

Ameliorating Effects of *Annona Muricata* Leaf Extract on Lipid Profile in Male Wistar Rats

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ABSTRACT

This study aims to determine the ameliorating effects of *annona muricata* leaf extract in lipid profile in male wistar rats. A total of 78 adult male rats obtained from the laboratory animal production unit of the Department of Physiology, Abia State University, Uturu were used for the different segments of the study. Eighteen of the rats were used for acute toxicity/lethal dose evaluation of the extract while the remaining sixty (60) were used for the testosterone-induced hyperplasia study. Prostate hyperplasia was induced in the rats via daily sub-cutaneous administration of testosterone propionate (5 mg/kg body weight) for 28 days. Sixty rats used for this segment of the study were assigned to 6 groups of 10 rats and treated as follows: Group 1, Normal control, no treatment. Group 2 Testosterone propionate (5 mg/kg) only and was the BPH control. Group3, Testosterone propionate (5 mg/kg bw) + Finasteride (3 mg/kg body weight). Group 4, Testosterone propionate (5 mg/kg bw) + *Annona Muricata* Leaf extract (300 mg/kg body weight). Group 5, Testosterone propionate (5 mg/kg bw) + *Annona Muricata* Leaf extract (600 mg/kg body weight). Group 6, *Annona Muricata* Leaf extract (600 mg/kg bw) only. At the end of treatment, animals were sacrificed and blood samples were collected into EDTA and plain bottles for Lipid profile analysis respectively. For the later (blood contained in plain bottles), the blood was allowed to clot and retract within two hours and then centrifuged at 3000 rpm for 10 minutes to obtain clear sera samples which were used for the various lipid profile (Total Cholesterol, High Density Lipoprotein, Triglycerides and Very Low Density Lipoprotein) analyses. All lipid profile parameters were determined using the respective commercial test kits and following protocols for the parameters. Data obtained were subjected to Statistical Package for Social Sciences (SPSS, Version 20.0, IBM SPSS Inc, Chicago, IL) was used for data analysis. Level of significance was calculated by One Way Analysis of Variance (ANOVA). Data were analyzed using Duncan Multiple Range Test and complemented with Student's *t* test for post-hoc test for comparisons of the means of the various doses and fractions. *P*- values less than 0.05 were considered statistically significantly different between the test and control groups as well as among test groups for measured value. *Annona muricata* leaf extract exhibited ameliorating effects on lipid profiles level contributing to overall metabolic health.

Keywords: *Annona Muricata* leaf extract, testosterone-induced hyperplasia, ameliorating effects, lipid profile and wistar rats

INTRODUCTION

Maintaining a healthy lipid profile is crucial for overall metabolic health, as abnormalities in lipid levels can increase the risk of cardiovascular diseases and metabolic syndrome. *Annona muricata* has been investigated for its potential effects on lipid metabolism, particularly in improving lipid profiles [3,5]. Lipid profile, consisting of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG), serves as a key indicator of cardiovascular risk [1]. Dyslipidemia, characterized by elevated levels of TC, LDL-C, and TG, along with decreased levels of HDL-C, is a common metabolic abnormality associated with increased cardiovascular morbidity and mortality [8]. Several studies have explored the impact of *Annona muricata* on lipid metabolism [1,5,7]. According to Aderibigbe *et al.* (2009), administration of *Annona muricata* extracts resulted in significant improvements in lipid profiles in animal models of metabolic disorders. Specifically, supplementation with *Annona muricata* led to reductions in TC, LDL-C, and TG levels, along with increases in HDL-C levels. The antioxidant activity of *A. muricata* leaf extract was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The results showed that the extract exhibited significant antioxidant activity, which could contribute to its lipid-lowering effects [6]. The potential mechanisms underlying the lipid-lowering effects of *Annona muricata* are multifaceted. One proposed mechanism involves the modulation of hepatic lipid metabolism. *Annona muricata* extracts may inhibit key enzymes involved in cholesterol synthesis, such as 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase), leading to decreased cholesterol production in the liver [1]. Additionally, the plant's antioxidant properties may protect against

lipid peroxidation and oxidative damage, thereby preserving the integrity of lipoproteins and preventing the formation of atherosclerotic plaques[1,2].

MATERIALS AND METHODS

Materials

Equipments used include sensitive weighing balance, Electric weighing balance, Manual blender, Dissecting set, Dissecting board, 5ml syringes, 1ml syringes, test tubes, serum bottles, Sample bottles, EDTA bottles, whatman No 1 filter paper, Measuring cylinders, Pasteur pipette, Cotton wool, Micro pipette, Rat cages, Feeding and water trough, Oral gavage, Glass column, Microscope, Glass TLC plates, microscope slides, Glass wool, funnel, Spatula, Retort stand, Hand gloves, TLC tank, beaker, Oven, freezer, Refrigerator, Spectrophotometer(722N, Mindray, MC Quijing, China), Centrifuge, Rotary evaporator, Soxhlet extractor, GC-MS machine, Chemoilluminoscene machine and Haematology analyser (BC-2300, Mindray, MC Quijing, China).

ETHICAL APPROVAL

This study was approved by the research and ethical committee of the Department of Physiology, Abia State University, Uturu.

Animals

A total of 78 adult male rats obtained from the laboratory animal production unit of the Department of Biochemistry, Abia State University, Uturu were used for the different segments of the study. Eighteen of the rats were used for acute toxicity/lethal dose evaluation of the extract while the remaining sixty (60) were used for the testosterone-induced hyperplasia study. The animals were housed in aluminum cages, under standard conditions 12/12h light-dark cycle at temperature of $25\pm 2^{\circ}\text{C}$ and were fed with standard grower pellets (Grand Cereals Ltd, Abia State) and had access to clean drinking water for seven days of acclimatization before the

initiation of experiment. All experiments were carried out in accordance with International Guideline for Biomedical Research (CIOMS/OMS, 1985) and as approved by the Department of Physiology, Abia State University, Uturu.

Experimental Design for the Hyperplasia Study

Prostate hyperplasia was induced in the rats via daily sub-cutaneous administration of testosterone propionate (5 mg/kg body weight) for 28 days. Sixty rats used for this segment of the study were assigned to 6 groups of 10 rats and treated as follows:

- Group 1 - Normal control, no treatment
- Group 2 - Testosterone propionate (5 mg/kg) only and was the BPH control
- Group 3 - Testosterone propionate (5 mg/kg bw) + Finasteride (3 mg/kg bw)
- Group 4 - Testosterone propionate (5 mg/kg bw) + AML extract (300 mg/kg bw)
- Group 5 - Testosterone propionate (5 mg/kg bw) + AML extract (600 mg/kg bw)
- Group 6 - AML extract (600 mg/kg bw) only

At the end of treatment, animals were sacrificed and blood samples were collected into EDTA and plain bottles for Lipid profile analysis respectively. For the later (blood contained in plain bottles), the blood was allowed to clot and retract within two hours and then centrifuged at 3000 rpm for 10 minutes to obtain clear sera samples which were used for the various lipid profile analyses.

Preparation of Plant Extracts

Plant extract were prepared in accordance with the method used by Oriek *et al.*, (2019). The freshly collected leaves of *Anona muricata* were air dried under shade

for within 14 days and were thereafter pulverized into coarse powdered form using a locally fabricated grinding machine. Two solvents, ethanol and N-hexene were used for the preparation of the extracts. A known quantity (300g) of the powdered plant material was macerated in 1.5 litres of ethanol (AR) for 48 hours, been vigorously shaken every 2 hours within the period and was thereafter filtered with Whatman No. 1 filter paper to obtained a filtrate which was the extract in solution. The filtrate so obtained was concentrated at low temperature to obtain a solid dark-green extract which weighed 13.91 g and represented 4.64% extract yield. The extract was preserved in the refrigerator at -4 °C until needed and is hereafter referred to as *Anona muricata* leaf (AML) extract.

Determination of Lipid Profile Parameters

All lipid profile parameters were determined using the respective commercial test kits and following protocols for the parameters as was reported by Ugbogu *et al.*, (2017).

Total Cholesterol (TC)

Kit: RANDOX

Reagent: R1 (a combination of 80 mmol/L of pipes buffer at P^H 6.8, 0.25 mmol/L of 4-Aminoantipyrine, 6 mmol/L of phenol, 0.5 U/ml of peroxidase, 0.15 U/ml of cholesterol esterase and 0.10 U/ml of cholesterol oxidase).

Principle: After oxidation and enzymatic hydrolysis, cholesterol is often measured. In the presence of phenol and peroxidase, hydrogen peroxide and 4-aminoantipyrine combine to generate the indicator quinoneimine, the amount of which is correlated with the concentration of cholesterol.

Procedure: For each sample, three test tubes labelled test, blank and standard were set up and 1.0 ml of the R1 reagent

was pipetted into each of the test tubes. Then 10µl of the test sample was added into the test tube labeled test. The same volume of the standard reagent was added to the test tube labeled standard and the same volume of distilled water to the blank test tube. The mixtures were incubated at 20-25°C for 10 minutes or at 37°C for 5 minutes before reading the absorbance of the test and standard on a spectrophotometer after zeroing with the blank at wavelength 546 nm. Total cholesterol for the sample was then calculated using the expression:

$$\text{Total cholesterol} = \frac{\text{Absorbance of test} \times \text{concentration of standard}}{\text{Absorbance of standard}}$$

Where concentration of standard = 206 mg/dl

High Density Lipoprotein-Cholesterol (HDL-C)

Kit: RANDOX

Reagent: R1 (A combination of 0.55mmol/L of Phosphotungstic acid and 25 mmol/L of Magnesium Chloride).

Principle: Low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the high density lipoprotein fraction which remains

in the supernatant is then determined according to the normal method for determining the concentration of total cholesterol.

Procedure (Stage 1): For a each sample, a test tube containing 1000µl of R1 and 500µl of the sample were set up, mixed and allow to sit for 10 minutes at 25°C. The clear supernatant was then recovered after centrifuging this for two minutes at 4000 rpm. Using the methods listed below, the cholesterol content of this supernatant—now known as high density lipoprotein cholesterol—was ascertained and set up for total cholesterol:

Procedure (Stage 2): Three test tubes labeled test, blank and standard were set up and 1.0 ml of the R1 reagent for total cholesterol was pipetted into each of the test tubes. The test tube with the test label was then filled with 10µl of the previously obtained supernatant. The standard test tube received the same volume of the standard reagent for total cholesterol, whereas the blank test tube received the same volume of distilled water. The mixtures was incubated at 20-25°C for 10 minutes or at 37°C for 5 minutes before reading the absorbance of the test and standard on a spectrophotometer after zeroing with the blank at wavelength 546 nm. High density lipoprotein cholesterol for the sample was calculated using the expression:

$$\text{HDL - C concentration in mg/dl} = \frac{\text{Absorbance of test} \times \text{concentration of standard}}{\text{Absorbance of standard}}$$

Where concentration of standard = 206 mg/dl

Triglycerides

Kit: RANDOX

Reagent: R1a (a combination of 40 mmol/L of pipes buffer at pH 7.6, 5.5 mmol/L of 4-chloro-phenol and 17.5 mmol/L of magnesium ions) and R1b

(composing of 0.5mmol/L of 4-aminophenazone, 1 mmol/L of ATP, 150 U/ml of lipases, 0.4 U/ml of glycerol-kinase, 1.5 U/ml of glycerol-3-phosphate oxidase and 0.5 U/ml of peroxidase)

Principle: Hydrogen peroxide, 4-aminophenazone, and 4-chlorophenol are converted into the indicator quinoneimine through the catalytic action of peroxidase. The quantity produced is correlated with the triglyceride levels.

Procedure: 15 ml of reagent R1a was reconstituted with one vial of reagent R1b, and the mixture was thoroughly mixed. Three test tubes with the labels test, blank, and standard were prepared for every sample. Then 1.0 ml of the reconstituted reagent was pipetted into each of the test

tubes. Exactly 10µl of the test sample was added into the test tube labeled test. The same volume of the standard reagent was added to the test tube labeled standard and the same volume of distilled water to the blank test tube. After zeroing with the blank at 546 nm, the solutions were incubated at 20–25 °C for 10 minutes or at 37 °C for 5 minutes. The absorbance of the test and standard was then measured using a spectrophotometer. Triglycerides value for the sample was calculated using the expression:

$$\text{Triglycerides concentration} = \frac{\text{Absorbance of test} \times \text{concentration of standard}}{\text{Absorbance of standard}}$$

Where concentration of standard = 206 mg/dl

Very low density lipoprotein cholesterol and Low density lipoprotein cholesterol are calculated using the expressions:

- Very low density lipoprotein cholesterol (VLDL-C) = $\frac{\text{Triglycerides}}{5}$
- Low density lipoprotein cholesterol (LDL-C) = Total cholesterol - (HDL + VLDL-C)

Data analysis

Statistical Package for Social Sciences (SPSS, Version 20.0, IBM SPSS Inc, Chicago, IL) was used for data analysis. Level of significance was calculated by One Way Analysis of Variance (ANOVA). Data were analyzed using Duncan Multiple Range Test and complemented

with Student's t test for post-hoc test for comparisons of the means of the various doses and fractions. P- values less than 0.05 were considered statistically significantly different between the test and control groups as well as among test groups for measured value.

RESULTS

Table 1: Lipid profile parameters.

Treatments	Normal control	BPH control (Testosterone only)	Finesteride (3 mg/kg) + Testosterone	AML extract (200 mg/kg) + Testosterone	AML extract (400 mg/kg) + Testosterone	AML extract (800 mg/kg) + Testosterone
Total cholesterol (mg/dl)	104.53 ±5.58 ^{a,b}	141.00±7.29 ^c	115.73±11.02 ^b	113.30±4.09 ^b	106.50±6.10 ^{a,b}	97.63±2.90 ^a
HDL-C (mg/dl)	59.17±1.32 ^a	53.87±3.03 ^b	58.59±1.12 ^b	59.95±0.43 ^b	59.14±1.24 ^b	61.48±0.72 ^b
TAG (mg/dl)	78.06±2.39 ^a	93.53±2.20 ^c	84.40±0.99 ^b	81.62±1.05 ^{a,b}	80.43±1.07 ^a	79.94±1.49 ^a
LDL-C (mg/dl)	29.76±4.91 ^{a,b}	68.42±4.26 ^c	40.26±10.73 ^b	37.02±4.01 ^b	31.27±5.38 ^b	20.17±2.34 ^a
VLDL-C (mg/dl)	15.61±0.48 ^a	18.71±0.44 ^c	16.88±0.20 ^b	16.32±0.61 ^{a,b}	16.09±0.21 ^a	15.99±0.30 ^a

Values are presented as mean $\pm$ standard deviation ($n = 3$); and means with different letter superscripts are significantly ($P < 0.05$) different from paired mean across the row.

Table 4.1 Show the results of the lipid profile parameters across various treatment groups. The BPH control (Testosterone only) group shows significantly elevated levels of total cholesterol (141.00 ± 7.29 mg/dl), LDL-C (68.42 ± 4.26 mg/dl), HDL-C (53.87 ± 3.03 mg/dl), TAG (93.53 ± 2.20 mg/dl), and VLDL-C (18.71 ± 0.44 mg/dl) compared to the normal control group (total cholesterol: 104.53 ± 5.58 mg/dl; LDL-C: 29.76 ± 4.91 mg/dl; HDL-C: 59.17 ± 1.32 mg/dl; TAG: 78.06 ± 2.39 mg/dl; VLDL-C: 15.61 ± 0.48 mg/dl). Treatment with Finasteride (3 mg/kg) + Testosterone and AML extract at various doses (200 mg/kg, 400 mg/kg, and 800 mg/kg) + Testosterone generally improves these lipid profiles compared to the BPH control group, with reductions observed in total cholesterol, LDL-C, TAG, and VLDL-C levels, and increases or maintenance of HDL-C levels. The highest dose of AML extract (800 mg/kg) + Testosterone shows the most favorable lipid profile, with values closer to those of the normal control group.

DISCUSSION

The lipid profile results from the study underscore the potential of *Annona muricata* leaf (AML) extract in improving lipid metabolism, a critical factor in mitigating cardiovascular risk associated with conditions like benign prostatic hyperplasia (BPH) (Alwan *et al.*, 2020; Florence *et al.*, 2014). The BPH control group (Testosterone only) exhibited dyslipidemia characterized by elevated levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), and very low-density lipoprotein cholesterol (VLDL-C), alongside reduced high-density lipoprotein

cholesterol (HDL-C). These lipid abnormalities are commonly linked with increased cardiovascular risk (Aderibigbe *et al.*, 2009; Toh *et al.*, 2024). Studies investigating AML extract have consistently shown beneficial effects on lipid profiles. For instance, Aderibigbe *et al.* (2009) demonstrated that *Annona muricata* supplementation led to significant reductions in TC, LDL-C, and TG levels, while increasing HDL-C levels in animal models of metabolic disorders. The antioxidant properties of AML extract, highlighted by its ability to scavenge free radicals and inhibit lipid peroxidation, likely contribute to these lipid-lowering effects (Hasmila *et al.*, 2019). Mechanistically, AML extract may modulate hepatic lipid metabolism by inhibiting enzymes such as 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase), which is crucial in cholesterol synthesis (Aderibigbe *et al.*, 2009). Moreover, the presence of flavonoids, phenolic acids, and alkaloids in AML extract enhances its antioxidant capacity, protecting against oxidative stress-induced damage to lipoproteins and thereby reducing atherogenic risk [4,9]. In the context of the current study, treatment with Finasteride (3 mg/kg) + Testosterone and AML extract at varying doses (200 mg/kg, 400 mg/kg, and 800 mg/kg) + Testosterone demonstrated improvements in lipid profiles compared to the BPH control group. These treatments resulted in decreased TC, LDL-C, TG, and VLDL-C levels, while maintaining or increasing HDL-C levels, with the highest dose of AML extract (800 mg/kg) + Testosterone showing the most favorable lipid profile outcomes comparable to the normal control group (Alwan *et al.*, 2020; Florence *et al.*, 2014).

CONCLUSION

Annona muricata leaf extract exhibited ameliorating effects on lipid profiles level contributing to overall metabolic health.

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