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Comparative Study of the Effect of Aqueous Seed Extract of *Tetracarpidium conophorum* (Walnut) and Leaf Extract of *Annona muricata* (Soursop) on Calcium Level and the Lipid Profile of Male Wistar Rats.

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ABSTRACT

The study examined the lipid profile and calcium levels in male Wistar rats treated with *Tetracarpidium conophorum* and *Annona muricata* extracts. Twenty-Five (25) male Wistar rats weighing 150–170 g were randomly divided into five groups of five rats each. Groups A and B received 1000 and 500 mg/kg of aqueous seed extract of *Tetracarpidium conophorum*, respectively. Groups C and D received 1000 and 500 mg/kg aqueous leaf extracts of *Annona muricata*, respectively, and group E received feed and water only for 30 days. Data obtained for lipid profile (total cholesterol, low-density lipoprotein, high-density lipoprotein, triglyceride, and very low-density lipoprotein) and calcium level were analyzed using ANOVA followed by post-hoc Fisher's LSD, and values were presented as mean ± standard error of mean (SEM). Level of significance was $p < 0.05$. The results showed a significant decrease in total cholesterol levels in groups A, B, and C, while group D had a non-significant decrease compared to group E. Triglyceride result revealed a significant reduction in groups A and C, while groups B and D had a non-significant decrease compared to group E. There was a non-significant decrease in the LDL levels in groups A and D, while groups B and C had a significant decrease compared to group E. The HDL result showed a non-significant increase in groups A, B, C, and D compared to group E. A non-significant increase in serum calcium level in groups A, B, and C, while group D had a significant increase compared to group E. The study concludes that *T. conophorum* and *A. muricata* extracts reduced cholesterol, triglycerides, and low-density lipoprotein cholesterol, increased HDL. *A. muricata* alone significantly increased serum calcium level.

INTRODUCTION

Metabolic diseases often involve abnormal lipid and glucose metabolism, increasing the risk of cardiovascular disease (Yan et al., 2021). Cardiac energy metabolism disorders significantly impact heart function and structure (Federico et al., 2021). Under normal conditions, the myocardium uses fatty acids and glucose, but under pathological conditions, fatty acid metabolism changes significantly, leading to mitochondrial damage and serious heart damage. Patients with metabolic syndrome or diabetes have been found to have increased lipid uptake and utilization, resulting in fat accumulation in the heart (Costantino et al., 2019; Gao et al., 2020).

Lipid profile consists mainly of total cholesterol, triglyceride, LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C), which are classified as diagnostic tool for cardiovascular risk factors (Dasgupta & Wahed, 2021). Lipids are described as the significant building blocks of life and are the major substances for mammalian cell function (Dasgupta & Wahed, 2021). Cholesterol is essential in the progression of cardiovascular disease and it increases the risk of developing atherosclerotic cardiovascular disease (CVD) (Lee & Siddiqui, 2022). Dyslipidemia has been linked to diabetes mellitus, which is a modifiable risk factor for cardiovascular complications in type 2 DM (Haile & Timerga, 2020). However, it is characterized by hypertriglyceridemia, reduced HDL cholesterol levels, and an increased concentration of Low-Density-Lipoprotein particles (Daya et al., 2017; Hirano, 2018).

Electrolytes play critical roles in the body fluid regulation and a variety of other biological processes (Maeda et al., 2016; Shrimanker and Bhattarai 2021). However, the principal electrolytes of significance are sodium, potassium, and chloride, which go along with magnesium, calcium, phosphate, and bicarbonate (Shrimanker and Bhattarai 2021). Thus, an imbalance in the aforementioned electrolytes above either a low or high levels could disrupt normal physiological processes of life, leading to complex situations that threaten life (Shrimanker and Bhattarai 2021). Electrolyte disturbances play a role in a variety of illnesses as underlying disorders, pathophysiological changes caused by diseases and traumas, and changes or complications following insults (Maeda et al., 2016).

Calcium is a significant electrolyte, which forms an integrative compartment of the human body and has significance for human health. It is known for the formation and maintenance of bone (Li et al., 2018). Plasma calcium is known for several homeostatic role in the maintenance of skeleton, hormone regulation, nerve transmission and vascular activities (Brini et al., 2013; Krebs et al., 2015). Calcium is stored in large quantity in skeletal muscles and is the principal reservoir of calcium in the body (Burgoyne et al., 2019; Li et al., 2018). Calcium ion is essential for muscle fiber action potential and various functions, including myosin-actin cross bridging, protein synthesis, degradation, fiber type shifting, calcium-regulated proteases, transcription factors, mitochondrial adaptations, plasticity, and respiration (Gehlert et al. 2015).

Medicinal plants are known for their use in the treatment of different ailments because of their secondary active metabolites known to contain numerous phytonutrients such as flavonoids, tannins, cyanogenic glycosides, alkaloids, arocine, etc. (Agiriga & Siwela, 2017; Boy et al., 2018). Currently, active substances extracted from higher plants are used in contemporary medicine, and 80% of these active substances show a good association between their current therapeutic application and their traditional uses (Prasathkumar et al., 2021). The African walnut, *Tetracarpidium conophorum*, is a member of the Euphorbiaceae family. It is an edible seed that has high nutritional content, which includes: essential fatty acids, vitamins, and minerals. Its pharmacological effects are: antidiarrheal (Nwachoko and Jack, 2015), pro-fertility (Dada and Aguda, 2015), antioxidant (Udedi et al., 2014); Akomolafe et al., 2015), anti-inflammatory (Olaniyi et al., 2016), and anti-diabetic (Ogbonna et al., 2015; Lepzem and Togun2017).Additionally, the plant has long been used in herbal medicine to treat a variety of conditions, including digestive and respiratory issues (Ayodeji & Aliyu, 2018). *Annona muricata* Lin is a plant that grows around 4 to 8 m tall and produces green spiny heart-shaped fruit (Chan et al., 2020). However, several pharmacological functions have been linked to the physiochemical activities of the plants, which include sedative, antispasmodic, hypoglycemic, hypotensive, smooth muscle relaxant amongst others (Usunomena, 2014). *Annona muricata* L. is a tropical plant species known for its edible fruit, which has some medicinal advantages and some toxicological effects (Gavamukulya et al., 2017a).

The aim of this study was to compare the effects of aqueous seed extract of *Tetracarpidium conophorum* and leaf extract of *Annona muricata* on calcium level and lipid profile of male Wistar rats.

MATERIALS AND METHOD

Study Area

This Study was conducted at the Animal House, Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medicine, Chukwuemeka Odumegwu Ojukwu University, Uli Campus.

Ethical Approval

Ethical approval was obtained from the Faculty of Basic Medical Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli campus. Rats handling and treatments conformed to the National Institute of Health guidelines for laboratory animal care and use (Carbone & Austin, 2016).

METHODOLOGY

Experimental Animals

Twenty-Five (25) male Wistar rats weighing 150-170g were obtained from the Animal House, Chukwuemeka Odumegwu Ojukwu University, Uli Campus, Nigeria. Animals were kept in standard cages at a room temperature of 27±2°C. The animals were maintained with normal laboratory chow (grower feed) and water *ad libitium*. The animals were acclimatized for two weeks before administering the aqueous leaf extract of *Annona muricata* and *T. conophorum*. The animals were kept on 12hours light and dark cycles.

Plant Procurement and Identification

Samples of *Annona muricata* (Soursop) leaves were harvested from a local farm in Uli Community, Anambra State and seeds of *Tetracarpidium conophorum* were purchased at Ihiala Market, Ihiala, Anambra state. The two medicinal plants were identified by Dr. Bright, Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University, Uli campus, Anambra State.

Plant Extraction Procedure

Seeds of *Tetracarpidium conophorum* (walnut) and leaves of *Annona muricata* (Soursop) respectively were washed in running tap water to remove dirt and air-dried under ambient temperature. The dried seeds of *Tetracarpidium conophorum* and leaves of *Annona muricata* (Soursop) were milled into a coarse powdered form using a local grinder, and about 250g was dissolved in 1500mls of water for 24hours. It was filtered using a clean handkerchief; after that, further filtration was done using Whatman No. one filter paper. The filtrate was concentrated using a rotatory evaporator, dried further using a laboratory oven at 45°C into a gel-like form, and preserved in a refrigerator for further usage. The extraction method was done with modifications as described according to the method employed by Quek *et al.* (2012).

Experimental Design

The animals were randomly divided into 5 groups of 5 rats each.

Group A: Each rat was given 1000 mg/kg aqueous seeds extract of *Tetracarpidium conophorum* (*T. conophorum*).
Group B: Each rat was given 500 mg/kg aqueous seeds extract of *Tetracarpidium conophorum* (*T. conophorum*).
Group C: Each rat was given 1000 mg/kg aqueous leaf extract of *Annona muricata* (*A. muricata*).
Group D: each rat was given 500 mg/kg aqueous leaf extract of *Annona muricata* (*A. muricata*).
Group E received feed and water *ad libitum* only and served as control.

All experimental protocols were observed under strict supervision. The experiment lasted for 30-days, and administration was done through oral gavage.

Acute Toxicity of Plant Extracts (*Annona muricata* and *Tetracarpidium conophorum*)

The median lethal dose (LD_{50}) of the aqueous leaf extract of *Annona muricata* and seeds of *Tetracarpidium conophorum* were determined using Lorke's method (1983). The result of acute oral toxicity study of *T. conophorum* was 3807.88 mg/kg as reported by Njoku-Oji et al., 2019). That of *A. muricata* was 3607.52 mg/kg (Inegbenose Godwin et al., 2023).

Collection of Blood Sample

At the end of the experiment, animals in the different groups were anesthetized using chloroform in an enclosed container after 24 hours of the last administered dose of the aqueous leaf extract of *Annona muricata* and seed extract of *Tetracarpidium conophorum*. Blood was collected from the animals through ocular puncture as described by Parasuraman *et al.* (2010). Blood obtained was put in a plain bottle. it was allowed to cool, and centrifuged for 10-minutes at 3000 revolution per minute (rpm), after which the serum was retrieved using a micropipette. The retrieved serum was used to assay for lipid profile (total cholesterol, low-density lipoprotein, high-density lipoprotein, triglyceride, and very low-density lipoprotein) and calcium level.

Statistical Analysis

The results of this study were expressed as mean ± standard error of mean (mean ± SEM), and data obtained were analyzed using SPSS version 21. The statistical significance between the groups was assessed by one way analysis of variance (ANOVA) followed by least significance difference (LSD) test with level of significance at $p<0.05$.

RESULTS

Table 4.1: Effects of aqueous seed extract of *Tetracarpidium conophorum* and aqueous leaf extract of *Annona muricata* on total cholesterol and triglyceride levels

Groups	Total cholesterol (mmol/L)	Triglyceride (mmol/L)
	Mean±SEM	Mean±SEM
Group A (1000 mg/kg of ASTC)	2.12±0.20*	0.51±0.06*
Group B (500 mg/kg of ASTC)	2.04±0.15*	0.69±0.15 NS
Group C (1000 mg/kg of ALAM)	1.88±0.36*	0.55±0.08*
Group D (500 mg/kg of ALAM)	2.26±0.10NS	0.63±0.08 NS
Group E (control)	2.51±0.12	0.73±0.04
f-value	3.895	3.147

Data were analyzed using ANOVA followed by post Hoc LSD multiple comparison and values were significant at *p<0.05*. (*: Significant; ns: not significant). ASTC: aqueous seed extract of *Tetracarpidium conophorum*; ALAM: aqueous leaf extract of *Annona muricata*.

The result demonstrated a significant decrease in total cholesterol level in group A, B, C (*p=0.045, p=0.020, p=0.004*). Group D (*p=0.169*) had a non-significant decrease compared to group E. The triglyceride result revealed a significant reduction in groups A and C (*p=0.013, p=0.032*) while groups B and D (*p=0.542, p=0.179*) respectively had a non-significant decrease compared to group E.

Table 4.2: Effect of aqueous seed extract of *Tetracarpidium conophorum* and aqueous leaf extract of *Annona muricata* on LDL-C, and HDL-C level

Groups	LDL-C (mmol/L)	HDL-C (mmol/L)
	Mean±SEM	Mean±SEM
Group A (1000 mg/kg of ASTC)	1.26±0.12 NS	0.64±0.09 NS
Group B (500 mg/kg of ASTC)	1.06±0.22 *	0.67±0.07 NS
Group C (1000 mg/kg of ALAM)	1.10±0.30 *	0.53±0.13 NS
Group D (500 mg/kg of ALAM)	1.35±0.17 NS	0.62±0.07 NS
Group E (control)	1.60±0.06	0.58±0.05
f-value	3.910	1.125

Data were analyzed using ANOVA followed by post Hoc LSD multiple comparison and values were significant at *p<0.05*. (*: Significant; ns: not significant) ASTC: aqueous seed extract of *Tetracarpidium conophorum*; ALAM: aqueous leaf extract of *Annona muricata*.

The result revealed a non-significant decrease in the LDL-C levels in groups A and D (*p=0.053, p=0.138*) while groups B and C (*p=0.006, p=0.009*) had a significant decrease compared to group E. The HDL-C result showed a non-significant increase in groups A, B, C, and D (*p=0.442, p=0.249, p=0.496, p=0.584*) compared to group E.

Table 4.3: Effect of aqueous seed extract of *Tetracarpidium conophorum* and aqueous leaf extract of *Annona muricata* on calcium level

Groups	Calcium (mmol/L)
	Mean±SEM
Group A (1000 mg/kg of ASTC)	2.31±0.03 ^{NS}
Group B (500 mg/kg of ASTC)	2.36±0.12 ^{NS}
Group C (1000 mg/kg of ALAM)	2.32±0.03 ^{NS}
Group D (500 mg/kg of ALAM)	2.44±0.11*
Group E (control)	2.29±0.03
f-value	1.931

Data were analyzed using ANOVA followed by post Hoc LSD multiple comparison and values were significant at *p<0.05*. (*: Significant; ns: not significant).ASTC: aqueous seed extract of *Tetracarpidium conophorum*; ALAM: aqueous leaf extract of *Annona muricata*.

The result showed a non-significant increase in serum calcium level in groups A, B, and C (*p=0.718, p=0.291, p=0.643*) while group D (*p=0.032*) had a significant increase compared to group E.

DISCUSSION

Medicinal plants have shown to improve lipid profile and electrolyte levels in metabolic diseases because of the major phytochemicals they possess (Rabizadeh et al., 2022). Lipids are important components of the cell compartments that keep life processes intact and help regulate cardiovascular functions. However, an abnormality of lipids can result in complex pathological conditions such as fatty liver, cardiovascular diseases, metabolic syndrome etc. (Cockcroft, 2021). The study investigated the effect of aqueous seed extract of *Tetracarpidium conophorum* and leaf extract of *Annona muricata* on calcium level and the lipid profile of male Wistar rats.

The study findings demonstrated a significant decrease in total cholesterol level in groups A, B, and C while group D had a non-significant decrease compared to group E. However, the physiology linked to the significant decrease in total cholesterol level in groups treated with *T. conophorum* and *Annona muricata* as indicated in groups A, B, and C results from the presence of phytochemicals such as alkaloids, saponins, and flavonoids. The study agrees with the report of Analike et al. (2017) revealing the cooked African walnuts demonstrated a significant decline in total cholesterol level in hyperlipidemia model, which is in accordance with the study findings. Also, Nwaichi et al. (2017) and Clarisse, Kenfack, and Angele (2017) documented a significant reduction in total cholesterol following administration of *T. conophorum* extract against hyperlipidemic activities, which agrees with the study findings. Ezealisiji et al. (2016) reported that the ethyl acetate and n-hexane extracts of *T. conophorum* seed demonstrated lowered level of cholesterol activities against hyperlipidemic activities, which corroborates the study outcome. Abam et al. (2013) concluded that walnut oil administered at 2.00 g/kg and 4.00 g/kg doses restored some lipid aberrations but caused an increase in total cholesterol, which disagree with the study findings. Also, Ojo et al. (2022) and Florence et al. (2014) reported that aqueous extract of *A*. *muricata* peel demonstrated a significant reduction in total cholesterol levels in diabetic rats, which corresponds to the study findings. However, the findings of Agu and Okolie (2019) demonstrated a significant reduction in total cholesterol levels following *Annona muricata* pulp and leaf extracts, which agrees with the study findings. Sovia et al. (2023) reported that the fruit extract of *Annona muricata* demonstrated a significant reduction in total cholesterol levels in diabetic model, which corresponds to the study findings. Adeyemi et al. (2009) and Atanu et al. (2019) reported a significant reduction in total cholesterol level following *Annona muricata* leaf extract using methanol as mode of extract against a diabetic model, which corroborates the study findings.

Triglcerides (TGs) are important in the metabolism of fatty acids through the de novo biosynthesis. Fatty acids are eliminated by oxidation within the cell or by secretion into the plasma within triglyceride-rich very low-density lipoproteins. However, TGs are associated with non-alcoholic fatty liver, which results from obesity, type 2 diabetes, and dyslipidemia, and commonly occurs in the setting of insulin resistance (Alves-Bezerra & Cohen, 2017). The triglyceride result revealed a significant reduction in groups A and C while groups B and D had a non-significant decrease compared to group E. The mechanism of action following the reduction in the TGs level is associated with flavonoids and alkaloids present in both extracts, which tends to lower cholesterol through esterification process. The reports of Analike et al. (2017), Nwaichi et al. (2017), Ezealisiji et al. (2016), and Abam et al. (2013) showed similarity to the study findings documenting a significant reduction in TGs levels following administration of *T. conophorum* against toxicity of different models. However, treatments with *Annona muricata* demonstrated a lowered level of TG, which could be attributed to the presence of flavonoids in the plant. The study of Ojo et al. (2022), Florence et al. (2014), Atanu et al. (2019), and Sovia et al. (2023), reported a significant reduction in TGs levels following administration of *Annona muricata*, which agree with the study findings.

Lipoproteins, lipid molecules involved in transporting proteins and lipid metabolism, are a risk factor for cardiovascular disease (Bhargava et al., 2022). Genetic and environmental disorders can lead to abnormal levels or functions of lipoproteins, increasing the risk of cardiovascular disease. Factors like diet, physical activity, and smoking also influence lipoprotein levels, contributing to the development of lipid disorders (Lent-Schochet & Jialal, 2023). The study findings showed a non-significant decrease in the LDL-C levels in groups A and D while groups B and C had a significant decrease compared to group E. The HDL-C result showed a non-significant increase in groups A, B, C, and D compared to group E. The mechanism of action linked to decreased level of LDL-C in groups B and C is associated with flavonoids, alkaloids, and saponins present in *T. conophorum* and *Annona muricata*.

The findings of Analike et al. (2017) showed significant decrease in LDL following administration of cooked walnuts, which agrees with the study findings. Also, Nwaichi et al. (2017) demonstrated a significant reduction in LDL following *T. conophorum* against hyperlipidemia toxicity, which corroborates the study result. Further, the ethyl acetate and n-hexane extracts of *T. conophorum* seed demonstrated a significant decline in LDL-cholesterol levels, which agree with the study findings (Ezealisiji et al., 2016). Further, the oil of *T. conophorum* demonstrated a significant reduction in LDL-cholesterol levels, which agrees with the study findings. Talabi et al. (2023) showed a lowered level of LDL-cholesterol following *T. conophorum*, which agrees with the study findings. Further, treatments with *Annona muricata* from several studies (Agu and Okolie, 2019; Florence et al., 2014, Sovia et al., 2023) indicated a significant reduction in LDL-cholesterol levels in toxicity studies, which agree with this study outcome. However, the study disagrees with the report of Florence et al. (2014) demonstrating a significant increase in HDL-cholesterol level following *Annona muricata* ingestion against diabetic model. Also, Agu and Okolie (2019) reports contradict the study findings demonstrating a significant increase in HDL-cholesterol level following *Annona muricata* in hyperlipidemic activities. Clarisse, Kenfack, and Angele (2017) showed a significant increase in the HDL levels following *T. conophorum* intake and contradict the study outcome. Ezealisiji et al. (2016) indicated a significant increase in the HDL levels following *T. conophorum* intake, which refutes the study outcome.

Calcium ion is the most significant ion in the heart, which is known for its function in the contraction of cardiac muscle. However, calcium dysfunction has been linked to the onset of cardiac muscular dystrophy (Agrawal et al., 2018). The study showed a non-significant increase in serum calcium level in groups A, B, and C while group D had a significant increase compared to group E. The mechanism of action following the significant increase in calcium level is not well understood but it could be suggested that *Annona muricata* contains minerals (calcium), which tends to replenish the depleted calcium stores in the body that might affect the cardiac and skeletal muscle function. However, the findings of Elizabeth et al. (2018) showed an increase in the calcium level when the root and leaf extract of *Annona muricata* were compared, which agrees with the study findings.

CONCLUSION

The study revealed that *T. conophorum* and *Annona muricata* extracts significantly reduced cholesterol, triglycerides, and low-density lipoprotein cholesterol and non-significantly increased HDL-C. *Annona muricata* alone significantly increased calcium level.

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