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Original Research Article

Antiatherogenic and Antiobesity Effects of Aqueous Stem Bark Extract of *Grewia mollis* Juss (Malvaceae)

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ABSTRACT

Grewia mollis Juss (Malvaceae) commonly described as "Dargaza" is a shrub, popularly used in folk medicine to treat obesity, diarrhoea and to ease childbirth. However, there are no established reports on its antiobesity activity in literature, hence, the aim of the study was to evaluate the antihyperlipidemic and antiobesity effects of *Grewia mollis* (*GM*) in rodents. Hyperlipidemia was induced by 200 mg/kg of triton WR-1339 and the rats were orally pre-treated with extract (150-600 mg/kg) and atorvastatin (25 mg/kg) for 3 days. Obesity was induced with dietary cholesterol (600 mg/kg) for 10 weeks and rats were orally treated with extract (150-600 mg/kg). The effects of the extract on body weight, total food consumption, relative organ weight, Lee index, lipid profile, atherogenic index, coronary risk index and histopathological indices were evaluated. Preliminary phytochemical screening showed presence tannins, saponins, alkaloids, flavonoids and steroidal nucleus. The extract significantly ($p<0.05$) decreased total cholesterol, triglyceride, low-density lipoprotein cholesterol, very low-density lipoprotein cholesterol (VLDL), non-high-density lipoprotein cholesterol, atherogenic index, coronary risk index in hyperlipidemic rats. In the obese rat, there was significant ($p<0.05$) decrease in relative liver weight, body weight, Lee index, triglycerides, VLDL. Atherosclerotic changes in the coronary artery and aorta were ameliorated by the extract. The reduction in lipid profile and amelioration of atherosclerotic lesions indicate a possible anti-atherogenic property. These effects appear to be mediated via inhibition de novo triglyceride and cholesterol biosynthesis. The extract shows therapeutic promise in the management of obesity, hyperlipidemia and in the prevention of atherosclerosis.

Keywords: *Grewia mollis*, atherogenic, hyperlipidemia, obesity

Introduction

Obesity is a chronic medical disorder characterized by excess accumulation of fat in various parts of the body or its organs resulting in adverse effects on the individual's health [1]. Body mass index (BMI), a measurement based on weight and height, defines people as overweight when their BMI is between 25 kg/m^2 and 30 kg/m^2, and obese when it is over 30 kg/m.[1] Over 36% of the world's population is obese and the factors responsible for increased prevalence of obesity include economic growth, modernization, high calorie diet including processed foods higher in fats and refined sugars and sedentary life, and the globalization of food markets. [2] Other factors include familial susceptibility, endocrine disorders, and environmental factors, with women being suggested to be especially at risk.

Being overweight is deleterious to one's health, an individual who is obese may develop several life-threatening diseases including different forms of cancer, diabetes mellitus, hypertension, various heart diseases, as well inflammatory conditions, infertility and mental disorders.[3]

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Ways of controlling overweight and obesity include modification of lifestyle for example, dieting and physical exercise, drugs used to manage obesity by enhancing satiety or decrease fatty acids absorption and gastric-bypass and other weight loss surgeries to decrease absorption of nutrients from food.[4] However, all these interventions moderately bring about decrease in weight loss, hence, there is a clear need for alternate ways of sourcing for drugs and methods in treating obesity. To achieve an effective and reliable anti-obesity therapy with very minimal side effects, potential medicinal plants are constantly being evaluated.[5] There are several established experimental animals' models for evaluating potential anti-obesity and anti-hyperlipidemic drugs' efficacy such as the diet-induced obesity model and the triton-induced hyperlipidemia respectively.[6] Previous reports have shown that several plant extracts of *Cucumis melo*, [7] *Achyranthes aspera*, [3] *Gnidia glauca*, *Morinda citrifolia*, *Anthocleista vogelii* and *Silybum marianum* displayed potent anti-obesity and anti-atherogenic activities.[7] However, there are still several medicinal plants with anti-obesity and anti-atherogenic potentials that are used in folklore medicine that are yet to be investigated and *Grewia mollis* is among them.

Grewia mollis Juss (Malvaceae) is a shrub commonly found in tropical Africa and widely distributed in Northern Nigeria and it is called 'Dargaza' in the Hausa. Phytochemical studies on the leaves and stem bark of the extract indicate the presence of flavonoids, saponins, tannins, glycosides, phenols, steroids, alkaloids and volatile oils. [8] Two new triterpene compounds: lup-20-en-2-ol and 1,3-hexyloxacyclotridec-10-en-2-one, have been isolated from the roots.[9] Luteolin (a known flavonoid compound) and two known steroids identified as *β*-sitosterol and *β*-sitosterol-3-*O*-glucoside have been isolated from the aerial parts.[10] Acute and subacute oral administration of stem bark for 28 days suggests it was relatively safe in rodents. [11] The stem bark and leaves are used to treat ulcers, rheumatism, and

snakebites, diarrhea, obesity.[12] Anti-inflammatory, antimicrobial, antioxidant and hepatoprotective activities have been reported for the ethanol and methanol extracts of the plant parts. [13,14]
Despite the diversified utilizations of preparations of *G. mollis* in folk medicine, there is no established scientific reports on the therapeutic potential of the plant in the management of obesity and hyperlipidemia. Thus, this study was aimed at evaluating the antiatherogenic and antiobesity effects of the aqueous stem bark extract of *G. mollis* in rodents.

Materials and Methods

Chemicals
Chemicals Dietary cholesterol was procured from Sigma Chemical (St Louis, MO, USA). Atorvastin (Teva, UK limited) was purchased from a local Pharmacy in Benin city, Nigeria. Diagnostic kits for cholesterol, triglycerides and high-density lipoprotein–cholesterol (HDL-C) precipitants were procured from Randox laboratories, UK. All other reagents were of analytical grade and high quality

Plant material
The stem bark of *G. mollis* Juss (Malvaceae) was collected in May 2015 from Suleja, Niger State, Nigeria. The collected plant parts were identified and authenticated by Mallam Ibrahim Muazzam of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja with a voucher number; NIPRD/H/6785. A herbarium sample was deposited at the herbarium for future references.

Extraction
The stem bark was sun dried and then powdered using a mechanical grinder. The powdered material (200 g) was boiled in 2.2 L of distilled water for 30 min, and allowed to cool. The mixture was filtered with WhatMan No 1 filter paper (24cm). The filtrate was then concentrated using Rotary Evaporator (Model RE52A, China) to 10% of its original volume at 37 ^{o}C – 40 ^{o}C. and then concentrated to complete dryness in water bath, to produce a percentage yield of 23.38% (w/w). The dried extract was preserved in an air tight clean glass container and kept in a refrigerator maintained at -4°C until use.

Phytochemical Analysis of aqueous extract of Grewia mollis
The plant extract was subjected to preliminary qualitative phytochemical screening for phyo-chemical constituents of the extract using established methods.[15,16]

Animals
Wistar rats of both sexes (180–200 g) were obtained from the Laboratory Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. The rats were kept in plastic cages and housed under natural light. They were fed with standard feed and water ad libitum. All experiments were carried out in accordance with the Institute for Laboratory Animal Research Guidelines for the Care and Use of laboratory Animals[17] and handling of experimental animals approved by the Ethics Committee of the Faculty of Pharmacy, University of Benin, Nigeria (EC/FP/016/11)

Induction and Treatment of Triton-induced Hyperlipidemia
Overnight fasted male rats were randomly divided into 6 groups of 5 rats. Group I rats were orally pre-treated with 10 mL/kg of distilled water once daily for 3 consecutive days before intraperitoneal injection of 1 mL/kg of distilled water. Group II rats were orally pre-treated with 10 mL/kg of distilled water once daily for 3 consecutive days before intraperitoneal injection of 200 mg/kg of triton WR-1339. Groups III, IV, V, and VI were orally pre-treated respectively with 25 mg/kg of atorvastatin (ATV), 150, 300 and 600 mg/kg of extract once daily for 3 consecutive days before intraperitoneal injection of 200 mg/kg of triton WR-1339[18,19]. Twenty-four hours after treatment, the rats were anaesthetized with ketamine and blood samples collected by cardiac puncture for serum analysis. The heart, liver, and abdominal aorta were surgically dissected for histopathological studies.

Biochemical measurement of Lipid Profile
Blood samples were collected into plain bottles, allowed to clot at room temperature, thereafter, they were centrifuged at 4000 rpm for 10 mins using Hettich Centrifuge (Rototix 32A, Germany). The sera separated were used for evaluation of total cholesterol (TC), triglyceride (TG) and high-density lipoprotein cholesterol (HDL-c) using standard diagnostic test kits (Randox laboratories, UK) following the manufacturer's instructions.

Total cholesterol
Total cholesterol was determined after enzymatic hydrolysis and oxidation, in the presence of an indicator, quinoneimine. 1 ml of R_1 (reagent) was added to 0.01 ml of serum sample in a test tube. The 1 ml of R_1 was also added to 0.01 ml of the standard in a test tube. 1 ml of R_1 added to 0.01 ml of distilled water used as blank. The solutions in the test tubes were mixed and incubated for 5 min at 37^{0}C. The absorbance of the samples and standard was measured spectrophotometrically within 60 min at 500 nm.
Cholesterol concentration was calculated from the formula:

$$\text{Total cholesterol (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 200$$

Triglycerides
The triglyceride level was determined after enzymatic hydrolysis with lipases. The indicator was a quinoneimine.1 ml of R_1 (reagent and buffer) was added to 0.01ml of serum sample in a test tube. 1ml of R_1 was added to 0.01 ml of standard in a test tube. 1 ml of the R_1 was used as the reagent blank. The solutions in the test tubes were mixed and incubated for 5 min at 37^{0}C. The absorbance of the samples and standard was measured spectrophotometrically within 60 min at 500 nm.
Triglyceride concentration was calculated using:
T

$$\text{riglyceride (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 200$$

Lipoproteins
Addition of phosphotungstic acid in the presence of magnesium ions to serum precipitates Low Density Lipoproteins (LDL and VLDL) and chylomicrons. After centrifugation, the cholesterol concentration in the HDL fraction, which remains in the supernatant, was determined.
0.5 ml of the precipitant R_1 was added to 0.2 ml of serum sample in a test tube. 0.5 ml of the precipitant R_1 was added to 0.2 ml of standard in a test tube. The solutions in test tubes were mixed and allowed to sit for 10 min at room temperature and thereafter, centrifuged at 4000 rpm for 10 min. The clear supernatant layer was separated off within two hours and the cholesterol content was determined by the CHOD-PAP method.
HDL concentration was calculated as:

$$\text{HDL concentration (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 200$$

Serum low-density lipoprotein cholesterol (LDL-C) was calculated using Frieldwann's equation: $\text{LDL-c} = \left\{\text{TC} - \left(\text{HDL-c} + \frac{\text{TG}}{5}\right)\right\}$
VLDL-c and Non HDL cholesterol (Non HDL-c) were calculated as:
$\text{VLDL-c} = \text{TC} - (\text{HDL} - \text{c} + \text{LDL} - \text{c})$
$\text{Non-HDL cholesterol} = \text{LDL} - \text{c} + \text{VLDL} - \text{c}$
Atherogenic index (AI) and coronary risk index (CRI) were calculated as: LDL-c (mg/dl)/HDL-c (mg/dl) [20] and TC (mg/dl)/HDL-c (mg/dl).[21]

Histopathology
The heart, liver, and abdominal aorta were fixed in 10% formol saline. Fixed tissues were completely dehydrated in ascending concentrations of alcohol (70, 90, 96 and 100%). The tissues were placed in xylene to remove the alcohol, impregnated and embedded with molten paraffin wax. They were allowed to solidify before sectioning into 4 µm using a microtome (Leica RM 2235, UK). The 4 µm sections were placed on slides and stained with hematoxylin-eosin dye.[22] Stained slides were

viewed using an optical photomicroscope (Leica MC170 HD, Leica Biosystems, Germany) at ×400 magnification.

Induction of obesity and experimental design
The experimental protocol for this model was a modified method.[23] Animals were randomly divided into six groups of five rats. The control (Group I) rats were orally administered daily with 10 ml/kg of coconut oil for 10 weeks. Group II rats were orally administered daily with 60 mg/0.3 ml (600 mg/kg) of dietary cholesterol powder in coconut oil for 10 weeks. Groups III, IV, V and VI rats were orally treated daily with 25 mg/kg of atorvastatin and graded doses of extract (150-600 mg/kg) respectively before oral administration of 60 mg/0.3 ml (600 mg/kg) of dietary cholesterol powder in coconut oil for 10 weeks.
The body weight of each rat was measured on days 14, 42 and 70. The percentage weight change (% Δ wt) on day 14 was calculated as:

$$\%\Delta\text{wt on day 14} = \frac{\text{Body weight on day 14 - Body weight on day 1}}{\text{Body weight on day 1}} \times 100$$

The % Δ wt on days 42 and 70 was calculated using the above formula.
On day 70, the rats were anaesthetized with ketamine and blood samples collected by cardiac puncture for serum analysis. The lipid profile, atherogenic index (AI) and coronary risk index (CRI) were evaluated and calculated.
The heart, liver, kidney and spleen were surgically dissected out and weighed in grams for the determination of relative organ weight (ROW). ROW was calculated as follows:

$$\text{ROW} = \frac{\text{Absolute organ wieght (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100$$

The heart, liver, kidney and abdominal aorta were fixed in 10% formol saline for histopathological studies.

Determination of adiposity levels
The adiposity of each treated and obese rat was determined by the Lee index (LI), calculated as the cubic root of body weight in grams divided by the naso-anal length in millimeters multiply by 10^4. [24]

$$\text{LI} = \left[\frac{\sqrt[3]{\text{Body wieght (g)}}}{\text{Naso-anal length (mm)}}\right] \times 10^4$$

Statistical analysis
The results obtained were expressed as mean ± S.E.M. and analyzed by one-way analysis of variance followed by Tukey's post-hoc test (GraphPrism® 6, San Diego, USA). $p<0.05$ was considered significant.

Results and Discussion

Phytochemical constituents of aqueous extract of Grewia mollis
Phytochemical tests showed that the extract gave positive reaction for carbohydrates (sugars), hydrolysable tannins, saponins, alkaloids, flavonoids and steroidal nucleus (Table 1)

Effect of aqueous extract of Grewia mollis on serum lipids in hyperlipidemic rats
Graded doses of extract caused a significant ($p<0.05$) decrease in TC, TG, LDL-c, VLDL-c, Non HDL-c, AI and CRI when compared to the triton-only group. Triton WR-1339 caused a significant ($p<0.05$) increase in TC, TG, LDL-c, VLDL-c, Non HDL-c, AI and CRI and a significant ($p<0.05$) decrease in HDL-c when compared to the control group (Table 2 and 3).

Effect of aqueous extract of Grewia mollis on organ tissue architecture in hyperlipidemic rats
There was amelioration of triton induced coronary intimal ulceration (patchy), mural infiltrates of chronic inflammatory cells and asymmetric media hypertrophy in the rat heart on oral pre-treatment with extract. Mild kupffer cell activation and normal vascular intima was produced by the extract in the rat liver. Focal vascular ulceration and mild periportal inflammation induced by triton WR-1339 in the rat liver were ameliorated by the extract. (Figure 1 - 3).

Effect of aqueous extract of Grewia mollis on average daily food consumption in obese rats
There were no significant ($p>0.05$) differences in average daily food consumption among the animals treated with extract or control group as shown in Figure 5.

Table 1: Phytochemical constituents of *Grewia mollis* stem bark

Phytochemical constituents	Inference
Carbohydrate	+
Tannins	+
Saponins	+
Alkaloids	+
Flavonoids	+
Steroidal nucleus	+
Cardiac glycosides	-

Present (+) /Absent (-)

Table 2: Effect of extract on serum lipid profile in triton WR-1339 induced hyperlipidemic rats

Treatments	TC (mg/dl)	TG (mg/dl)	HDL-c (mg/dl)	Non HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
Control	75.80 ± 2.57	30.40 ± 4.34	62.80 ± 3.18	13.00 ± 1.92	6.92 ± 1.41	6.08 ± 0.86
Triton WR-1339	375.20 ± 9.64[a]	519.00 ± 24.38[a]	22.40 ± 3.73[a]	352.80 ± 7.18[a]	249 ± 6.30[a]	103.8 ± 4.87[a]
GM 150 mg/kg	149.20 ± 5.75[b]	183.60 ± 20.66[b]	22.60 ± 3.07	126.60 ± 8.42[b]	89.88 ± 5.37[b]	36.72 ± 4.13[b]
GM 300 mg/kg	176.60 ± 10.66[b]	262.60 ± 13.87[b]	22.00 ± 1.14	154.60 ± 11.62[b]	142.60 ± 11.42[b]	12.00 ± 0.67[b]
GM 600 mg/kg	195.00 ± 16.76[b]	272.20 ± 27.64[b]	24.00 ± 3.22	171.00 ± 14.77[b]	116.6 ± 16.59[b]	54.44 ± 5.52[b]
ATV 25 mg/kg	182.60 ± 8.76[b]	335.40 ± 20.98[b]	17.60 ± 3.38	165.00 ± 11.16[b]	103.90 ± 8.67[b]	61.08 ± 7.77[b]

Data represents mean ± SEM; n=5 rats; [a] p< 0.01, when compared to control group; [b]p< 0.05, when compared to triton WR-1339 group;. ATV: atorvastatin; *GM*: *Grewia mollis.* TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol: Non-HDL-C: non high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol: VLDL-C: very low-density lipoprotein cholesterol

Table 3: Effect of extract on coronary risk index and atherogenic index in triton WR-1339 induced hyperlipidemic rats

Treatments	Coronary risk index	Atherogenic index
Control	1.21 ± 0.03	0.11 ± 0.02
Triton WR-1339	18.51 ± 2.67[a]	12.38 ± 1.93[a]
GM 150 mg/kg	7.64 ± 1.96[b]	4.69 ± 1.35[b]
GM 300 mg/kg	8.19 ± 0.84[b]	6.64 ± 0.80[b]
GM 600 mg/kg	8.45 ± 0.92[b]	4.92 ± 0.59[b]
ATV 25 mg/kg	11.96 ± 2.19	6.71 ± 1.21[b]

Data represents mean ± SEM; n= 5 rats. [a] p<0.01, when compared to control group; [b]p<0.05, when compared to triton WR-1339 group. ATV: atorvastatin; *GM*: *Grewia mollis*

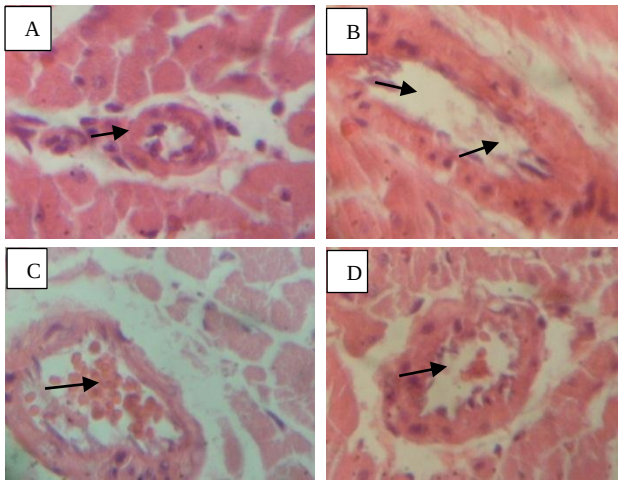

Figure 1: Histological analysis of heart sections in triton WR-1339 induced-hyperlipidemic rats. Heart tissues were stained with H & E (× 400). A, (control): showing normal heart architecture; B (rats treated with triton only): showing assymetric media hypertrophy, patchy intimal ulceration, mural infiltrates of fibroblast, monocyte, lymphocyte; C, (rats treated with triton and atorvastatin, 25 mg/kg): showing patchy intimal erosion, focal mural infiltrates of chronic inflammatory cells; D, (rats treated with triton and *Grewia mollis* extract, 300 mg/kg): showing normal intima and mild congestion.

Effect of aqueous extract of Grewia mollis on body weight and Lee index in obese rats

Dietary cholesterol caused a significant (p<0.05) increase in percentage body weight change at days 14, 42 and 70 when compared to the control group. The extract (150, 300 and 600 mg/kg) caused a significant (p<0.05) decrease in percentage change in body weights (Table 4) and Lee index when compared to the cholesterol-only group (Figure 4). There was also no significant reduction in relative organ weights (Table 5)

Effect of aqueous extract of Grewia mollis on serum lipids in obese rats

The extract caused a significant (p<0.05) decrease in TG and VLDL-c when compared to the cholesterol-only group. Atorvastatin caused a significant (p<0.05) decrease in TG, VLDL-c, and CRI when compared to the cholesterol-only group (Table 6 and 7).

Effect of aqueous extract of Grewia mollis in organ tissue architecture in obese rats

There was amelioration of severe vascular stenosis, ulceration and mild infiltrate of chronic inflammatory cells in the vascular walls on oral pre-treatment with the extract. The vascular obstruction and asymmetric medial hypertrophy observed in the rat heart was ameliorated by extract. However, mild perivascular infiltrates of chronic inflammatory cells was observed with atorvastatin (Figure 6). The severe ulceration and obstruction of the vascular wall induced by dietary cholesterol in the longitudinal section of the aorta was ameliorated by extract. The normal vascular wall and lumen was observed on treatment with the extract and atorvastatin (Figure 7). In the kidney, a normal renal tubule was observed with the extract and atorvastatin. Dietary cholesterol through its vascular changes induced patchy area of tubular necrosis (Figure 8). The extract and atorvastatin ameliorated the vascular ulceration, microvesicular steatosis and activated of sinusoidal kupffer cells in the liver (Figure 9).

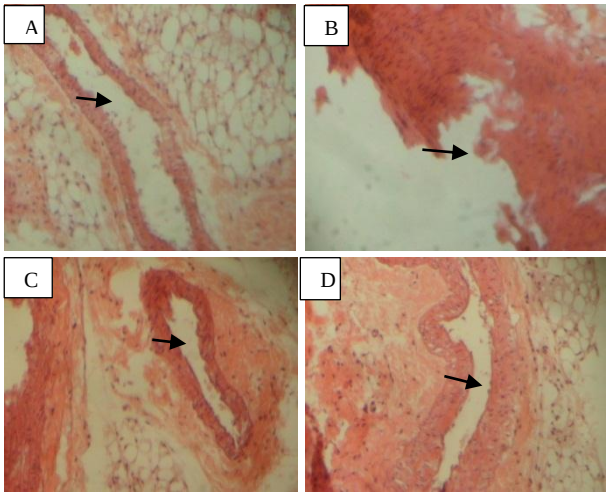

Figure 2: Histological analysis of aorta sections in triton WR-1339 induced-hyperlipidemic rats. Aorta tissues were stained with H & E (× 400). A, (control): showing normal aorta architecture; B, (rats treated with triton only); C, (rats treated with triton and atorvastatin, 25 mg/kg): showing patchy intimal erosion; D: (rats treated with triton and *Grewia mollis* extract, 300 mg/kg): showing mild intima erosion.

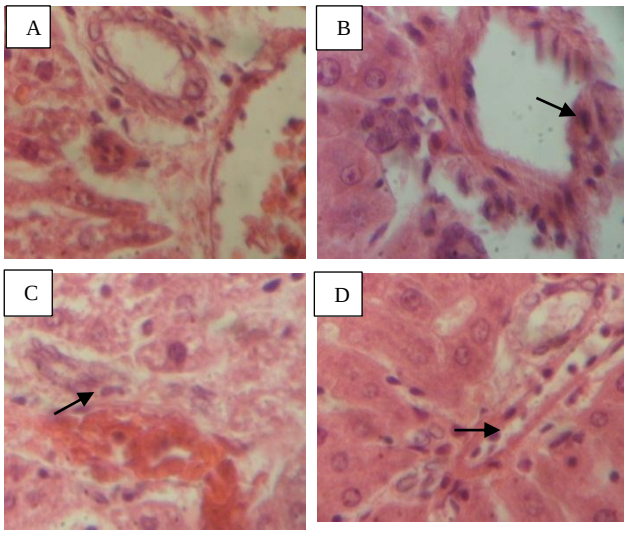

Figure 3: Histological analysis of liver sections in triton WR-1339 induced-hyperlipidemic rats. Liver tissues were stained with H & E (× 400). A, (control): showing normal liver architecture; B: (rats treated with triton only): showing focal vascular ulceration and mild periportal inflammation; C, (rats treated with triton and atorvastatin, 25 mg/kg): showing mild

vascular congestion and moderate kupffer cell activation. D: (rats treated with triton and *Grewia mollis* extract, 300 mg/kg) showing mild kupffer activation.

Table 4: Effect of extract on the percentage body weight change (% Δ wt) in cholesterol fed rats

Treatments	% Δ wt on day 14	% Δ wt on day 42	% Δ wt on day 70
Control	3.09 ± 1.75	12.77 ± 2.76	22.16 ± 3.32
Cholesterol only	16.45 ± 2.35[a]	29.64 ± 2.30[a]	42.87 ± 1.78[a]
*GM 1*50mg/kg	7.32 ± 1.07[b]	19.48 ± 2.42	25.00 ± 3.40[b]
*GM 3*00mg/kg	9.82 ± 0.81[b]	19.53 ± 2.00	26.63 ± 3.50[b]
*GM 6*00mg/kg	6.85 ± 1.47[b]	17.67 ± 3.54[b]	23.54 ± 4.45[b]
ATV 25 mg/kg	5.94 ± 0.67[b]	18.26 ± 1.18[b]	24.81 ± 1.76[b]

Data represents mean ± SEM; n= 5 rats. [a]p<0.05, when compared to control group; [b]p<0.05, when compared to cholesterol-fed group. ATV: atorvastatin; *GM*: *Grewia mollis.*

Table 5: Effect of extract on relative organ weight in cholesterol fed rats

Treatments	Liver	Spleen	Heart	Kidney
Control	3.10 ± 0.22	0.24 ± 0.01	0.28 ± 0.01	0.25 ± 0.01
Cholesterol only	4.09 ± 0.08	0.19 ± 0.00	0.29 ± 0.01	0.25 ± 0.01
GM 150 mg/kg	3.17 ± 0.17	0.18 ± 0.00	0.26 ± 0.01	0.20 ± 0.00
GM 300 mg/kg	3.14 ± 0.07	0.21 ± 0.00	0.28 ± 0.01	0.26 ± 0.02
GM 600 mg/kg	3.33 ± 0.09	0.21 ± 0.01	0.28 ± 0.00	0.24 ± 0.00
ATV 25 mg/kg	3.21 ± 0.09	0.20 ± 0.00	0.28 ± 0.01	0.23 ± 0.00

Data represent mean ± SEM; n = 5. ATV: atorvastatin; *GM*: *Grewia mollis*

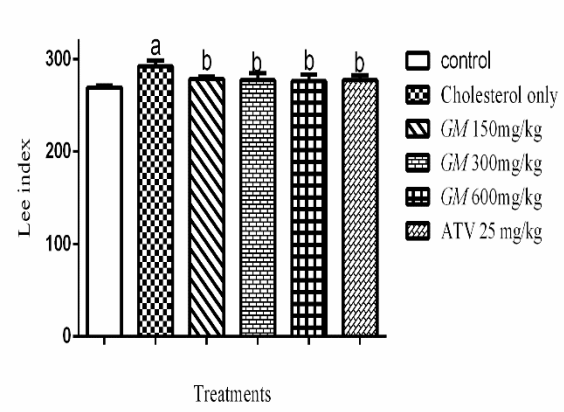

Figure 4: Effect of extract on Lee index in obese rats. Data represent mean ± SEM; n=5 rats. [a]p<0.05, when compared to control group; [b]p<0.05, when compared to cholesterol only group. ATV: atorvastatin; *GM*: *Grewia mollis.*

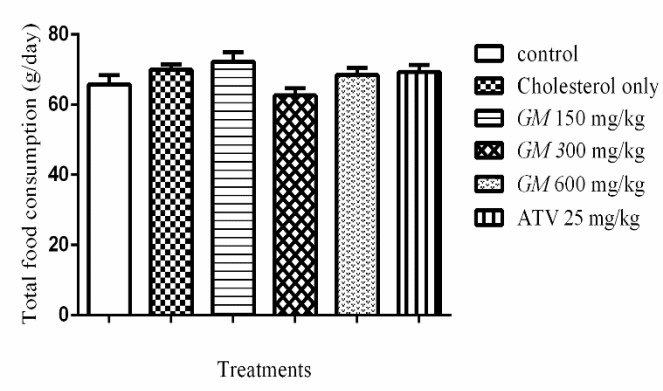

Figure 5: Effect of extract on average daily food consumption in cholesterol only group. Data represent mean ± SEM; n=5 rats. ATV: atorvastatin; *GM*: *Grewia mollis.*

Majority of obese people in the world live in developing countries, and Nigeria, the most populous country in Africa has over 12 million obese persons in the year 2020, making it possibly the most affected country in Africa.[25] Drugs used to induce weight loss may reduce appetite or increase satiety, reduce the absorption of nutrients, or increase energy expenditure.[26] Currently, there is a lack of high-quality evidence from long-term studies of both the efficacy and safety of pharmacological interventions for obesity.[26]

Triton WR-1339, a nonionic surfactant, has been widely used to induce hyperlipidemia in experimental animals. It is also an investigative tool for studying lipid metabolism and for investigating metabolic interrelationship between plasma lipoproteins.[27,28] Intraperitoneal or intravenous administration of Triton WR-1339 induce hyperlipidemia in two phases: Phase I is reported to be due to increased hepatic cholesterol biosynthesis by increasing 3-hydroxy-3-methylglutaryl–coenzyme A (HMG-CoA) reductase activity and interfering with the tissue uptake of plasma lipids,[29,30] while Phase II involves triton's interference with cholesterol excretion and metabolism.[31,32] Triton causes structural changes in the circulatory lipoproteins and inhibits lipases activity, in particular, lipoprotein lipase thus preventing or delaying the uptake of circulating lipids by extra hepatic tissues. [33] In this study, the increase in serum TG, TC, LDL-c, VLDL-c and decrease in serum HDL-c induced by triton WR-1339 corroborates these findings. Agents inhibiting cholesterol biosynthesis (Phase I) are known to be active within the first 24 h of triton injection while agents that are known to inhibit cholesterol metabolism and excretion (Phase II) are active after 24 h of triton induction.[27,32] In the present study, the extract decreased TC, TG, LDL-c and VLDL-c levels within the first 24 h of triton WR-1339 administration, suggesting it could be mediating antihyperlipidemic activity via inhibition of hepatic cholesterol biosynthesis rather than through cholesterol metabolism and excretion. This effect was comparable with atorvastatin, a competitive antagonist of HMG-CoA reductase enzyme.

The high triglyceride levels after induction with triton WR-1339 in this study indicates that a significant fraction of Non-HDL-c is contained in VLDL. The non-HDL-c is highly correlated with total apolipoprotein B which is major atherogenic lipoproteins [34] and VLDL-c is highly correlated with atherogenic remnant lipoproteins. The National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III recommended that the non-HDL-c (atherogenic cholesterol) should be a secondary target of therapy in persons with high triglycerides (>200 mg/dL). The profound reduction in major atherogenic lipoproteins such as LDL-c, non-HDL-c and VLDL-c strongly suggest the antiatherogenic effect of the extract. Atherogenic and coronary artery index are reliable indicators and independent assessors on uptake, metabolism and excretion of cholesterol and higher reference values indicate increased risk of developing coronary artery disease.[35, 36] The reference values for values for atherogenic index and coronary artery index in human should not be greater than 4 and 2.5, respectively. [37] The reduction in atherogenic and coronary indices by the extract strongly suggests its cardioprotective potential.

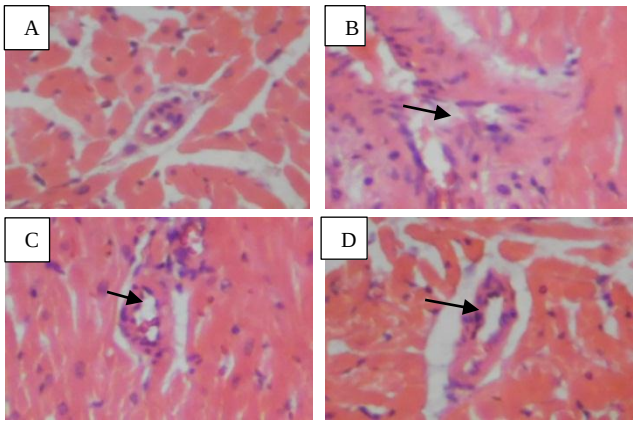

Figure 6: Histological analysis of heart sections in cholesterol fed rats. Heart tissues were stained with H & E (× 400). A, (control): showing normal heart architecture; B, (cholesterol fed rats only): showing vascular obstruction, asymmetric medial hypertrophy and mild mural infiltrates of chronic inflammatory cells; C, (cholesterol fed rats treated with atorvastatin, 25 mg/kg): showing mild perivascular infiltrates of chronic inflammatory cells and normal coronary artery; D, (cholesterol fed treated with *Grewia mollis* extract, 300 mg/kg) showing mild perivascular infiltrates of chronic inflammatory cells and normal vascular wall.

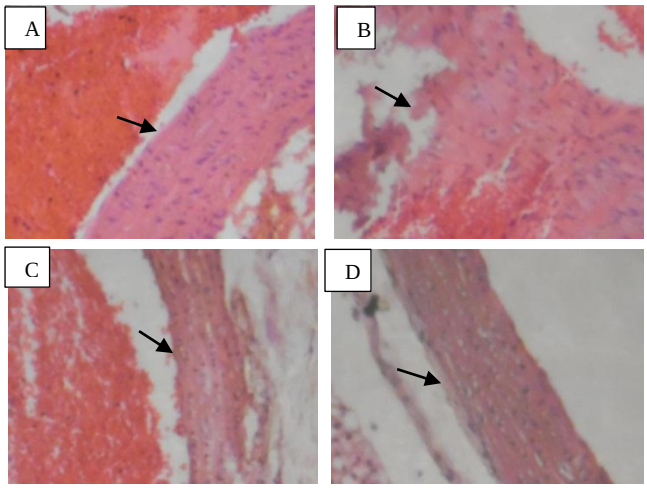

Figure 7: Histological analysis of aorta sections in cholesterol fed rats. Aorta tissues were stained with H & E (× 400). A, (control): showing normal aorta architecture; B, (cholesterol fed rats only): showing severe vascular ulceration and obstruction; C, (cholesterol fed rats treated with atorvastatin, 25 mg/kg): showing normal lumen and normal arterial wall; D: (cholesterol fed with *Grewia mollis* extract, 300 mg/kg): showing normal lumen and normal arterial wall.

Histopathology is regarded as the gold standard for evaluating therapy related pathological changes in tissues and organs. [38] Triton WR-1339 induced vascular changes to selected organs ranging from atherosclerotic changes in the coronary artery of the heart with the mild devitalization of adjacent myocardium and mural infiltrates of chronic inflammatory cells to the mucosal ulceration in the aorta as well as mild periportal hepatitis in the liver. These results are in line with other studies[31] which showed significant increase in wall thickness in triton WR-1339 treated animals indicating atherosclerosis. There was amelioration of patchy intimal erosion produced by triton WR-1339 to an appreciable degree as well as activation of effector phase of the local immune system of the liver (kupffer cells) on pre-treatment with atorvastatin and extract. These results corroborate with studies by Abdou et al. [31]

Dietary cholesterol has been reported to contribute significantly to elevation of plasma cholesterol levels and the presence of increased cholesterol levels in the diet has been shown to increase serum and aortic tissue cholesterol and, as such, increase aortic atherosclerosis. [39] There is a linear correlation between dietary cholesterol intake and mortality from coronary heart disease. [40] Also, feeding dietary cholesterol has been shown to accelerate the formation of aortic plaques in the apolipoprotein E null mutant mouse.[40]

The lipid profile analysis for the cholesterol-fed rats in this study showed atherogenic dyslipidemia which is a common form of dyslipidemia characterized by three lipid abnormalities: elevated triglycerides, small LDL particles, and reduced HDL-c.[41,42] Atherogenic dyslipidemia commonly occurs in persons with premature congestive heart failure (CHD) and strongly associated with abdominal obesity, obesity in general, and physical inactivity. Thus, in the present study, the considerable reductions in the weight gain pattern, Lee index and serum lipids by extract treatment after chronic feeding of animals with dietary cholesterol are indicative of the antiobesity and antiatherogenic property of the plant extract. This is consistent with previous works evaluating antiatherogenic and antiobesity properties of medicinal plants.[23,43]

From histopathological examination, dietary cholesterol induced vascular damage and secondary parenchyma injuries to the heart, aorta, kidney and liver. Atherosclerotic and cardiovascular disease (CVD) changes observed in heart and aorta such as severe vascular stenosis, ulceration, infiltration of chronic inflammatory cells, media hypertrophy and vascular obstructions and renal tubular necrosis observed in the kidney have been associated with hypertension, CHD, stroke and myocardial infarction in dyslipidemia and obesity.

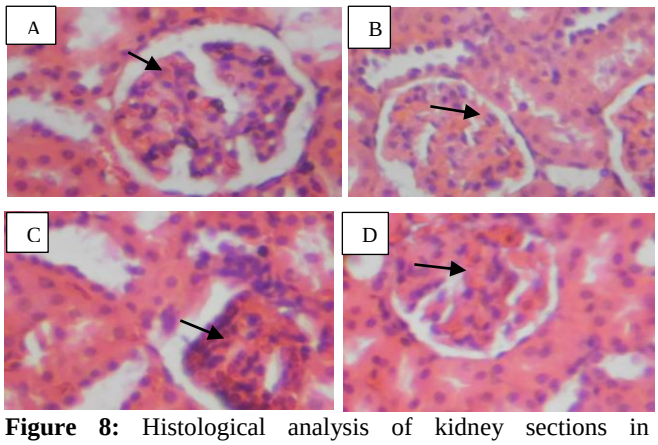

Figure 8: Histological analysis of kidney sections in cholesterol fed rats. Kidney tissues were stained with H & E (× 400). A, (control): showing normal kidney architecture; B, (cholesterol fed rats only): showing focal tubular necrosis; C, (cholesterol fed rats treated with atorvastatin, 25 mg/kg):

showing patchy tubular necrosis; D, (cholesterol fed rats treated with *Grewia mollis* extract, 300 mg/kg): showing mild tubular necrosis.

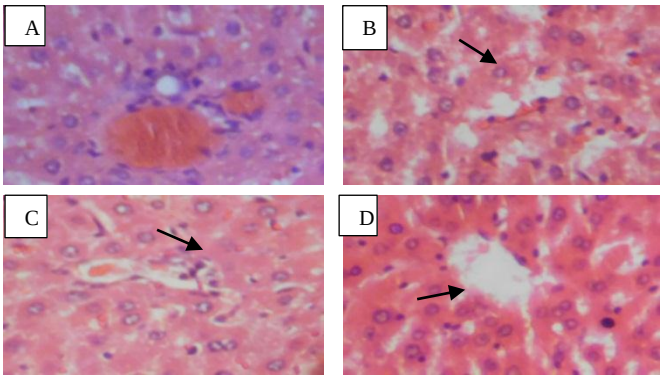

Figure 9: Histological analysis of liver sections in cholesterol fed rats. Liver tissues were stained with H & E (× 400). A, (control): showing normal liver architecture; B, (cholesterol fed rats only): showing focal microvesicular steatosis; C, (cholesterol fed rats treated with atorvastatin, 25 mg/kg): showing mild kupffer cell activation; D, (cholesterol fed rats treated with *Grewia mollis* extract, 300 mg/kg): showing mild kupffer cell activation

In the liver, the presence of numerous small vesicles of fat in the cytoplasm of hepatocyte that does not displace the nucleus termed as microvescicular steatosis was observed in the present study. [3] Microvescicular steatosis has been associated with non-alcoholic fatty liver disease in insulin resistance and obesity.[3] The improvement in the histological features of myocardium, aorta and amelioration of the microvescicular steatosis in the liver strongly corroborates the biochemical evidence of the antihypergtriglyceridaemic and antiobesity activities of the extract as reported in previous studies.[44]

Phytochemicals, such as flavonoids has been shown to decrease lower serum lipids levels by inhibiting the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase enzyme and up-regulating peroxisome proliferators-alpha and gamma expressions in the liver.[45] Flavanoids prevent the oxidation of low-density lipoprotein, lowers the blood lipid levels thereby reducing the risk for the development of atherosclerosis. It has been suggested that consumption of tannin containing food and beverages, can prevent a variety of ailments such as heart related diseases. [46] Similarly, it has been reported by Ramchoun et al[47] that saponins mediate antihyperlipidemic activity by inhibiting or delaying absorption of lipids in the intestines through a resin-like action and by inhibiting pancreatic lipase activity. In addition, saponins mediate their action by promoting enterohepatic excretion of cholesterol in the bile acids. [47,48] Previous phytochemical studies on the extract revealed the presence of alkaloids, flavonoids, tannins and saponins.[8] Thus, the presence of these phytochemicals could be responsible for the antiobesity and antiatherogenic effect of *Grewia mollis* stem bark. The present study revealed that the plant extract did not significantly alter the total food intake of the treated animals, thus suggesting that the antiobesity and antiatherogenic property of the extract are not mediated via inhibition of appetite. This result is consistent with the findings of Flavel et al,[49] where polyphenol-rich sugarcane extract significantly reduced the body weights of obese C57/BL6J mice fed on high calory diet without altering the food intake in the animals.

Conclusion

Grewia mollis stem bark extract has shown great promise in the management of atherosclerosis and obesity as a result of the amelioration of disease risk factors as shown in this study. The favourable reduction in lipid profile and amelioration of atherosclerotic lesions in the animals indicate a possible anti-atherogenic property and these effects appear to be mediated via inhibition de novo triglyceride and cholesterol biosynthesis. However, further study is needed to isolate the active constituents and evaluate the molecular mechanisms responsible for the anti-atherogenic activity, with the aim of developing drug candidates in the management of cardiovascular related diseases.

Table 6: Effect of extract on serum lipids and lipoproteins in cholesterol fed rats

Treatments	TC (mg/dl)	TG (mg/dl)	HDL-c (mg/dl)	Non HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
Control	114.80 ± 6.85	189.60 ± 10.61	26.4 ± 2.56	88.4 ± 5.88	52.36 ± 4.49	36.04 ± 2.22
Cholesterol-only	139.4 ± 7.14	311.00 ± 23.76[a]	27.0 ± 3.20	112.4 ± 6.85	50.20 ± 9.00	62.20 ± 4.75[a]
GM 150 mg/kg	126.8 ± 2.37	178.2 ± 11.53[b]	26.0 ± 0.94	100.8 ± 2.08	65.16 ± 2.03	35.64 ± 2.30[b]
GM 300 mg/kg	121.4 ± 7.45	189.6 ± 20.23[b]	31.6 ± 4.53	89.8 ± 10.71	51.88 ± 11.84	37.92 ± 4.04[b]
GM 600 mg/kg	127.0 ± 6.79	186.0 ± 7.24[b]	32.6 ± 1.16	94.4 ± 6.21	57.20 ± 5.17	37.20 ± 1.44[b]
ATV 25 mg/kg	105.8 ± 2.57	137.2 ± 13.95[b]	32.8 ± 1.46	73.0 ± 3.70[b]	45.56 ± 5.41	27.44 ± 2.79[b]

Data represent mean ± SEM; n = 5; [a]p<0.01, when compared to control group, [b]p<0.05, when compared to cholesterol only group. ATV: atorvastatin; *GM*: *Grewia mollis*. TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol: Non-HDL-C: non high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol: VLDL-C: very low-density lipoprotein cholesterol

Table 7: Effect of extract on coronary risk index and atherogenic index in cholesterol fed rats

Treatments	Coronary risk index	Atherogenic index
Control	4.46 ± 0.39	2.07 ± 0.30
Cholesterol only	5.37 ± 0.50	2.00 ± 0.44
GM 150 mg/kg	4.89 ± 0.14	2.52 ± 0.14
GM 300 mg/kg	4.16 ± 0.61	1.85 ± 0.53
GM 600 mg/kg	3.89 ± 0.17	1.75 ± 0.14
ATV 25 mg/kg	3.26 ± 0.20[b]	1.42 ± 0.22

Data represent mean ± SEM; n = 5. [b]p<0.05,when compared to cholesterol only group.
ATV: atorvastatin; *GM*: *Grewia mollis*

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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