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Anti-Atherosclerosis and Anti-Hyperlipidemia Functions of *Terminalia catappa* Fruit

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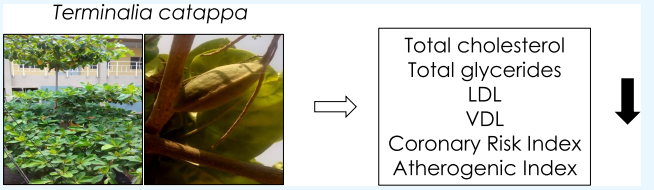
ABSTRACT: *Background*: Atherosclerosis is a chronic pathological condition that has remained clinically silent for decades, and the epidemic has continued to be on the rise due to risk factors, including diet, lifestyle, hyperlipidemia, pathogenic microorganisms, and aging. Using various synthetic drugs in treating atherosclerosis is associated with a high risk of myositis, angioedema, myoglobinuria, and acute renal failure. Various side effects of the available drugs have been reported; attempts are underway to explore natural sources with antiatherosclerotic activity. *Aim and objective*: Using a diet-induced atherosclerosis rat model, the current study tested the hypothesis of antiatherosclerotic and antihyperlipidemic roles of *Terminalia catappa* fruit extracts. *Materials and Methods*: Atherosclerosis in Wistar rats was induced using an atherogenic diet. Total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (AP), creatine kinase (CK), and lactate dehydrogenase (LDH) were determined using analytical kits. *Results*: Quantitative phytochemical analysis of the extracts demonstrated that the plant had flavonoids, saponins, tannins, terpenoids, alkaloids, cardiac glycosides, sterols, phenols, and anthraquinones. Diet-induced atherogenic Wistar rats showed a significant ($p < 0.05$) increase in total cholesterol, triglyceride, low-density lipoprotein cholesterol, and very low-density lipoprotein cholesterol compared to the healthy control group; however, the atherogenic lipid profile was reversed by the treatment of *T. catappa* fruit extracts. The biochemical experiments demonstrate that *T. catappa* fruit extracts have an antihyperlipidaemic effect, shown by a decreased coronary risk index and the atherogenic index, and an increased cardioprotective index, compared to disease control. *Conclusion*: The current study indicates that *T. catappa* fruit extracts may contain bioactive molecules to treat atherosclerosis.



INTRODUCTION

Atherosclerosis is a blood vessel disease of lipid accumulation in the artery.[1] Primary complications of atherosclerosis include angina pectoris, myocardial infarction, and stroke.[2] Atherosclerosis is an inflammatory condition leading to the development of ischemic heart diseases, cerebrovascular diseases, and peripheral vascular diseases.

Hyperlipidemia represents the early development of atherosclerosis and its cardiovascular complications.[3] The inflammatory signs of atherosclerosis are accompanied by incipient lipid accumulation in the artery wall.[4] Several factors determine endothelial modifications through a primary inflammatory response followed by a local prothrombotic balance.[5] Low-density lipoprotein cholesterol (LDL) oxidation is a leading cause of endothelial injury and induces the expression of pro-inflammatory molecules in endothelial cells.[6] Thus, removing modified LDL is the necessary treatment of the inflammatory response.[7] Other risk factors include diabetes developing in the early stages of insulin-dependent and insulin-resistant patients. Diabetes is clinically associated with impaired endothelial function, worsened by severe hyperglycemia.[8]

When plaques are damaged and rupture, prothrombotic molecules are exposed to the coagulation system to inhibit blood flow.[9] Statins are widely used in the treatment of atherosclerosis due to their excellent efficacy in reducing low-density lipoprotein levels.[10] Statins competitively inhibit the 3-hydroxy-3-methylglutaryl coenzyme A reductase as catalysis of the rate-limiting step in cholesterol biosynthesis.[11] Many antihyperlipidemic agents, including statin, fibrates, niacin, bile acids, and ezetimibe, reduce cholesterol levels under different conditions.[12]

Terminalia catappa Linn belongs to the Combretaceae family as a considerable (25−40 m height) deciduous tree with smooth gray bark and whorled branches that form a canopy in tropical and subtropical regions of Asia and Africa.[13] It is often found in coastal vegetation, growing at the edges of mangrove swamps or on rocky shores. It is widely planted throughout the tropics as an ornamental tree for shade and edible nuts in Africa. The tree loses its leaves twice yearly in most places,

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turning a brilliant red to yellow before leaf shedding.[14] Traditionally, people used *T. catappa* as a dysentery and hepatitis medicine in Asia. The dried leaves are used for fish pathogen treatment as an alternative to antibiotics. The leaves have antioxidant, anticlastogenic, and antihyperglycemic properties.[15] The leaves and bark extracts of *T. catappa* are anticancer, anti-HIV reverse transcripts, hepato-protective, anti-inflammatory, antihepatitis, antidiabetic, and aphrodisiac.[16] The fallen leaves of *T. catappa* have been used in the management of sickle cell disorders.[17]

Atherosclerosis has remained asymptomatic and clinically silent for decades, and the epidemic has continued to rise due to environmental risk factors, including diet, lifestyle, hyperlipidemia, pathogens, and aging.[18] Eighteen synthetic drugs, including statins, are associated with a higher risk of myositis, angioedema, myoglobinuria, and acute renal failure in treating the above conditions. Due to the side effects of the available medicine, attempts are underway to explore natural sources with antiatherosclerotic activity.[19] The current study aims to determine the antiatherosclerotic effects of *T. catappa* extracts in a murine model.[20] Our in vivo study demonstrated that *T. catappa* fruit extracts could regulate total cholesterol, triglyceride, low-density lipoprotein cholesterol, and very low-density lipoprotein cholesterol. In addition, metabolic biomarkers, including alanine transaminase, aspartate aminotransferase, alkaline phosphatase, creatine kinase, and lactate dehydrogenase, decreased using *T. catappa* fruit extracts. Histology confirmed that the aorta, heart, and liver improved cholesterol deposits in the lumen of the blood vessels by the extracts. The current biochemical experiments show that *T. catappa* fruit extracts contain bioactive molecules to treat atherosclerosis.

MATERIALS AND METHODS

Materials. Cholesterol, cholic acid, and Atorvastatin were commercially available (Sigma-Aldrich Chemical Co., Milwaukee, Wisconsin). Analytical kits were purchased from Randox Laboratories Limited, UK, to determine lipids and biomarkers, including total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (AP), creatine kinase (CK), and lactate dehydrogenase (LDH). All other chemicals were of analytical grade.

Plant Materials. *T. catappa* Linn ripe fruits were collected from Jimeta, Yola-North local government area of Adamawa State, Nigeria (February 2015, latitude 11055'N and longitude 11011'), and were authenticated in the Department of Plant Science, Modibbo Adama University of Technology, Yola, Nigeria. The plant taxonomists Bristone B. and Chimbe Konje identified and confirmed the herbarium specimens by the taxonomic literature. *T. catappa* fruit was photographed in the live form at the time of collection and was preserved after the allotment of the voucher number. The *T. catappa* fruit was deposited in the herbarium of Modibbo Adama University, Yola (voucher number MAU/YL/BT/0420). The herbarium specimen of *T. catappa* fruit was a pressed, mounted sample with collection data deposited for future studies.

Animals. Albino rats of the Wistar strain (200−250 g) were purchased from the Veterinary Research Institute, Vom, Jos, Nigeria. The animals were in a group of five rats per cage and were maintained under standard laboratory conditions. The animals were fed for 6 weeks with a regular diet from Vital Feeds (Jos, Nigeria) and freshwater ad libitum. All of the animals were acclimatized to laboratory conditions for a week before the commencement of the experiment.

An atherogenic diet was used to induce atherosclerosis in Wistar rats.[21] The atherogenic diet was prepared by mixing cholesterol 1.25%, cholic acid 0.5%, fat 15%, and standard pellet 83.25%.[22] All animal experiments were followed in accordance with the Guide for the Care and Use of Laboratory Animals (8th edition, 2006, NIH).

Protocols for animal experiments were approved by the Animal Experimental Ethics Committee of the Modibbo Adama University of Technology with approval number UAEC/YMAU/YL/0100 on the fourth of January, 2016, in compliance with the National Institutes of Health guidelines for the care and use of laboratory animals.

***T. catappa* Fruit Extraction.** For the ethanol extraction (ETC), ripe fruits of *T. catappa* were sliced and shade-dried. After moisture was removed, fruits were pulverized using an electric blender (BL335, Kenwood Company) to obtain a powder. The powdered sample (250 g) was macerated in absolute ethanol (95%, 1000 mL) for 72 h at room temperature. The mixture was wrung in a neat cloth and filtered by using Whatman No. 1 filter paper. The filtrate was concentrated at 40 °C using a rotary evaporator to obtain a semidried brown solid (22%). ETC was stored in a tightly closed container and inside a refrigerator (4 °C). For the aqueous extraction of *T. catappa* (ATC), the dry powder was macerated in distilled water (1000 mL) for 72 h at room temperature. The filtrate was concentrated using a drying oven (40 °C, 3 days) to obtain a brown solid (21%). ATC was kept in a sterilized container in a refrigerator at 4 °C.

Two different postextraction drying methods were used, such as lyophilization or oven-drying routinely. All filtrated were lyophilized using Labconco FreeZone (36 h, −100 °C, 4.5 L) or a drying oven (48−72 h, 40−60 °C). Aqueous/EtOH extracts were dried in the oven (72 h, 40 °C or 48 h, 60 °C) or a lyophilizer (36 h, −100 °C).

Phytochemical Screening of *T. catappa* Fruit. The ethanol and aqueous extracts were analyzed qualitatively through phytochemical screening of alkaloids, saponins, tannins, steroids, flavonoids, phenols, terpenoids, anthraquinones, and cardiac glycosides. Phenolic and flavonoid molecules were determined by UV−vis spectrophotometric analysis.

METHODS

Quantitative Phytochemistry. A five-point calibration curve using standard molecules, including gallic acid or quercetin, was prepared by using the peak area against different standard concentrations to obtain the concentration of the phytochemicals. The phenol concentration in the extracts was analyzed by the modified Folin−Ciocalteu method using gallic acid equivalent (mg/g).[23] Total phenolics were expressed using the regression equation between gallic acid standards and A765.

The total flavonoid concentration was analyzed using the aluminum chloride method.[24] The flavonoid content was expressed as quercetin equivalents (mg/g) from the calibration curve of the standard. The extracts (10 mg) were dissolved in ultrapure hexane (2 mL) as a nonpolar solvent or chloroform/MeOH/H_2O (8:2:1) as a polar solvent system. The fractions were filtered using a micro chromatography syringe filter (0.25 μm) into a vial (1 mL). The prepared organic layer (hexane or chloroform) was injected into a high-performance liquid

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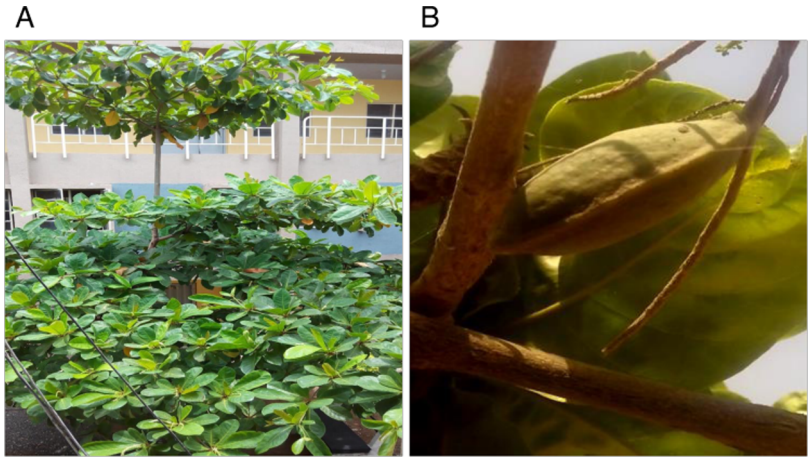

Figure 1. *T. catappa* tree (A) and fruit (B). Pictures were taken in Yola, Nigeria.

chromatography (HPLC, Buck Scientific BLC10/11) with a fluorescence detector (excitation at 295 nm and emission at 325 nm) and an analytical column (25 cm × 4.6 mm, stainless steel, 5 μm). The mobile phase was hexane: tetrahydrofuran: isopropanol (1000:60:4 = v/v/v) at a 1.0 mL/min flow rate with a sample injection volume of 20 μL. Positive controls, including quercetin (flavonoid), oleanolic acid (saponin), lanatoside C (cardiac glycoside), gallic acid (phenols), caffeine (alkaloids), corosolic acid (terpenoids), 6β-hydroxycortisone (steroids), and anthraquinone (anthraquinones), were used to analyze plant metabolites for quantitative HPLC analysis. Stock solutions of each standard were prepared separately in methanol at 1.0 mg/mL. The highest calibrator was prepared by mixing appropriate volumes of each stock solution and diluting it to obtain a final concentration. The HPLC sample was purified to remove any contaminants by solid-phase extraction with Phenomenex Strata C_{18} cartridges. The identification of the different compounds in the chromatographic profile of the extracts was compared by their retention time and UV spectra of positive controls.

Antiatherosclerotic Roles of *T. catappa* Fruit In Vivo. Fifty-five male rats (200−250 g) were divided into 11 groups (five rats/cage).[25] Standard control group (I) received a regular pellet diet; disease control group (II) received an atherogenic diet; positive control groups (III) received an atherogenic diet and Atorvastatin (10 mg/kg), ethanol extract-treated group (IV, V, VI) received 100, 200, and 300 mg/kg of *T. catappa* fruit ethanol extracts (ETC), respectively.[26] Aqueous extract-treated groups (VII, VIII, IX) received an atherogenic diet and 100, 200, and 300 mg/kg of aqueous extracts of *T. catappa* fruit (ATC), respectively. The highest dose group (X and XI) received a regular diet and the highest dose of ETC/ATC extracts (300 mg/kg), respectively. Extracts were administered to different groups daily for 6 weeks by oral gavage. At the end of the experiment, the rats were fasted overnight and euthanized under ether, isoflurane, or pentobarbital anesthesia, showing no difference. In all cases, animals were euthanized using ether. Blood was collected by cardiac puncture in plain tubes and allowed to stand for an hour. The clotted sample was centrifuged, followed by serum collection, and stored at −20 °C for biochemical analysis. The liver, heart, and aorta were removed and stored in 10% formaldehyde solution for histopathological examinations.[27]

Determination of Lipid Profile Biomarkers. Total cholesterol was determined using enzymatic hydrolysis and oxidation protocol.[28] Triglycerides were analyzed using the colorimetric method, and HDL-cholesterol was measured using the precipitation method.[29] Low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol were determined using Friedewald's method.[30] Transaminases (AST/ALT) were analyzed using the colorimetric method,[31] and creatine kinase activity was assayed by the Nelson method. The p-nitrophenol protocol was used to evaluate ALP activity using the Randox test kit.[32] Atherogenic, cardioprotective, and coronary risk indices were calculated as previously.[33] In brief, the atherogenic index (AI), coronary risk index (CRI), and cardioprotective index (CPI) were calculated as AI = Log [TG/HDL-C], CRI = TC/HDL-C, and CPI= [HDL-C/LDL-C], respectively.

Histopathological Determination. Aorta, heart, and liver specimens were fixed in a 10% buffered formaldehyde solution, sectioned, and embedded in paraffin wax using conventional techniques for histopathological studies. The aortas were cut transversely at the arch and midthoracic level; the heart and liver were also cut, followed by fixing in Bruin's fluid for 24 h, and were processed for routine histopathological examination by passing through graded alcohols. Tissue sections (6−8 μm thickness) were obtained by using a microtome, transferred on glass slides, and stained with hematoxylin and eosin. The stained sections were examined under a light microscope and photographed.

Statistical Analysis. One-way ANOVA (Analysis of Variance) with Tukey's multiple comparison tests were performed using GraphPad Prism version 5.02 for Windows (GraphPad Software, San Diego, California, www.graphpad.com). All values were expressed as means ± standard deviation of the mean (SEM). Differences were considered to be significant at $p < 0.05$.

RESULTS

Phytochemical Screening. Phytochemical screening of *T. catappa* fruit (Figure 1A, 1B) demonstrated the presence of alkaloids, flavonoids, tannins, saponins, cardiac glycosides, phenols, terpenoids, anthraquinones, and steroids in the ethanol and aqueous extracts.

The phytochemicals were further analyzed quantitatively to show that ethanol and aqueous extracts of *T. catappa* fruit contain large amounts of phenols, alkaloids, cardiac glycosides, and flavonoids using a UV−vis spectrophotometer (Table 1).

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Table 1. Quantitative Analysis of Phytochemical Components of *T. catappa*[a,b]

phytochemicals	ethanol (mg/g)	H_2O (mg/g)
alkaloids	182.7 ± 0.35[c]	176.5 ± 0.25
flavonoids	63.27 ± 0.15[c]	43.93 ± 0.25
saponins	28.50 ± 0.10	36.57 ± 0.15[d]
terpenoids	0.31 ± 0.015[c]	0.22 ± 0.030
steroids	8.07 ± 0.015[c]	6.24 ± 0.025
cardiac glycosides	71.31 ± 0.03[c]	61.37 ± 0.02
phenols	304.8 ± 0.20[c]	213.9 ± 0.25
tannins	0.15 ± 0.020[c]	0.11 ± 0.015
anthraquinones	0.08 ± 0.002[c]	0.06 ± 0.002

[a]Results are shown as mean ± SD (standard deviation) of 3 determinations. [b]All quantitative analysis was performed by UV−vis spectrophotometer and confirmed by HPLC using positive controls. [c]$P < 0.05$ is significantly higher than aqueous extracts. [d]$P < 0.05$ is significantly higher than ethanol extracts.

Ethanol solubilizes better for alkaloids, flavonoids, cardiac glycosides, and phenols, whereas saponin is well-soluble in aqueous extraction. All components were confirmed by HPLC using positive controls with standard curves.

The lipid profile of the atherogenic rat model was estimated, as shown in Table 2 (Supporting Information Figure S1−S5). Hyperlipidemia was evident in rats fed with an atherogenic diet only (Group II) by the significant increase ($P < 0.05$) in serum levels of total cholesterol (TC), total glyceride (TG), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) when compared to the values of standard diet control (Group I). The antiatherogenic and antihyperlipidemic potential of ETC was observed in Groups IV, V, and VI as concomitant administration of ETC along with an atherogenic diet showed significant ($P < 0.05$) decrease in the serum levels of TC, TG, LDL, and VLDL when compared to values of negative control (Group II).

The hypolipidemic effect of ETC was observed in rats (Group X) treated with 300 mg/kg of ETC under normal diet conditions, showing a significant ($P < 0.05$) decrease in TG and VLDL when compared to standard diet control (Group I).

The antiatherogenic and antihyperlipidemic potential of ATC was observed in Groups VII, VIII, and IX as concomitant administration of ATC along with an atherogenic diet showed a significant ($P < 0.05$) decrease in the serum levels of TC, TG, LDL, and VLDL when compared to atherogenic group (Group II). The highest doses of ATC (Group IX) showed no significant difference ($P < 0.05$) in serum levels compared to the values of standard diet control (Group I), implying its ability to suppress atherogenesis. The antiatherosclerotic activity of ETC at the highest dose (300 mg/kg) is evident, as an atherogenic diet effectively reduced LDL serum levels. The hypolipidemic activity of ATC was also observed in Group XI, which shows a significant ($P < 0.05$) decrease in TG and VLDL when compared to standard diet control (Group I). Standard drug Atorvastatin (Group III) at 10 mg/kg was able to effectively suppress atherogenesis, showing a significant decrease ($P < 0.05$) of TC, TG, LDL, and VLDL, compared to the atherogenic diet control (Group II) and no significant difference when compared to standard diet control (Group I).

Antiatherosclerotic Roles of *T. catappa* Fruit In Vivo. Next, ETC and ATC efficacy on enzyme markers of atherosclerosis was analyzed (Table 3, Supporting Information Figures S6−S10). The results are shown as mean ± SEM (standard error of the mean) of five animals from each group. The activity of ALT, AST, ALP, CK, and LDH were significantly ($P < 0.05$) increased under atherogenic conditions (Group II) when compared to standard diet control (Group I). Coadministration of ETC with an atherogenic diet in Group IV, V, and VI significantly ($P < 0.05$) decreased the activity of ALT, AST, ALP, and CK compared to the atherogenic group (Group II). *T. catappa* (aqueous extract, Group VI) significantly ($P < 0.05$) decreased the activity of LDH when compared to atherogenic control (Group II). Concomitant administration of ATC with an atherogenic diet (Groups VII, VIII, IX) decreased the activities of ALT, AST, ALP, and LDH significantly ($P < 0.05$) compared to the atherosclerosis model (Group II). The highest dose (300 mg/kg) of *T. catappa* fruit reversed the activity of ALT and AST significantly ($P < 0.05$). The positive control as a standard atherosclerosis drug (Atorvastatin 10 mg/kg, Group III) effectively reversed the activity of enzyme markers compared to the atherogenic group (Group II).

Determination of Lipid Profile Biomarkers. Next, the coronary risk index (CRI), cardioprotective index (CPI), and atherogenic index (AI) were analyzed quantitatively (Table 4, Supporting Information Figures S11−S13).[34]

Table 2. Lipid Profile of Atherogenic Rat Model[a,b]

group	total cholesterol (mg/dL)	total glyceride (mg/dL)	HDL (mg/dL)	VLDL (mg/dL)	LDL (mg/dL)
I. standard diet (SD)	74.50 ± 1.49	76.62 ± 2.30	42.73 ± 0.78	15.32 ± 0.46	16.45 ± 2.20
II. atherogenic diet (AD)	116.20 ± 4.07[c]	149.90 ± 4.63[c]	34.33 ± 1.12	29.98 ± 0.92[c]	51.85 ± 3.03[c]
III. AD + 10 mg/kg atorvastatin	80.05 ± 0.67[c,d]	85.89 ± 2.58[c,d]	55.45 ± 4.78[e]	17.18 ± 0.52[d]	7.42 ± 5.17[d,f]
IV. AD + 100 mg/kg ETC	92.68 ± 1.13[c,d]	136.60 ± 2.89[c,d]	38.28 ± 1.93	27.24 ± 0.56	27.06 ± 1.32
V. AD + 200 mg/kg ETC	87.14 ± 4.15[c,d]	123.70 ± 0.59[c,d]	40.60 ± 2.15	24.74 ± 0.12[c,d]	21.79 ± 6.22[c,d]
VI. AD + 300 mg/kg ETC	86.49 ± 2.06[c,d]	112.60 ± 4.07[c,d]	51.68 ± 5.17	22.52 ± 0.81[c,d]	12.29 ± 6.38[d,f]
VII. AD + 100 mg/kg ATC	99.38 ± 2.58[c,d]	134.30 ± 0.78[c,d]	38.03 ± 2.70	26.86 ± 0.16	34.49 ± 5.10[d]
VIII. AD + 200 mg/kg ATC	91.52 ± 5.46[c,d]	105.10 ± 2.21[c,d]	38.91 ± 3.93	21.02 ± 0.44[c,d]	31.59 ± 7.58[d]
IX. AD + 300 mg/kg ATC	80.30 ± 4.80[d]	73.92 ± 2.83[d]	41.64 ± 1.79	14.78 ± 0.56[d]	23.88 ± 5.85[d]
X. SD + 300 mg/kg ETC	71.41 ± 3.68	45.61 ± 1.79[f]	43.18 ± 7.98	9.12 ± 0.35[f]	19.11 ± 10.52[d]
XI. SD + 300 mg/kg ATC	71.41 ± 1.51	49.30 ± 1.18[f]	36.16 ± 1.67	9.86 ± 0.24[f]	25.39 ± 1.83[d]

[a]Values are expressed as mean ± SEM (standard error of the mean). [b]SD = standard diet, AD = atherogenic diet, ATC= aqueous extract of *T. catappa* fruit, ETC= ethanol extract of *T. catappa* fruit. [c]$P < 0.05$ is significantly higher compared to the standard diet control. [d]$P < 0.05$ is considerably lower compared to atherogenic control. [e]$P < 0.05$ is markedly higher compared to atherogenic control. [f]$P < 0.05$ is significantly lower compared to the standard diet control.

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Table 3. Effects of ETC and ATC on the Tissue Enzyme Markers of Atherosclerosis[a,b]

group	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	CK (IU/L)	LDH (IU/L)
I. standard diet (SD)	21.53 ± 0.11	105.90 ± 1.02	338.00 ± 19.31	174.60 ± 36.46	210.30 ± 46.36
II. atherogenic diet (AD)	102.30 ± 4.36[c]	182.20 ± 6.33[c]	680.70 ± 18.48[c]	479.20 ± 19.30[c]	577.40 ± 90.51[c]
III. AD + 10 mg/kg atorvastatin	20.27 ± 2.04[d]	81.14 ± 6.37[d]	394.30 ± 6.64[d]	230.30 ± 4.29[d]	340.30 ± 99.34
IV. AD + 100 mg/kg ETC	66.38 ± 10.10[c,d]	149.50 ± 3.57[c,d]	573.00 ± 20.79[c,d]	271.00 ± 10.56[d]	527.60 ± 110.4
V. AD + 200 mg/kg ETC	41.92 ± 3.64[d]	126.70 ± 2.50[d]	562.70 ± 10.40[c,d]	252.60 ± 19.65[d]	415.50 ± 86.78
VI. AD + 300 mg/kg ETC	28.97 ± 3.36[d]	94.90 ± 6.37[d]	478.00 ± 16.37[c,d]	237.70 ± 12.87[d]	221.80 ± 30.91[d]
VII. AD + 100 mg/kg ATC	47.22 ± 4.03[c,d]	126.20 ± 3.15[d]	579.00 ± 6.65[c,d]	445.70 ± 90.07[c]	225.60 ± 19.87[d]
VIII. AD + 200 mg/kg ATC	29.72 ± 5.14[d]	112.20 ± 3.84[d]	557.70 ± 16.46[c,d]	401.20 ± 64.34[c]	198.80 ± 17.66[d]
IX. AD + 300 mg/kg ATC	22.65 ± 3.94[d]	97.28 ± 2.23[d]	519.00 ± 11.85[c,d]	300.90 ± 23.59	233.20 ± 24.28[d]
X. SD + 300 mg/kg ETC	19.84 ± 1.85[d]	84.87 ± 3.35[d]	396.00 ± 8.083[d]	237.70 ± 8.57[d]	267.60 ± 79.47
XI. SD + 300 mg/kg ATC	17.77 ± 1.60[d]	112.20 ± 2.36[d]	398.30 ± 6.64[d]	315.00 ± 15.01	244.70 ± 8.83

[a]Values are expressed as mean ± SEM (standard error of the mean). [b]SD = standard diet, AD = atherogenic diet, ATC = aqueous extract of *T. catappa* fruit, ETC = ethanol extract of *T. catappa* fruit. The groups treated with ETC and ATC only resulted in no significant ($P < 0.05$) changes in enzyme markers when compared to the normal control (Group I). [c]$P < 0.05$ is significantly higher compared to standard diet control. [d]$P < 0.05$ is significantly lower compared to atherogenic control.

Table 4. Coronary Risk and Cardioprotective and Atherogenic Indices of *T. catappa* Using an Atherosclerosis Model[a,b]

group	coronary risk index (CRI)	cardioprotective index (CPI)	atherogenic index (AI)
I. standard diet (SD)	1.74 ± 0.05	2.73 ± 0.46	0.25 ± 0.01
II. atherogenic diet (AD)	3.39 ± 0.13[c]	0.67 ± 0.05[d]	0.64 ± 0.02[c]
III. AD + 10 mg/kg atorvastatin	1.57 ± 0.12[e]	3.06 ± 0.27[f]	0.23 ± 0.02[e]
IV. AD + 100 mg/kg ETC	2.50 ± 0.03	1.43 ± 0.15	0.55 ± 0.02[c]
V. AD + 200 mg/kg ETC	2.17 ± 0.23[e]	2.19 ± 0.57	0.48 ± 0.03[c,e]
VI. AD + 300 mg/kg ETC	1.86 ± 0.13[e]	3.09 ± 0.78[f]	0.38 ± 0.03[e]
VII. AD + 100 mg/kg ATC	2.65 ± 0.23	1.19 ± 0.29	0.55 ± 0.03[c]
VIII. AD + 200 mg/kg ATC	2.41 ± 0.33	1.40 ± 0.35	0.39 ± 0.06[e]
IX. AD + 300 mg/kg ATC	1.94 ± 0.16[e]	1.98 ± 0.48	0.25 ± 0.01[e]
X. SD + 300 mg/kg ETC	1.77 ± 0.36[e]	1.46 ± 0.33	0.09 ± 0.01[d,e]
XI. SD + 300 mg/kg ATC	1.98 ± 0.09	1.45 ± 0.15	0.13 ± 0.02[d,e]

[a]Values are shown as mean ± SEM (standard error of the mean). [b]SD = standard diet control; AD = atherogenic diet disease control; ATC= aqueous extract of *T. catappa* fruit; ETC = ethanol extract of *T. catappa* fruit. Values were calculated as follows: CRI = TC/HDL-C, CPI= [HDL-C/LDL-C], AI = Log [TG/HDL-C]. [c]Significantly higher compared to the standard diet control. [d]Significantly lower compared to the standard diet control. [e]Significantly lower compared to the atherogenic diet. [f]Significantly higher compared to atherogenic control.

The levels of coronary risk index significantly ($P < 0.05$) increased with an atherogenic diet (Group II). The antiatherogenic and antihyperlipidemic effects of the *T. catappa* fruit extract were observed with coadministration of ETC (Groups V and VI) with an atherogenic diet, as shown by the decreased coronary risk index significantly ($P < 0.05$) when compared to atherogenic diet group (II). *T. catappa* fruit treatment in 300 mg/kg ATC group (IX) with an atherogenic diet significantly ($P < 0.05$) decreased levels of coronary risk index when compared to the disease model.

The atherogenic index significantly increased following the administration of an atherogenic diet (Group II) compared to the standard diet control (Group I). Coadministration of ETC with an atherogenic diet (Groups V and VI) significantly ($P < 0.05$) decreased the atherogenic index when compared to disease control. The antiatherogenic effect of ATC was observed (Groups VIII and IX), which received *T. catappa* and an atherogenic diet, as shown by a decreased atherogenic index compared to the disease model (Group II).

The cardioprotective index of the atherogenic murine model (Group II) significantly ($P < 0.05$) decreased compared to the standard diet control (Group I). The increased cardioprotective index of *T. catappa* fruit was observed with the administration of ETC with an atherogenic diet (Group VI), compared to that of the disease model (Group II). The Atorvastatin (positive control, standard drug, 10 mg/kg) showed no significant ($P < 0.05$) changes in the CRI, CPI, and AI indices compared to the standard diet control (Group I). However, it showed a significant ($P < 0.05$) decrease in coronary risk and atherogenic index and a significant ($P < 0.05$) increase in the cardioprotective index compared to atherogenic control (Group II).

Histopathology. Under a standard diet control, tissues showed a major arterial vessel with a free lumen containing blood cells and intima of the endothelium free of any lipid deposit surrounding the adventitial layer (Figure 2A). The plaques in the atherogenic group occlude over 40% of blood passage (Figure 2B). Administration of standard drug (Figure 2C, Atorvastatin 10 mg/kg) under atherogenic conditions showed a lumen containing much-reduced cholesterol deposits with blood cell clusters. Administration of *T. catappa* fruit extracts (300 mg/kg ethanol extracts) and an atherogenic diet (Figure 2D) show major arterial vessels with endothelium, fibrous media, and adventitia layers. In addition, the lumen contains much-decreased levels of cholesterol plaques, with a 5% narrowing of blood passage.

DISCUSSION

The current experiments demonstrated that aqueous and ethanol extracts of *T. catappa* fruit might have antiatherosclerotic and antihyperlipidemic effects, as indicated by coronary risk and cardioprotective and atherogenic indices,

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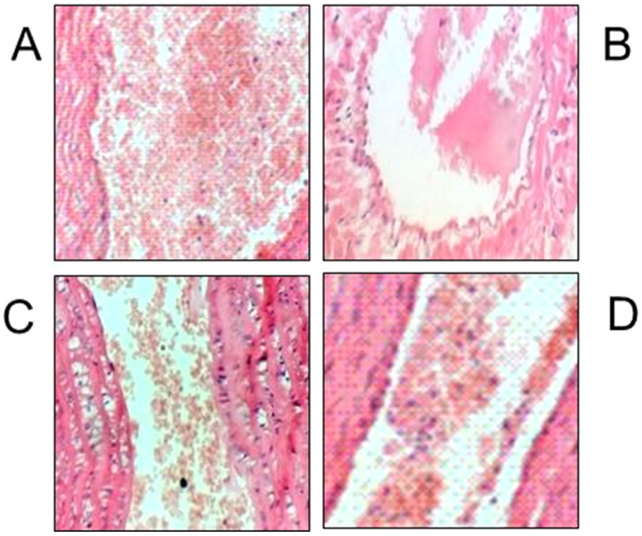

Figure 2. Aorta tissues were examined histologically using hematoxylin and eosin staining (H&E) under the atherogenic diet/plant extract-treated conditions. Standard diet condition demonstrated a free lumen containing blood cells and intima of the endothelium free of any lipid deposit (A). The atherogenic conditions occlude over 40% of blood passage by lipid plaques (B). Atorvastatin as positive control showed much-reduced cholesterol deposits (C, Atorvastatin 10 mg/kg). *T. catappa* fruit extracts under an atherogenic condition showed much-decreased levels of lipid deposit (D, 300 mg/kg ethanol extracts).

in a murine model in vivo. *T. catappa* is one of Africa's most popular medicinal plants, possessing diverse biological activities and pharmaceutical functions, including the hypoglycemic effect.[13] The quantitative phytochemical screening of *T. catappa* fruit revealed specific amounts of alkaloids, flavonoids, tannins, saponins, glycosides, phenols, terpenoids, anthraquinones, and steroids. Phenolic phytochemicals exhibit potent antioxidant activity, neutralizing reactive oxygen species. Table 1 shows that *T. catappa* contains the highest concentrations of phenolic compounds in ethanol and aqueous extracts, indicating that the fruit might have an antioxidative effect.

Previously, it was reported that flavonoids from alcoholic extracts might include an activity of lecithin cholesterol acyl transferase (LCAT), which regulates blood lipids by incorporating free radicals into HDL and transferring it back to VLDL and LDL, which are taken back into the liver cells.[35] The antiatherosclerotic effect of *T. catappa* could be attributed to high levels of cardiac glycosides.[36]

Saponins act as antihyperlipidemic by binding with cholesterol in the intestinal lumen so that cholesterol is less readily absorbed. Decreased bile acids in their extrahepatic circulation increase the metabolism of cholesterol to sterols through their fecal secretion. Saponins increase lipoprotein lipase activity (LPL) in the fast removal of free fatty acids from circulation, causing a decrease in total cholesterol.[37] The antihyperlipidemic activity of the *T. catappa* fruit could be attributed to the significant amount of saponins in the extracts. Atherosclerosis is characterized by endothelial dysfunction and vascular inflammation. *T. catappa* contains tannins as anti-inflammatory agents to reduce the risk of atherosclerosis.[38]

Improving atherosclerosis and coronary heart disease is related to lowering serum cholesterol levels. As shown in Table 2, TC was significantly decreased in animals treated with *T. catappa* extracts, demonstrating their ability to improve atherosclerosis. The endothelial damage, which ultimately resulted in atheroma and plaque formation, is characterized by high cholesterol, lipid concentration, and free radical oxidative stress.[39]

LDL oxidation in vessel walls initiates an inflammatory cascade that activates many atherogenic pathways.[40] LDL oxidation is an immediate reaction of endothelial injury and induces the expression of pro-inflammatory molecules in endothelial cells, so removing modified LDL is a critical therapy to treat inflammatory diseases. Our experiments showed that LDL was decreased significantly (200 and 300 mg/kg) by *T. catappa* fruit, suggesting efficacy in inhibiting atherogenic pathways (Table 2). In addition, statins are widely used as the clinical treatment for atherosclerosis, reducing the low-density lipoprotein (LDL) level as it is shown that Atorvastatin treatment significantly decreased LDL levels compared to atherogenic control.

Our data showed that *T. catappa* treatment for 6 weeks had regression effects in atherosclerosis. ETC decreased serum levels of LDL, consequently reducing the risk of atherosclerosis. In contrast, cholesterol transported in HDL particles, known as antiatherogenic cholesterol, protects against metabolic diseases.[41] HDL inhibits the uptake of LDL by the arterial wall and facilitates cholesterol transport from peripheral tissue to the liver, where they are catabolized.[42] Our results showed that HDL increased using ETC, suggesting the antiatherosclerotic activity of *T. catappa*. Atorvastatin (10 mg/kg body weight) was chosen carefully to compare with the activity of the plant extracts as a positive drug control.

The hypolipidemic activity of ETC and ATC suggests that a protective mechanism exists against the development of atherosclerosis. The activity of enzyme markers, including ALT, AST, ALP, CK, and LDH, was increased in the atherogenic model compared to the standard diet control (Table 3). Besides, ETC and ATC treatment decreased the activities of enzyme markers, suggesting the cytoprotective mechanism of *T. catappa*. ETC and ATC treatment showed no changes in enzyme activity compared to standard diet control. In addition, our experiments show the cardioprotective roles of *T. catappa* fruit (Table 4). For example, rats treated with ETC and ATC (300 mg/kg) significantly decreased the coronary risk and atherogenic index, while the cardioprotective index was significantly increased compared to the atherogenic diet control.

The antiatherogenic and antihyperlipidemic effects of the *T. catappa* extract were observed with ETC with an atherogenic diet, as shown by the decreased CRI and AI. The CRI, CPI, and AI determine a person's risk of developing cardiovascular disease. The CRI is a ratio of total cholesterol to high-density lipoprotein cholesterol (HDL-C) to measure a person's risk of developing coronary artery disease (CAD). The low CRI indicates a higher ratio of HDL-C to total cholesterol as cardioprotective. CI is a ratio of HDL-C to low-density lipoprotein cholesterol (LDL-C) to determine the level of cardioprotection. An increased CI indicates better cardioprotection.

The AI, on the other hand, is a ratio of total cholesterol to HDL-C, indicating atherogenicity, the potential of cholesterol to cause atherosclerosis and artery hardening. In addition, a higher AI indicates a higher risk of developing cardiovascular disease.

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The lower the CRI and AI values and the higher the CI value demonstrate, the lesser the risk of cardiovascular disease. These indices are indicators in evaluating overall cardiovascular health, but they must not be used in isolation as other risk factors like smoking, hypertension, and diabetes are equally critical in determining a person's risk of developing cardiovascular disease.

Based on the current data, the isolation and characterization of the active components for the antiatherogenic, antihyperlipidemic, and hypolipidemic activity are underway, including the mechanism of action.

CONCLUSIONS

T. catappa fruit exhibited antiatherosclerotic, antihyperlipidemic, and hypolipidemic activities in the atherosclerosis model in vivo. The extract treatment showed an improved lipid profile, enzyme biomarkers, histopathology, cardioprotection, and atherogenic index. In high amounts, *T. catappa* fruit contains polyphenols, flavonoids, cardiac glycosides, alkaloids, and saponins. Our data demonstrated the antiatherogenic and antihyperlipidemic effects of the *T. catappa* fruit extracts with an atherogenic diet, shown by the decreased CRI and AI, and increased CPI. The current study will guide further investigation to propose a clinical application to treat vessel diseases, including atherosclerosis.

ASSOCIATED CONTENT

Data Availability Statement

This published article and its Supporting Information files include all data generated or analyzed during this study.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c00685.

> Quantitative analysis of lipid profile of the atherogenic rat model with *T. catappa* extracts (Supporting Information Figures S1−S5); the efficacy on enzyme markers of atherosclerosis (Supporting Information Figures S6−S10); and quantitative analysis of the coronary risk index (CRI), cardioprotective index (CPI), and atherogenic index (AI) with *T. catappa* extracts (Supporting Information Figures S11−S13) (PDF)

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Author Contributions

D.T. participated in research design, experimental performance, manuscript drafting, and data analysis. D.D. participated in the research design, manuscript preparation, and revision. A.T.P. participated in research design, technical assistance, and manuscript preparation. W.J.J. participated in data analysis, manuscript preparation, and revision. All authors wrote, read, and approved the final manuscript.

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