02^{b}	2.27±0.01^{b}
4	OB + HFD + A.C (500mg/kg)	73.40±3.35^{b}	31.36±2.32^{b}	36.14±2.00^{bc}	34.82±5.27^{b}	42.87±2.96^{c}	1.35±0.16^{b}	0.06±0.03^{b}	2.35±0.16^{b}
5	OB + HFD + A.C (1000mg/kg)	61.99±1.76^{c}	38.09±2.63^{a}	30.88±4.10^{c}	17.73±1.69^{d}	61.39±1.44^{b}	0.63±0.04^{c}	-0.09±0.01^{c}	1.63±0.04^{c}

Results in Table 6 are presented as mean±SEM, n=6. Means with comparable superscript letter are not significantly distinct from one another ($p<0.05$). OB- obese rats; HFD- High-fat diet; A.C- *Alchornea cordifolia*. Total cholesterol (TC), High-density lipoprotein cholesterol (HDL-c), Triglyceride (TG), Low-density lipoprotein cholesterol (LDL-c), Atherogenic coefficient (AC), Atherogenic index (AI), Cardiac Risk Ratio (CRR).

TABLE 7:

Effect of *A.cordifolia* on triglyceride (TG), total cholesterol (TC), total lipids (TL), and phospholipids (PL) levels in the liver and adipose tissue of HFD-induced obese Wistar rats

Groups	Treatments	Liver				Adipose tissue			
		TG (mg/dl)	**TC (mg/dl)**	**TL (mg/g)**	**PL (mg/ml)**	**TG (mg/dl)**	**TC (mg/dl)**	**TL (mg/g)**	**PL (mg/ml)**
1	Normal control	168.46±39.86^{d}	77.16±14.71^{b}	0.04±0.00^{c}	12.38±0.42^{a}	150.36±0.00^{c}	86.52±0.25^{b}	0.04±0.01^{c}	11.79±0.25^{cd}
2	OB control	395.67±61.48^{a}	315.74±28.48^{a}	0.14±0.02^{a}	14.86±1.75^{a}	233.58±0.00^{a}	158.70±6.78^{a}	0.17±0.01^{a}	14.58±0.18^{a}
3	OB + HFD + Orlistat (30mg/kg)	253.36±11.09^{bc}	87.24±29.60^{b}	0.06±0.00^{b}	12.99±0.32^{a}	153.87±8.14^{c}	93.91±8.72^{b}	0.12±0.00^{b}	12.70±0.76^{b}
4	OB + HFD + A.C (500mg/kg)	294.71±5.35^{b}	75.93±8.56^{b}	0.05±0.01^{bc}	14.73±0.31^{a}	184.43±30.77^{b}	90.87±13.30^{b}	0.15±0.01^{a}	12.39±0.43^{bc}
5	OB + HFD + A.C (1000mg/kg)	211.15±20.79^{cd}	65.84±18.05^{b}	0.05±0.01^{bc}	13.07±0.18^{a}	181.51±21.86^{b}	89.86±7.91^{b}	0.05±0.02^{c}	11.34±0.04^{d}

Results in Table 7 are presented as mean±SEM, n=6. Means with comparable superscript letter are not significantly distinct from one another ($p<0.05$). OB- obese rats; HFD- High-fat diet; A.C- *Alchornea cordifolia*. Triglyceride (TG), Total cholesterol (TC), Total lipids (TL), and Phospholipids (PL)**.**

TABLE 8:

Effect of *A.cordifolia* on triglyceride (TG), total cholesterol (TC), total lipids (TL), and phospholipids (PL) levels in the heart of HFD-induced obese Wistar rats

Groups	Treatments	Heart TG (mg/dl)	Heart TC (mg/dl)	Heart TL (mg/g)	Heart PL (mg/ml)
1	Normal control	1.81 ± 0.05^{ab}	184.48 ± 10.88^{ab}	0.007 ± 0.001^{a}	1.63 ± 0.45^{a}
2	OB control	2.03 ± 0.02^{c}	242.76 ± 6.85^{c}	0.015 ± 0.001^{c}	6.29 ± 1.75^{c}
3	OB + HFD + Orlistat (30mg/kg)	1.88 ± 0.02^{bc}	207.82 ± 4.79^{b}	0.008 ± 0.001^{b}	1.99 ± 0.20^{ab}
4	OB + HFD + A.C (500mg/kg)	1.83 ± 0.01^{abc}	201.84 ± 6.48^{b}	0.010 ± 0.002^{b}	4.19 ± 0.24^{bc}
5	OB + HFD + A.C (1000mg/kg)	1.78 ± 0.01^{a}	163.79 ± 3.07^{a}	0.007 ± 0.000^{a}	1.82 ± 0.42^{ab}

Results in Table 8 are presented as mean±SEM, n=6. Means with comparable superscript letter are not significantly distinct from one another ($p<0.05$). OB- obese rats; HFD- High-fat diet; A.C- *Alchornea cordifolia*. Triglyceride (TG), Total cholesterol (TC), Total lipids (TL), and Phospholipids (PL)**.**

Furthermore, a significant ($p<0.05$) decrease in the percentage HDL-c/TC ratio (16.85±0.77) as well as a significant ($p<0.05$) elevation in TC-HDL-c/HDL-c (4.96±0.27), Log TC/HDL-c (0.77±0.01), and TC/HDL-c (5.96±0.27) values of the obese control group of animals fed with a HFD for 9weeks compared with that of normal control.

Additionally, administration of *A.cordifolia* 500mg/kg and 1000mg/kg b.w significantly ($p < 0.05$) increased the percentage HDL-c/TC ratio and decreased TC-HDL-c/HDL-c, Log TC/HDL-c, and TC/HDL-c values of both obese rats compared with obese control. The 1000mg/kg treatment of *A.cordifolia* had the most significant effect on lipid ratios and compared favorably with the conventional anti-obesity drug- Orlistat.

4.5 Effect of *A.cordifolia* on tissue lipids in high-fat diet (HFD) – induced obese rat model

Table 7 shows the changes in liver, adipose tissues and Table 8 heart tissues on triglyceride (TG), total cholesterol (TC), total lipids (TL), and phospholipids (PL) levels in HFD-induced obese rats administered with *A.cordifolia*. The obese (OB) control group had significantly ($p<0.05$) higher TG, TC, and TL concentrations in the liver, adipose and heart tissues than the normal group.

In the liver, both treatments with 500mg/kg and 1000mg/kg *A.cordifolia* significantly reduced ($p<0.05$) TG levels (294.71; 211.15 mg/dl) in obese rats compared with obese control rats (395.67 mg/dl). Similarly, for TC levels, treatment with both doses of *A.cordifolia* significantly reduced ($p<0.05$) TC content (75.93; 65.84 mg/dl) in the liver when compared to the obese control, and this effect was not profoundly distinct from that obtained from Orlistat administered group (87.24 mg/dl). Total lipids (TL) levels of 500mg/kg and 1000mg/kg of *A.cordifolia* treated obese rats significantly reduced, with 1000mg/kg b.w having the highest significant reduction.

In the adipose and heart tissues, both doses - 500mg/kg and 1000mg/kg of *A.cordifolia* administered to obese Wistar rats significantly ($p<0.05$) lowered TG, while Orlistat treated group showed more significant TG reduction compared with obese control in adipose tissue. The TC, TL and PL levels in the adipose tissue of obese rats were considerably and dose-dependently decreased as 1000mg/kg of *A.cordifolia* treatment showed the most significant reduction compared with all other treatment groups.

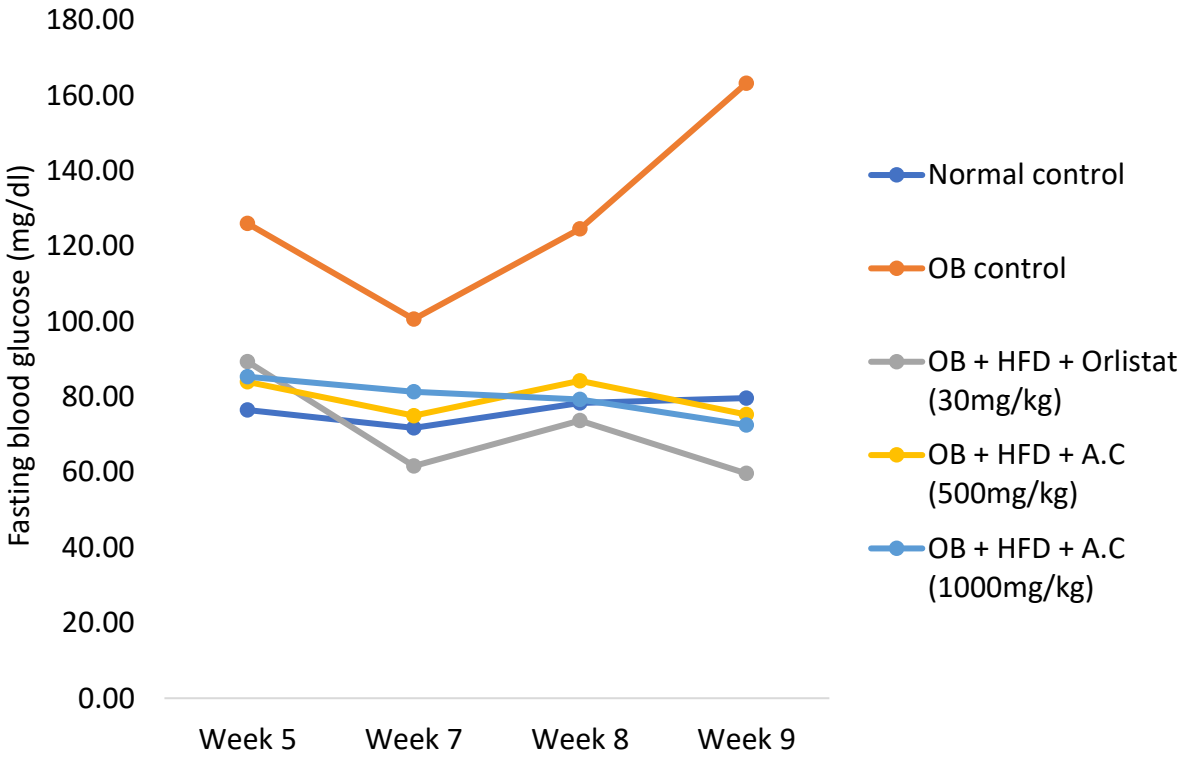


FIG 4: Graph depicting the effect of *A.cordifolia* on HFD-induced obese Wistar rats' fasting blood glucose (FBG).

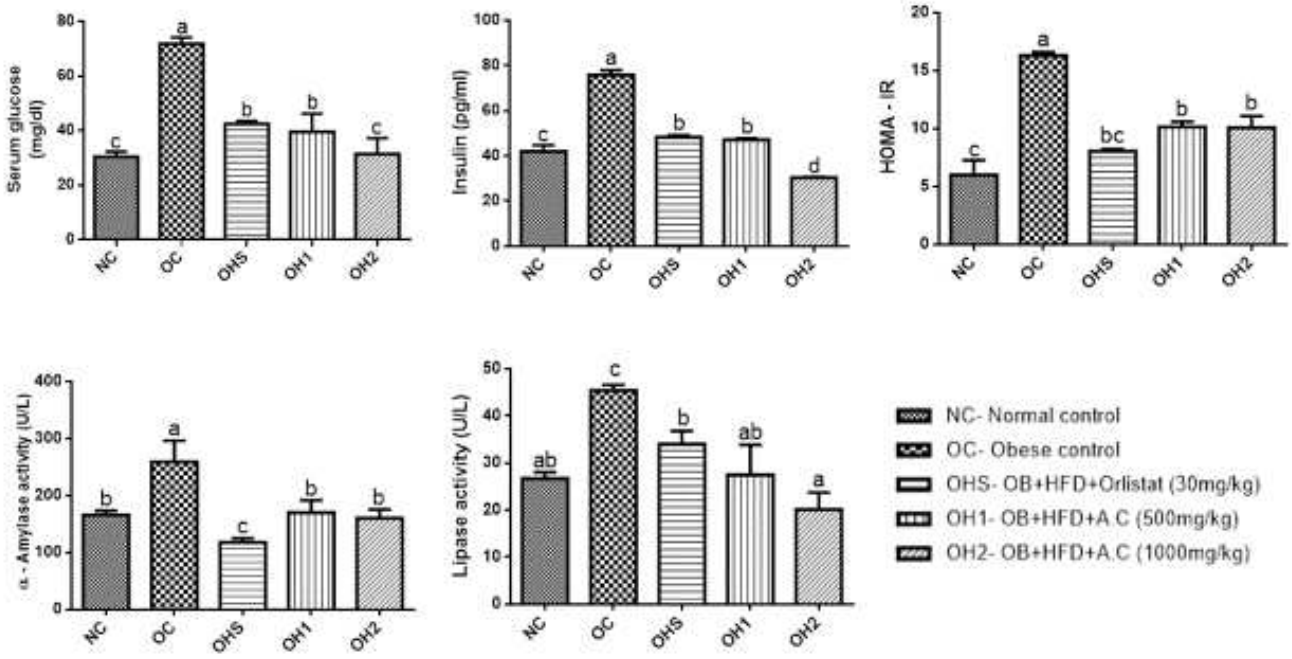


FIG 5: Bar charts showing the effect of *A.cordifolia* on serum glucose, insulin, HOMA-IR, amylase, and lipase activity of HFD-induced obese Wistar rats. Bars are presented as mean±SEM, n=6. Means with comparable superscript letter are not significantly distinct from one another ($p<0.05$). OB- obese rats; HFD- High-fat diet; A.C- *Alchornea cordifolia*

4.6 Hypoglycaemic potentials of *A.cordifolia* in high-fat diet (HFD) – induced obese rat model

4.6.1 Fasting blood glucose (FGB)

Figure 4 shows the effect of *A.cordifolia* on obese rats' fasting blood glucose (FBG) levels in comparison with control groups. Rats in the obese control group's fasting blood sugar levels showed significantly ($p < 0.05$) higher values in the course of the experiment, especially at week 7, where an astronomical increase began when compared with the normal control. Significant ($p<0.05$) reductions in fasting blood sugar levels of obese rats were observed after treatment with *A. cordifolia* extract (at doses of 500mg/kg and 1000mg/kg b.w) and the reference drug (Orlistat). These reductions were observed in a dose-independent manner compared to the obese control group at week 9 after treatment.

4.6.2 Effect on serum glucose, insulin, and HOMA-IR, amylase, and lipase activity

Figure 5 demonstrates the effect of *A.cordifolia* on serum glucose, insulin, HOMA-IR, amylase and lipase activity of HFD-induced obese Wistar rats. In comparison to the normal control group, obese control rats had a significant rise in serum glucose, insulin, HOMA-IR, amylase, and lipase values, respectively. Concurrent supplementation with both 500mg/kg and 1000mg/kg doses of *A.cordifolia* in obese rats reduced serum glucose levels, as well as ameliorated the excessively high insulin concentration and reduced significantly ($p<0.05$) HOMA-IR. The 1000mg/kg b.w treatment of *A.cordifolia* performed better as it reduced serum glucose, insulin levels, and HOMA-IR compared with other treatments and the obese control group. A dose-dependent decrease of amylase and lipase activities was observed after administering 500mg/kg and 1000mg/kg b.w of *A.cordifolia* to obese rats compared with obese control. Moreover, obese rats given the standard anti-obesity medication (Orlistat) showed the lowest amylase activity compared with other treatment groups.

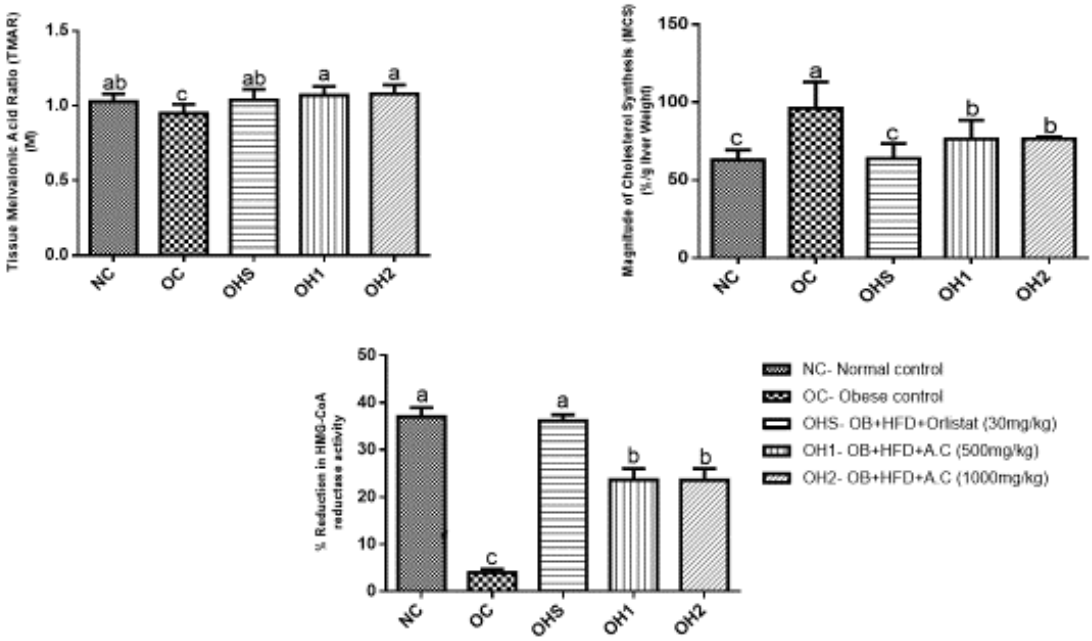


FIG 6: Effect of *A.cordifolia* on Hydroxylmethylglutaryl (HMG)-CoA reductase activity in liver homogenates of HFD induced obese Wistar rats. Bars are presented as mean±SEM, n=6. Means with comparable superscript letter are not significantly distinct from one another ($p<0.05$). OB-obese rats; HFD- High-fat diet; A.C- *Alchornea cordifolia*

TABLE 9:

Effect of *A.cordifolia* on malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), and glutathione peroxidase (GPx) levels in tissue homogenates of HFD – induced obese rats

Groups	Treatment	MDA (M)		SOD (mg/ml)		GSH (µM)		GPx (µM)	
		Liver	Adipose tissue	Liver	Adipose tissue	Liver	Adipose tissue	Liver	Adipose tissue
1	Normal control	0.16 ± 0.01^{b}	0.26 ± 0.06^{b}	30.16 ± 2.98^{b}	17.14 ± 1.28^{a}	6.75 ± 0.48^{a}	27.64 ± 7.01^{a}	4.17 ± 0.85^{a}	1.17 ± 0.07^{b}
2	OB control	1.01 ± 0.34^{a}	0.51 ± 0.08^{a}	15.91 ± 0.20^{d}	9.66 ± 1.35^{b}	1.79 ± 0.11^{c}	6.68 ± 2.84^{d}	0.39 ± 0.02^{c}	0.15 ± 0.01^{d}
3	OB + HFD + Orlistat (30mg/kg)	0.16 ± 0.03^{b}	0.14 ± 0.02^{c}	27.39 ± 0.12^{bc}	17.01 ± 1.35^{a}	4.48 ± 0.33^{b}	18.29 ± 1.11^{bc}	4.10 ± 1.20^{a}	0.71 ± 0.01^{c}
4	OB + HFD + A.C (500mg/kg)	0.23 ± 0.08^{b}	0.28 ± 0.05^{b}	26.01 ± 1.73^{c}	16.46 ± 1.42^{a}	4.83 ± 0.72^{b}	14.87 ± 1.35^{c}	1.40 ± 0.33^{bc}	1.48 ± 0.25^{a}
5	OB + HFD + A.C (1000mg/kg)	0.14 ± 0.03^{b}	0.24 ± 0.03^{b}	34.14 ± 0.38^{a}	18.74 ± 0.64^{a}	5.06 ± 0.56^{b}	23.78 ± 4.55^{ab}	2.00 ± 0.25^{b}	1.47 ± 0.10^{a}

Results in Table 9 are presented as mean±SEM, n=6. Means with comparable superscript letter are not significantly distinct from one another ($p<0.05$). OB- obese rats; HFD- High-fat diet; ND- Normal diet; A.C- *Alchornea cordifolia*; Malondialdehyde (MDA), Superoxide dismutase (SOD), Glutathione (GSH), and Glutathione peroxidase (GPx)

4.7 Effect of *A.cordifolia* on Hydroxylmethylglutaryl (HMG)-CoA reductase activity in liver homogenates of High Fat Diet (HFD) – induced obese rat

Figure 6 shows the effect of *A.cordifolia* on hydroxyl-methyl glutaryl (HMG)-CoA reductase activity in liver homogenates of HFD-induced obese Wistar rats. The Tissue Melvalonic Acid Ratio (TMAR) of HFD-fed rats was significantly ($p < 0.05$) reduced when compared with normal control. The 500mg/kg and 1000mg/kg per body weight of *A.cordifolia* administrated to the rats significantly ($p<0.05$) increased TMAR compared with obese control. Results showing the magnitude of cholesterol synthesis (MCS) per gram liver weight of the obese control group indicated that there was a significant ($p<0.05$) increase when compared with the normal control group. Opposite to TMAR results, administration of both 500mg/kg and 1000mg/kg body weight of *A.cordifolia* significantly ($p<0.05$) reduced MCS values compared with obese control. On the other hand, the percentage reduction in HMG-CoA reductase activity in HFD-fed rats was significantly ($p<0.05$) lowered when compared with the normal group of rats. Both 500mg/kg and 1000mg/kg treatment groups had similar percentage increases in HMG-CoA reductase activity compared with the obese control group.

4.8 Effect of *A.cordifolia* on oxidative stress indicators in tissue homogenates of high-fat diet (HFD) – induced obese rats

The effects of *A.cordifolia* treatment on enzymatic and non-enzymatic antioxidant levels in hepatic and adipose tissue of obese Wistar rats are shown in Table 9. Lipid peroxidation expressed by malondialdehyde (MDA) was significantly ($p < 0.05$) elevated in the liver (1.01±0.34 M) and adipose tissue (0.51±0.08 M) of obese rats' group in contrast to that of the normal group of Wistar rats (0.16±0.01; 0.26±0.06 M). However, administration of 1000mg/kg of *A.cordifolia* extract led to a substantial ($p < 0.05$) decrease of these MDA values of liver and adipose tissue (0.14±0.03; 0.24±0.03 M) to almost normal values in the liver (0.16±0.01 M) and adipose tissue (0.26±0.06 M) respectively. Superoxide dismutase (SOD) results showed that the activities of this enzyme were decreased in the liver (15.91±0.20 mg/ml) and adipose tissue (9.66±1.35 mg/ml) of the obese control group compared with normal control (30.16±2.98 mg/ml and 17.14±1.28 mg/ml). At the same time, *A.cordifolia* concerning glutathione (GSH), and glutathione peroxidase (GPx), significant reductions ($p < 0.05$) in these antioxidant enzymes' activity were seen in the liver (15.91±0.20 and 1.79±0.11 µM) and adipose tissue (9.66±1.35 and 6.68±2.84 µM) were seen in

obese control rats compared with their levels in the normal control rats. Moreover, as shown in Table 7, the activities of antioxidant enzymes were significantly ($p<0.005$) enhanced in a dose-dependent manner by administering *A.cordifolia* extract. The 1000mg/kg body weight treatment of *A.cordifolia* almost doubled the level of GSH in adipose tissue of 500 mg/kg treatment. In summary, an appreciable improvement in the concentration and activities of these antioxidants (non-enzymatic and enzymatic) was seen in the *A.cordifolia*-treated rats in contrast with the obese control group.

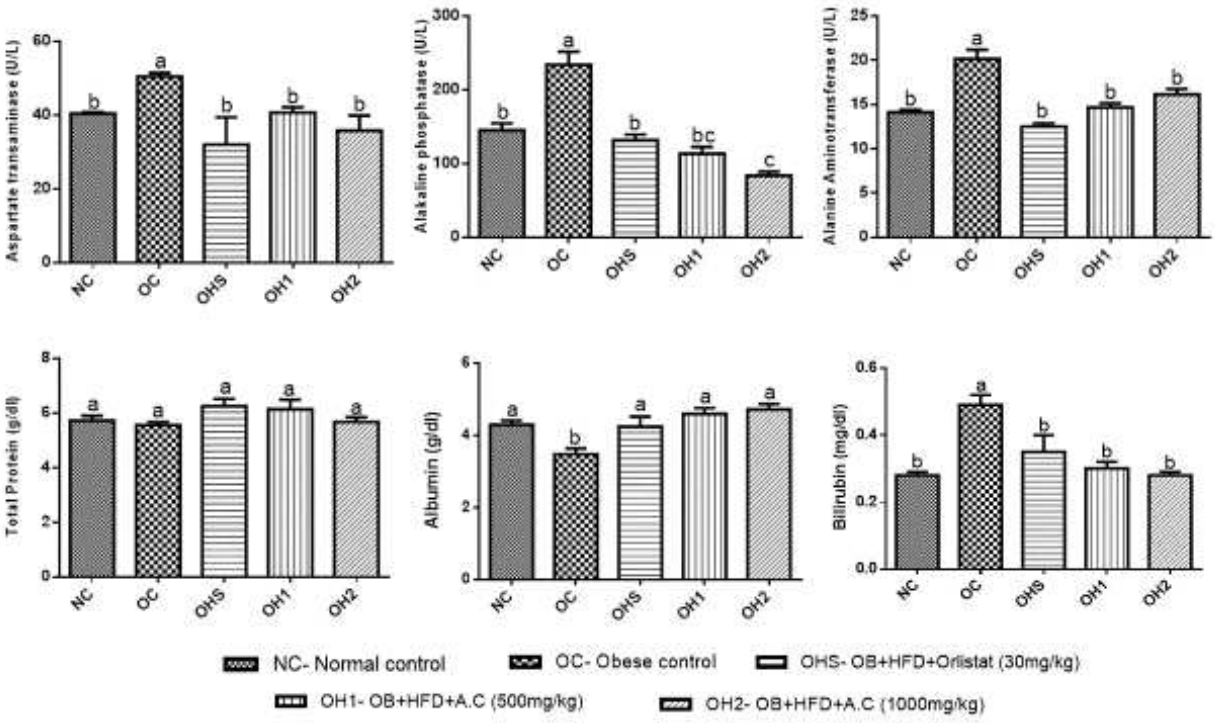


FIG 7: Effect of *A.cordifolia* on serum AST, ALT, ALP, total protein, albumin, and bilirubin activity of HFD induced obese wistar rats. Bars are presented as mean±SEM, n=6. Means with comparable superscript letter are not significantly distinct from one another ($p<0.05$). OB- obese rats; HFD- High-fat diet; A.C- *Alchornea cordifolia*

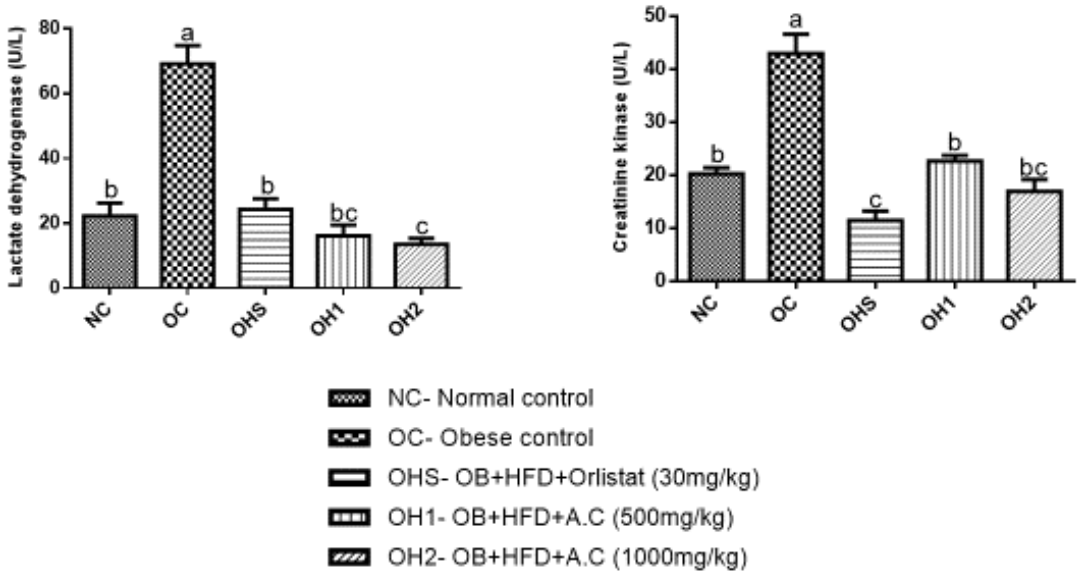
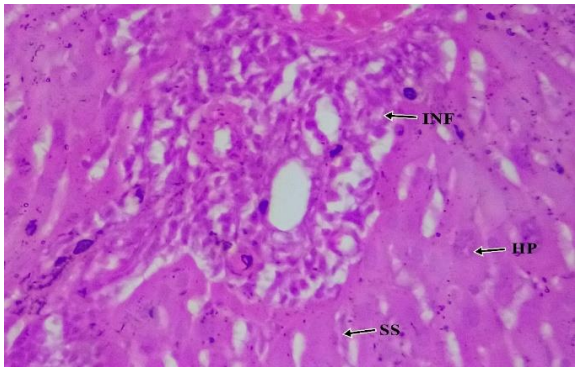
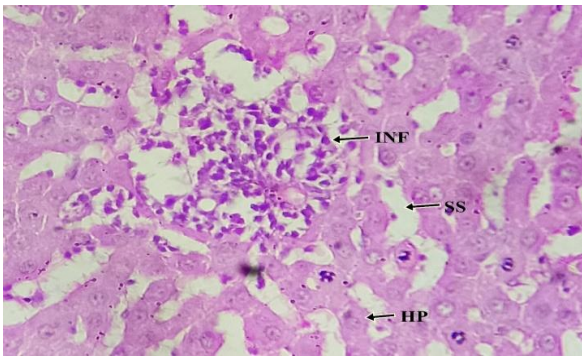


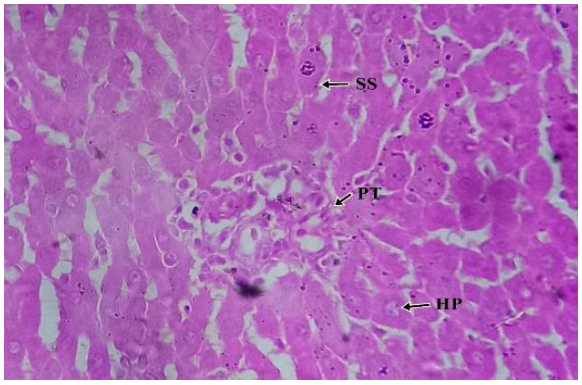
FIG 8: Effect of *A.cordifolia* on lactate dehydrogenase (LDH) and creatinine kinase-MB levels in HFD induced obese Wistar rats. Bars are presented as mean±SEM, n=6. Means with comparable superscript letter are not significantly distinct from one another ($p<0.05$). OB- obese rats; HFD- High-fat diet; A.C- *Alchornea cordifolia*



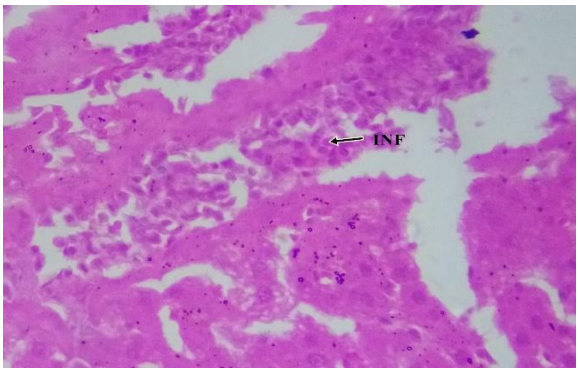
Group 1 – Normal Control (H&E X 400)



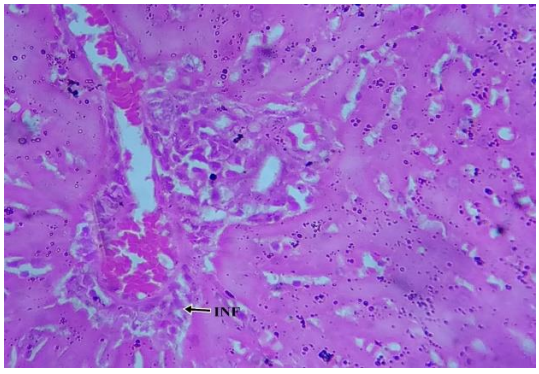
Group 2- Obese Control (H&E X 400)



Group 3: OB + HFD + Orlistat (30mg/kg) (H&E X 400)



Group 4- OB + HFD + A.C (500mg/kg) (H&E X400)



Group 5- OB + HFD + A.C (1000mg/kg) (H&E X 400)

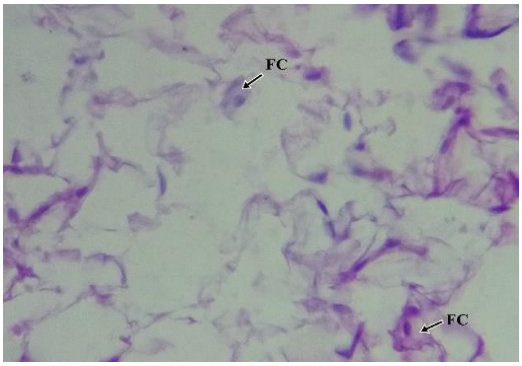
FIG 9: Liver histopathology section

TABLE 10:

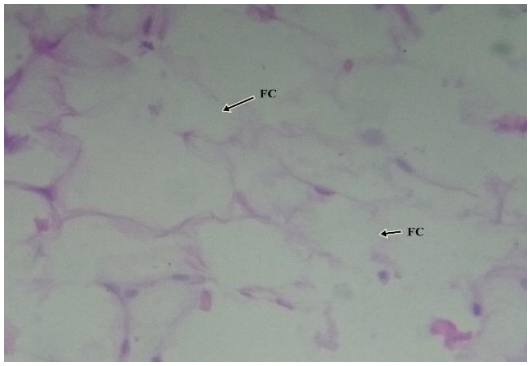
Effect of *A.cordifolia* on histopathology of the liver sections of obese rats

Group	Treatment	Characteristics	Inference
1	Normal control	HP – Normal hepatocytes, no ballooning observed INF – No inflammatory cells SS - Sinusoidal spaces are also dilated, congested and in normal condition	Liver section showed normal morphological appearance
2	Obese control	HP - a severe swelling of hepatocytes with loss of nucleus was observed INF – high accumulation of inflammatory cells was also observed SS - sinusoidal spaces are also dilated and congested and contains many sinusoidal mononuclear inflammatory infiltrates	Liver section showed highly inflammed liver indicating hepatic steatosis
3	OB + HFD + Orlistat (30mg/kg)	HP – Few number of ballooned hepatocytes were observed INF – Slight accumulation of inflammatory cells was observed SS - Sinusoidal spaces are also slightly dilated and congested and contains mild sinusoidal mononuclear inflammatory infiltrates	Liver section showed mild degree of necrosis and slight lymphocyte infiltration
4	OB + HFD + A.C (500mg/kg)	HP – Hepatocytes appeared ballooned with intact nucleus was observed. INF – Multiple Inflammatory cells were also noticed SS - Sinusoidal spaces are also slightly dilated and congested and contains mild sinusoidal mononuclear inflammatory infiltrates	A mild degree of necrosis and slight lymphocyte infiltration, almost comparable to those treated with 30mg/kg of Orlistat.
5	OB + HFD + A.C (1000mg/kg)	HP - Hepatocytes were well arranged with no swelling or loss of nucleus INF – Few inflammatory cells noticed SS – Well separated and dilated Sinusoidal spaces were observed.	Liver section showed significant improvement in hepatic tissues with almost normal hepatic strands

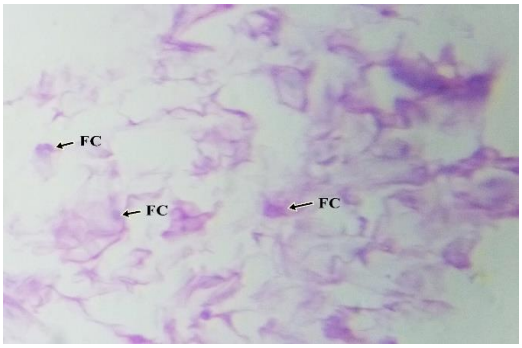
HP- hepatocytes; INF- inflammation; SS- sinusoidal spaces



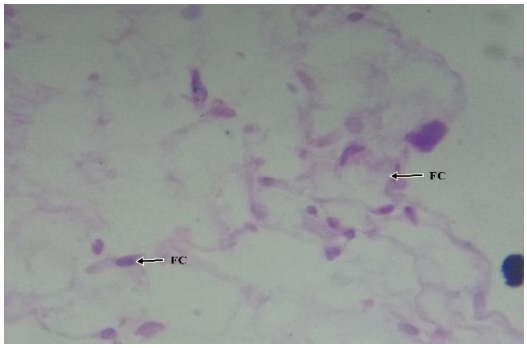
Group 1 - Normal Control (H&E X 400)



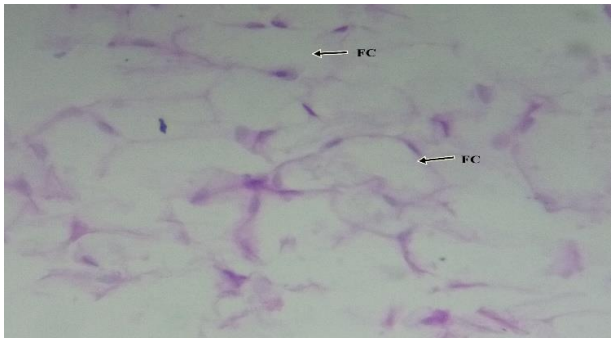
Group 2- Obese Control (H&E X 400)



Group 3: OB + HFD + Orlistat (30mg/kg) (H&E X 400)



Group 4- OB + HFD + A.C (500mg/kg) (H&E X400)



Group 5- OB + HFD + A.C (1000mg/kg) (H&E X 400)

FIG 10: Adipose tissue histpathology section

TABLE 11:

Effect of *A.cordifolia* on histopathology of the liver sections of obese rats

Group	Treatment	Characteristics	Inference
1	Normal control	FC- Adipose tissue session showed normal fat cells (FC) with normal arranged collagenous fibres	Normal adipose tissue architecture
2	Obese control	FC- Fat cell enlargement was observed. Fibro collagenous stroma consisting of irregular arranged collagenous fibers with scattered mature adipocytes; accumulation fat causing more fat deposits and consequently leading to the expansion of adipocytes' size	Multiple mature adipocytes indicating fat accumulation and obesity.
3	OB + HFD + Orlistat (30mg/kg)	FC- Orlistat caused fat cells to shrink considerably, reducing fat accumulation and expansion of adipocytes' size	Fat cells were reduced significantly, showing anti-obesity effect.
4	OB + HFD + A.C (500mg/kg)	FC- A mild effect on the fat cells was observed as there were still some matured adipocytes and slight fat accumulation.	Mild reduction in fat accumulation.
5	OB + HFD + A.C (1000mg/kg)	FC- Scattered scanty mature adipocytes was observed. There was general reduction in the adipocytes size as the fat cells seemed to have shrinked. This effect is on par with the Orlistat treatment effect.	Number and size of Fat cells were reduced significantly, indicating anti-obesity effect.

FC- Fat cell

4.9 Effect of *A.cordifolia* on tissue function parameters in High Fat Diet (HFD) – induced obese rat model

4.9.1 Effect on liver function parameters

The effects of *A.cordifolia* extract supplementation on the levels of serum liver function enzyme AST, ALT, ALP, total protein, albumin, and bilirubin of HFD-induced obese Wistar rats are shown in Figure 7. The results showed that AST, ALT, ALP, and bilirubin levels was elevated significantly ($p < 0.05$) in HDF-treated rats. In contrast, total protein and albumin levels decreased. However, only that of albumin was significant ($p < 0.05$) increased when compared with normal control. Administration of *A.cordifolia* extract to HDF-treated rats led to a significant decrease ($p < 0.05$) in AST, ALT, ALP enzymes, and bilirubin compared with the HFD group, which was not significantly different from the Orlistat-treated group. On the other hand, *A.cordifolia* extract treatment significantly increased albumin levels compared with the HFD-fed group. The high-dose *A. cordifolia* extract (1000mg/kg) administered to the treatment group of rats given with HFD and treatment with *A.cordifolia* extract to effectively lower the levels of AST, ALT, and ALP enzymes.

4.9.2 Cardiac function parameters

Figure 8 illustrates the effect of *A.cordifolia* extract on serum cardiac biomarkers- lactate dehydrogenase (LDH) and creatinine kinase-MB levels in HFD-induced obese Wistar rats. The rats that were fed a high-fat diet showed significantly ($p < 0.05$) elevated levels of serum LDH and CK-MB as compared to the control group that was on a normal diet. Furthermore, serum levels of the enzymes mentioned above were significantly ($p < 0.05$) decreased in *A.cordifolia*-treated rats (500mg/kg and 1000mg/kg body weight) on an HFD. The rats treated with *A. cordifolia* extract at a dosage of 1000mg/kg body weight experienced a larger reduction compared to those treated with *A. cordifolia* extract at a dosage of 500mg/kg body weight.

4.11 Histopathological investigations showing the effect of *A.cordifolia* hepatic and adipose tissue of high-fat diet (HFD) – induced obese rats

4.11.1 Histopathology of the liver

Figure 9 and Table 10 illustrate the effect of *A.cordifolia* extract on the liver histopathology of Wistar rats induced with a high-fat diet.

4.11.2 Histopathology of the adipose tissue

The effect of *A.cordifolia* on adipose tissue histopathology of high-fat diet (HFD) – induced obese rats was shown in Figure 10; Table 11.

5. Discussion

The HPLC results showed the copious presence of chlorogenic acid, benzoic acid, caffeic acid, coumaric acid, sinapic acid, luteolin, catechin, gallic acid, ellagic acid, quercetin, myricetin, and kaempferol in *A.cordifolia* leaves. Previous studies have commonly identified these compounds in ethanol, methanol, and ethyl acetate extracts of *A.cordifolia* [54][55][56][57][58]. Phytochemicals such as flavonoids (e.g. Quercetin, Catechin, Chlorogenic acid), phenolics (e.g. luteolin, myricetin, kaempferol), phytosterols, coumarins (coumaric acid), saponins, and terpenoids have been reported to have anti-obesity potentials [59][60]. They show their activities by reducing fat mass and body weight by decreasing adipocyte formation and lipid accumulation, increasing lipolysis, thermogenesis, energy expenditure, and suppressing inflammation and oxidative stress [61][62][63][64]. Several polyphenolic phytochemicals, such as kaempferol and quercetin, which are found in *A.cordifolia*, have also been identified to target the adipocyte life cycle [65][59]. Some studies have also suggested that the inhibition of adipocyte differentiation by phytochemicals is likely the safest and most effective strategy for treating obesity [65].

According to the findings of this study, continuous intake of the HFD diet by Wistar rats caused obesity, which was identified based on increased body weight, Lee's index, and other factors such as BMI, waist circumference, and fat mass (adipose tissue weight) with the development of hyperlipidemia. Experimental rats that are subjected to HFD-induced obesity studies are considered a reliable model as they have been demonstrated to mimic human obesity [66]. Adiposity, hyperglycemia, dyslipidemia, insulin resistance, and hepatic steatosis were all seen in

both mice and rats fed a lard-based HFD, all of which are associated with human obesity [67]. Interestingly, this study indicated that administering *A.cordifolia* extract (1000 mg/kg b.w. per day especially) considerably reduced body weight gain by about 21%, Lee's index, BMI, waist circumference, and fat mass compared with obese control rats after treatment. In addition, the 1000mg/kg b.w treatment group had the least percentage weight difference in bodyweight between weeks 4 and 9 compared with other treatment groups. The rats in all experimental groups received equal diets; therefore, the decrease in body weight was not due to a drop in food or energy intake. *A.cordifolia* treatment was responsible for the loss of body weight, as it significantly reduced the weight of subcutaneous and abdominal adipose tissue compared to the obese control group that did not receive any treatment. Adipose tissue expansion causes obesity through hypertrophic (cell size increase) and hyperplastic (cell number rise) processes [68]. The histopathological pattern of white adipocytes in the HFD group treated with 1000mg/kg of *A.cordifolia* was relatively regular, with adipocyte sizes comparable to those in the normal control group. It appears that *A.cordifolia* may have hindered the accumulation of lipids in various tissues, particularly in adipose tissue, and reduced both body weight gain and adipose tissue mass induced by HFD. In addition, flavonoids and phenols, e.g., quercetin, luteolin, apigenin, myricetin, and resveratrol present in *A.cordifolia* are known to have anti-obesity properties such as body fat reduction, reducing adipogenic transcription factors, and increasing fatty acid oxidation [64] which may have played a role in the reduction of body weight and fat mass.

Excessive intake of energy can cause obesity, leading to the accumulation of extra energy in adipose tissues and organs such as the liver, kidney, and heart [69]. The experimental rats that were fed a high-fat diet showed an increase in the weight of these organs. However, *A.cordifolia* treatment in a dose-dependent manner reduced the relative weight of adipose tissue compared to that of the liver and heart. The decrease in body weights of rats treated with the plant extract could be linked to the reduction in organ weights. The plant extract may have caused a decrease in the relative weights of the total visceral and subcutaneous fat-depot by inhibiting the formation of new adipocytes from precursor cells (adipocyte differentiation) or by reducing adipocyte size due to fat storage (adipocyte hypertrophy) [70]. Studies have shown that visceral fat mass can significantly predict metabolic disorders such as cardiovascular diseases, atherosclerosis, dyslipidemia, and hypertension [71].

Obesity is associated with dyslipidaemia and is usually illustrated by increased serum triacylglycerol (TGs), total cholesterol (TC), low-density lipoprotein (LDL), Phospholipids (PLs) and total lipids (TL), and a decrease in serum level of high-density lipoprotein (HDL) [72]. Thus, as a result, anomalies in lipid profiles can be used as an indicator of obesity. In this study, consistent consumption of HFD substantially elevated the lipid profile in the serum, liver, and adipose tissue of the obese Wistar rats. Our result showed TC increased by 54.44%, TG -63.42%, LDL- 89.92%, while HDL-c decreased by 47.04% compared with normal control. Consequently, the increase in fat granules and lipid accumulation (hepatic steatosis) seen in the histopathological evaluation of liver tissue may be a result of the alterations in the lipid profile of obese rats. Furthermore, it is possible that the changes in lipid profile caused by a high-fat diet (HFD) are related to the activation of gastric lipases and the absorption of fats in the intestines, as suggested by Saravanan et al. [73]. The risk of developing coronary heart disease increases with high levels of total cholesterol. Elevated levels of LDL-cholesterol are a risk factor for coronary heart disease, whereas high levels of HDL-C help move excess cholesterol to the liver for removal in bile [74]. The increase in TGs might be due to dietary cholesterol, which has been shown to decrease fatty acid oxidation, leading to higher levels of hepatic and serum triacylglycerol [75].

The serum lipid profiles of HFD-induced obese rats were normalized by oral administration of *A.cordifolia*, as observed by decreased concentrations of TC, TGs, and LDL, as well as elevated levels of HDL. Moreover, *A.cordifolia* administration led to a decrease in concentrations of TC, TGs, TL, and PLs in the liver, adipose and heart tissue of the obese rats. In obese rats, treatment with 500mg/kg and 1000mg/kg of *A.cordifolia* significantly reduced TC by 41% and 50%, respectively, and TG by 71% and 75%, respectively, while LDL was reduced by 56% and 77%, respectively, and HDL was increased by 33% and 45%, respectively. The effect of *A.cordifolia* was found to be comparable to the conventional medication Orlistat. These results were similar to those reported by Thomford *et al.* [76]. The dose-dependent suppression of the increment of serum and tissue lipids by *A.cordifolia* may have resulted from reducing the absorption of fat and cholesterol by inhibiting pancreatic lipase activity [77][78]. In addition, the *A.cordifolia* leaves contain flavonoids that have the ability to lower TC and LDL-c levels through the activation of β-adrenergic receptors, which help to burn fats and prevent the formation of fat cells by inducing apoptosis in preadipocytes found in mice [79][74][76]. Chlorogenic acid, luteolin, catechin, gallic acid, myricetin, and kaempferol, which were all present in *A.cordifolia* ethanol extract, usually

exhibit anti-hyperlipidemic effects by reducing serum total cholesterol and triacylglycerol [80][68][81]. Fat oxidation thermogenesis can be stimulated by catechins and squalene, which can lead to a reduction in body fat [82][83][84]. *A.cordifolia* is also reported to be rich in dietary fibre and is known to improve HDL-c levels and reduce LDL-c levels [85]. This may have contributed to the increase in HDL-c and decreased LDL-c levels observed in *A.cordifolia* treated obese rats.

Moreover, the observed effect of *A.cordifolia* treatment on the atherogenic and coronary artery risk indices of obese rats is significant. According to results from this study, *A.cordifolia* treatment resulted in significant reductions in both atherogenic and coronary indices in hyperlipidemic and obese rats induced by a high-fat diet. The results strongly suggest that this treatment has the potential for therapeutic use in the management of obesity, hyperlipidemia, and the prevention of atherogenic cardiovascular diseases. The atherogenic index (the ratio of TC to HDL-C) and the coronary artery index (the ratio of LDL-C to HDL-C) are both considered reliable indicators of cholesterol deposition or metabolism and excretion, according to Hartog et al. [86].

Following the induction of obesity, there is usually an increase in glucose levels [87]. The findings also demonstrated that obese rats had higher levels of fasting blood glucose (FBG), fasting serum blood glucose (FSBG), and insulin, compared to the normal control group. Obese control rats had a 58 per cent, 45 per cent, and 63 per cent rise in serum glucose, insulin, and HOMA-IR, respectively, when compared to the normal control group. Previous studies have explained that animals consuming a high-fat diet experience elevated blood glucose levels, as prolonged exposure to high levels of fatty acids induces increases in fatty acid oxidation and decreases in glucose oxidation [87][88]. Additionally, ingesting high saturated fat has been reported to induce an increase in hepatic liposynthetic gene expression and impair glucose metabolism. This is evident through impaired translocation of glucose transporter-4 activity, suppressed expression of glucose transporter-4, inhibited expression of hepatic glycolytic and lipogenic enzymes, and impaired insulin signalling [89][90]. The hypoglycemic effects of *A.cordifolia* treatment were observed by reducing FBG and FSBG levels. This may be due to the decrease in carbohydrate absorption into the hepatic portal circulation and the increase in insulin sensitivity [91][69]. Long-term exposure to a high-fat diet can lead to the accumulation of lipid metabolites (such as fatty acyl CoA, ceramide, and diacylglycerol) in various tissues like pancreatic beta cells, adipocytes, muscle, liver, and arterial tissues, which can lead to insulin resistance and beta-cell dysfunction [92][69]. From

our results, insulin resistance represented as HOMA-IR (used extensively for estimates of beta-cell function and insulin resistance) seen in HFD-induced obese rats was significantly reduced with *A.cordifolia* extract treatment. The 1000mg/kg b.w treatment of *A.cordifolia* performed better as it lowered serum glucose, insulin levels, and HOMA-IR by 56%, 60%, and 38%, respectively, compared with other treatments and the obese control group.

The hypoglycemic effect of *A.cordifolia* extract in this study compared favourably with results from another study by Thomford *et al.* [76], which suggested that *A.cordifolia* extract possesses anti-diabetic properties and significantly lowered blood glucose levels in diabetic rats. *A.cordifolia* contains many flavonoids and phenolic acids capable of eliciting hypoglycemic potentials. Luteolin found in *A.cordifolia* was reported in another study to have initiated insulin action and enhanced the expression of PPARγ (improved glucose tolerance and insulin sensitivity) target genes in primary mouse adipose cells [93]. It was also suggested that luteolin possesses anti-diabetic effects as it improves blood glucose, insulin, and HOMA-IR levels and inhibits lipid synthesis [94]. Different research demonstrated that luteolin had the ability to reverse insulin resistance and inflammation of adipose tissue by modifying the polarization of M1-like macrophages in adipose tissue [95]. Apigenin is also found in *A.cordifolia* and has been shown to decrease hyperglycemia, as well as serum cholesterol and G6Pase activities in the liver, according to Rauter et al. [96]. Hossain et al. [97] suggest that apigenin enhances GLUT4 translocation, indicating its ability to lower blood glucose levels. Kaempferol [98], caffeic acid [99], sinapic acid [100][101], chlorogenic acid [102] also present in *A.cordifolia* have been suggested to have several anti-diabetic effects. These phenolic acids and flavonoids exert their hypoglycemic potentials by reducing blood glucose levels, enhancing glucose uptake by the cells, modulating the activities of carbohydrate metabolizing enzymes, inhibiting glucose absorption, and improving glucose tolerance [101].

Obese rats induced by a high-fat diet showed an increase of about 41% and 36% in lipase and amylase activities, respectively, compared to normal control rats. Carbohydrate and lipid metabolism rely on amylase and lipase enzymes, which can be targeted for developing anti-diabetic and anti-obesity compounds. The absorption of triacylglycerol in the small intestine depends on pancreatic lipase, which is secreted by the pancreas and breaks down TGs into glycerol and free fatty acids [103]. Therefore, pancreatic lipase inhibitors are considered an effective

therapeutic option for treating diet-induced obesity in humans. Several anti-lipidemic drugs act as lipase inhibitors and are used clinically to prevent obesity and hyperlipidemia. On the other hand, α-Amylase, one of the digestive enzymes secreted from the pancreas and salivary glands, hydrolyzes dietary starch to provide energy to the body [74]. Oral supplementation of *A.cordifolia,* especially the 1000 mg/kg dose, normalised both lipase and amylase enzyme levels to near normal. Reduced secretion of pancreatic amylase may be a natural mechanism that helps decrease carbohydrate absorption by suppressing digestion, resulting in decreased energy intake, as suggested by Nakajima [104]. The inhibition of α-amylase activity can prevent the breakdown of carbohydrates, which might account for the weight loss observed in rats fed with HFD and treated with *A.cordifolia*. The α-amylase inhibitory activity of *A.cordifolia* in vitro is consistent with a previous report by Idakwoji and Uzuazokaro [105] and Ali et al. [106]. Plant flavonoids- luteolin, quercetin, and chlorogenic acid have been reported to show anti-lipase activity [107][108][109]. Plant alkaloids are known as lipase inhibitors according to Kim et al. [110]. Experimental studies have found that alkaloids such as caffeic acid, coumaric acid, and sinapic acid, as reported by Gupta et al. [107] and Maryana et al. [109], can also inhibit lipase. The reduction in pancreatic lipase levels resulting from *A.cordifolia* treatment in HFD-fed obese rats may lead to a decrease in lipid incorporation and uptake, which could ultimately result in lower caloric intake and promote weight loss as reported by Saravanan et al. [74]. In summary, the presence of flavonoids, polyphenols, and alkaloids in *A.cordifolia* may be responsible for inhibiting lipase activity in HFD-induced obese rats.

The liver homogenate of the HFD control group showed a decrease in HMG CoA reductase activity compared to the normal control group. *A.cordifolia* administration to the rats, at both 500mg/kg and 1000mg/kg body weight, significantly ($p<0.05$) increased tissue mevalonic acid Ratio (TMAR), reduced the magnitude of cholesterol synthesis (MCS) and decreased the percentage reduction in HMG-CoA reductase activity in the liver of obese rats. These findings confirmed that *A.cordifolia* administration effectively inhibits the activity of HMG COA reductase, which can be attributed to the phytochemical components of *A.cordifolia*, such as phenols and flavonoid compounds. Studies have also reported the in vivo inhibition of HMG CoA reductase activity by medicinal plants, including *Quercus infectoria*, *Rosa damascene*, *Myrtus communis*, *Andrographis paniculata*, *Anthocephalus indicus*, and *Ocimum sanctum* [111][112]. Clinical trials have shown improved endothelial function and reduced oxidative stress and/or upregulation of

nitric oxide activity in patients with coronary risk who were treated with HMG CoA reductase inhibitors [113].

Musolino et al. [114] conducted a study where they discovered that certain flavonoids and phenolic compounds present in plants can prevent the production of cholesterol in the body. This inhibition occurs as these compounds bind to the catalytic site of the HMG-CoA reductase enzyme, which is responsible for the biosynthesis of cholesterol. The glycosylated polyphenols, bruteridin and melitidin, act as endogenous HMG-CoA substrates and cause the inhibition of cholesterol synthesis. This study reported the presence of caffeic acid in *A. cordifolia*. Some studies have suggested that caffeic acid and its esters may contribute to lowering cholesterol levels by down-regulating HMGCR and up-regulating LDL receptor, as well as down-regulating ACAT2 [115][116]. Karthikesan and colleagues [115] discovered that the administration of chlorogenic acid for a duration of 45 days led to a significant reduction in HMGCR and ACAT activity in rat lipid metabolism.

Oxidative stress arises due to an imbalance between the levels of antioxidants and oxidants, where the oxidants outweigh the antioxidants in favour of the former [117]. This study showed that the group of rats given high-fat diet showed that there was a significant reduction in the antioxidant status of the liver and adipose tissues. This was evident from the increased levels of lipid peroxidation, as indicated by malondialdehyde (MDA), and the decreased activities of SOD, GSH, and GPx, as compared to the normal control group. The obese control group of rats exhibited a significant increase in lipid peroxidation by 84% and 49% in the liver and adipose tissue, respectively, compared to the normal control group. Additionally, the results of the SOD test demonstrated a reduction of this enzyme's activity in the liver and adipose tissue of the obese control group by 47% and 44% compared with the normal control group. Some studies suggest that a high-fat diet can alter metabolic patterns, leading to the generation of reactive oxygen species (ROS), which are responsible for several health complications, including atherosclerosis, diabetes, cancer, cardiovascular disorders, and obesity [118][119]. Mabrouki et al. [119] have highlighted that antioxidant enzymes such as glutathione (GSH), Glutathione peroxidase (GPx), and superoxide dismutase (SOD) play a crucial role in protecting biological systems against damage caused by free radicals. The liver's first line of defence against free radicals is the GSH, which also maintains protein thiols and acts as a substrate for GPx and GST [120]. Non-enzymatic

antioxidants such as reduced vitamins C and E, natural flavonoids, and other compounds, like GSH, work synergistically with the enzymatic antioxidant system and neutralize or scavenge free radicals by donating electrons (Mabrouki et al., 2020). In HFD-induced obese rats, administration of *A. cordifolia* reduced lipid peroxidation levels and restored antioxidant enzyme activities compared to obese control rats. The MDA values of liver and adipose tissue were almost normal in rats treated with 1000mg/kg of *A. cordifolia* extract, indicating lower lipid peroxidation levels. Overall, *A. cordifolia* extract administration increased antioxidant enzyme activity in the liver and adipose tissue, with the highest effect seen in the 1000mg/kg treatment group, resulting in a 53% rise in the liver and a 48% increase in adipose tissue. These findings suggest that *A.cordifolia* extract is effective in preventing tissue damage by reducing the peroxidation of lipids and maintaining the levels of ROS at acceptable concentrations within the cells. Our findings imply that the use of *A. cordifolia* leaf extract, which contains antioxidants, may alter cholesterol absorption and increase antioxidant levels [121][122]. Hyperlipidemia can reduce the efficacy of the body's antioxidant defence system, as noted by Wang et al. [39] and Zhang et al. [123]. Mehta et al. [124] have suggested that a high-fat diet (HFD) can cause liver damage and insulin resistance through oxidative stress. The results of the study indicate that one of the reasons why the ethanol extract of *A. cordifolia* has anti-obesity potential may be due to its ability to suppress oxidative stress in HFD-induced obese rats. The free radical scavenging properties of the phytoconstituents present in *A. cordifolia*, such as flavones and alkaloids, could restore the enzymatic and non-enzymatic antioxidant systems. The leaves of *A. cordifolia* also contain polyphenols like gallic acid, which is a powerful antioxidant and a scavenger of peroxyl radicals and superoxides. Antioxidants play a crucial role in regulating excess reactive oxygen species (ROS) and are therefore involved in obesity-related conditions by reducing obesity-related oxidative stress [125][126].

The evaluation of liver function can be done by measuring key indicators through liver function tests. The liver is an essential organ responsible for detoxifying compounds and general metabolism [68]. Obesity-induced by HFD is associated with higher concentrations of markers of liver damage, such as ALT, AST, ALP, and LDH [127][128]. Results from a study by Mopuri et al. [68] showed that in diet-induced obesity in rats, the liver displays characteristic features of hepatic steatosis, such as fat accumulation and swelling of rough endoplasmic reticulum and mitochondria in hepatocytes. Consuming a high-fat diet can play a crucial role in the pathogenesis

of fatty liver or hepatic steatosis associated with obesity depicted via ballooning degeneration. Elevated liver enzyme levels also signify hepatocellular damage and correlate with increased liver weight [129][130]. The results of this study further established that a high-fat diet causes hepatocellular damage, as demonstrated by the marked elevation of serum enzymes AST, ALP, ALT, and total bilirubin activities. However, treatment with *A. cordifolia* extract caused a significant reduction in the enzyme levels and reversed changes in hepatocytes, signifying the role of *A. cordifolia* in preventing liver damage caused by the high-fat diet. Albumin is a negative acute-phase protein and might decrease during inflammatory conditions, such as obesity [131]. Our results show that consistent HFD consumption by Wistar rats decreased serum albumin. However, *A. cordifolia* extract significantly reversed changes in albumin levels to compare favourably with normal rats.

Obesity has been found to be closely linked with changes in the structure and function of the heart in both humans and animals, according to Abel et al. [132]. Some of the cardiac consequences of obesity include cardiac remodellings, such as cardiac hypertrophy, cardiac fibrosis, and subclinical impairment of left ventricle systolic and diastolic function [133]. Elevated levels of Lactate Dehydrogenase (LDH) and Creatine kinase (CK) activity, along with other biomarkers such as troponin, are known to indicate skeletal muscle injury and disruption in cardiac metabolism [134][135]. Our results revealed that these enzymes were elevated in response to HFD, which is consistent with the findings of Pisoni et al. [136] and Oloyo et al. [137]. Leakage of these enzymes from the tissues into the serum reflects the disruption and loss of integrity of the cell membrane, according to Aulbach and Amuzie [138]. High-dose *A. cordifolia* supplementation (1000mg/kg) in HFD-fed rats was noted to be more effective in lowering these enzyme levels.

Additionally, histopathological examination of liver and adipose tissue was conducted to confirm the anti-lipogenic and adipogenic activity of *A. cordifolia* extract. Our results revealed the reduced size of adipocytes and lipids levels in the liver of groups treated with 1000mg/kg body weight *A.cordifolia* extract, indicating its possible anti-obesity activity. The major bioactive compounds which could be behind *A.cordifolia's* anti-obesity potential are phenolic compounds such as catechin, chlorogenic acid, coumaric acid, gallic acid, quercetin, resveratrol, and their derivatives which are present in ethanol extract of the leaves. Studies conducted previously have demonstrated

the anti-diabetic and anti-hyperlipidemic activity of these bioactive compounds from different plant sources [60].

6. Conclusion

This study demonstrated that HFD feeding induced physiological, biochemical, and histological changes. However, ethanol extract of *A.cordifolia* was able to evoke gradual weight loss, ameliorate increased blood glucose levels and lipid peroxidation, normalize insulin, amylase, lipase, HMG CoA reductase activities and regulate blood and tissue lipid profile in obese rats. Furthermore, *A.cordifolia* improved the enzymes involved in cardiac and liver function and reversed histopathological changes in the liver and adipose tissues induced by HFD. The substantial presence of phenolic and flavonoids contained in *A.cordifolia* is possibly responsible for weight reduction, anti-hyperlipidemic, anti-hyperglycemia, antioxidant and organ-protective activities in HFD-induced Wistar obese rats.

LIST OF ABBREVIATION

World Health Organisation (WHO)
High-fat diet (HFD)
Normal feed (NF)
Reactive oxygen species (ROS)
Low-density lipoprotein (LDL)
Very low-density lipoprotein (VLDL)
High-density lipoprotein (HDL)
Non-alcoholic fatty liver disease (NAFLD)
High-Performance Liquid Chromatography (HPLC)
Alchornea cordifolia (Schumach.) Müll.Arg (*A.cordifolia*)
Ultraviolet-Visible Diode Array Detector (UV-Vis DAD)
Normal control (NC)
Obese control (OC)
body weight (BW)
Insulin resistance (IR)
enzyme-linked immunoassay (ELISA)
total cholesterol (TC)
triacylglycerol (TG)
Lactate dehydrogenase (LDH)
Creatine kinase (CK)
Alanine transaminase (ALT)
Alkaline phosphatase (ALP)
Aspartate transaminase (AST)
Atherogenic Index of Plasma (AIP)
Cardiac risk ratio (CRR)
Castelli's Risk Index (CRI)

Atherogenic Coefficient (AC)
Amino-Napthosulphonic Acid (ANSA)
Malondialdehyde (MDA)
Superoxide Dismutase (SOD)
Catalase (CAT)
Reduced Glutathione (GSH)
Hydroxylmethylglutaryl (HMG)-CoA
Magnitude of cholesterol synthesis (MCS)
Fasting blood glucose (FGB)
Total lipids (TL)
Total lipids (TL)
Phospholipids (PL)
Tissue Melvalonic Acid Ratio (TMAR)

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Statements & Declarations

Ethical approval

Ethical approval for the research was obtained from the University of Calabar Ethical Review Committee, which is in accordance with the ethical standards as reviewed in the Principles of Laboratory Animal Care (NIH publication no. 8523, revised 1985).

Consent to participate

The study did not involve human participants; therefore, no informed consent was necessary.

Consent to publish

The researchers did not involve any human participants in their study. As a result, there was no need to seek consent from individuals before publishing their data in this journal.

Author Contributions

All authors contributed to the study's conception and design. Chinedum Martins Ekeleme, Item Justin Atangwho, Esien David-Oku: Conceptualization, Methodology, Software Chinedum Martins Ekeleme, Esien David-Oku: Data curation, Writing- Original draft preparation. Chinedum Martins Ekeleme, Diana Ochuole Odey, Chidinma Emmanuel Ibeneme: Visualization, Investigation. Godwin Eneji Egbung, Item Justin Atangwho, Esien David-Oku, Eyong Ubana Eyong*:* Supervision.: Edet Effiong Asanga: Software, Validation.: Chinedum Martins Ekeleme: Writing- Reviewing and Editing.

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