RESEARCH PAPER

In Vivo Anti-Atherosclerotic Activity of Ethanolic Leaf Extract of *Annona reticulata* and Atrabotrys hexapetalus on Albino Rats

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ABSTRACT

Background: Atherosclerosis is a chronic inflammatory and lipid-driven vascular disorder characterized by dyslipidemia, endothelial dysfunction, and plaque formation. Herbal medicines rich in bioactive phytoconstituents are increasingly explored for their cardioprotective potential. *Annona reticulata* and *Artabotrys hexapetalus* are traditionally used medicinal plants reported to possess antioxidant and lipid-modulating properties. However, their comparative and synergistic anti-atherosclerotic effects require scientific validation.

Aim: To evaluate the in vivo anti-atherosclerotic activity of ethanolic leaf extracts of *Annona reticulata* and *Artabotrys hexapetalus*, individually and in combination, in high-fat diet-induced atherosclerotic albino rats.

Methods: Atherosclerosis was induced in albino rats using a high-fat diet. Animals were divided into seven groups (n=6): normal control, disease control, atorvastatin (1 mg/kg), Annona reticulata extract (200 mg/kg, p.o.), *Artabotrys hexapetalus extract* (200 mg/kg, p.o.), combined extract (1:1), and vehicle control. Body weight changes, serum lipid profile parameters (total cholesterol, triglycerides, LDL, HDL), and histopathological examination of aortic tissue were assessed. Phytochemical screening was performed to identify bioactive constituents. Statistical analysis was conducted using one-way ANOVA followed by Dunnett's test.

Results: Phytochemical analysis revealed the presence of flavonoids, alkaloids, glycosides, steroids, and tannins in both extracts. The disease control group showed significant elevation in total cholesterol (279.2 mg/dL) and LDL (134.5 mg/dL) with marked reduction in HDL (23.67 mg/dL) compared to normal controls. Treatment with individual extracts significantly reduced total cholesterol (180 mg/dL) and LDL (100 mg/dL) while increasing HDL levels (63–67 mg/dL) ($p < 0.01$). The combined extract exhibited the most pronounced lipid-lowering and HDL-elevating effect, nearly comparable to atorvastatin. Histopathological findings demonstrated improved aortic architecture and reduced lipid deposition in treated groups.

Conclusion: Ethanolic leaf extracts of *Annona reticulata* and *Artabotrys hexapetalus* exhibit significant anti-atherosclerotic activity, with synergistic effects observed in combination therapy. The cardioprotective action may be attributed to their rich phytochemical profile and modulation of lipid metabolism.

Major Findings: The study demonstrated that ethanolic leaf extracts of Annona reticulata and Artabotrys hexapetalus significantly reduced serum total cholesterol and LDL levels while markedly increasing HDL concentrations in high-fat diet–induced atherosclerotic rats. The combined extract (1:1) exhibited a superior and synergistic lipid-lowering effect compared to individual treatments, producing results nearly comparable to atorvastatin. Phytochemical screening confirmed the presence of flavonoids, alkaloids, glycosides, steroids, and tannins, which likely contributed to antioxidant activity, improved lipid metabolism, and vascular protection. Histopathological analysis further revealed restoration of normal aortic architecture with reduced lipid deposition in treated groups, confirming the anti-atherosclerotic potential of both extracts.

Keywords: *Annona reticulata*; *Artabotrys hexapetalus*; Anti-atherosclerotic activity; Hyperlipidemia; Ethanolic leaf extract; LDL; HDL; Phytochemicals; Cardiovascular protection; In vivo study.

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1. INTRODUCTION

Throughout India, up to an elevation of 900 meters, it is both wild and cultivated. Completely naturalized, it grows gregariously and extensively in steep regions and wastelands [1,2]. About a 6-meter-tall tree with thin, gray bark, it possesses simple, alternate leaves measuring 3.5–8 × 1.5–4 cm^3. The fruit is round, 5–10 cm in diameter, initially covered with a glaucous bloom and turning yellowish-green upon maturity; it breaks easily into large segments. The areoles are clearly visible, and the flesh is white and palatable, containing numerous smooth, polished, arillate seeds that are brownish-black in color [34]. The plant flowers from March to July and bears fruits from August to January [3].

Traditionally, the plant is used for treating infestations; the leaves are employed in helminthiasis, while the bark acts as a strong astringent and tonic [4,5]. *Artabotrys hexapetalus* is native to tropical Asia, including India, Sri Lanka, and Southeast Asia, and is commonly cultivated in gardens as an ornamental plant in these regions [11].

It is a woody climber or shrub growing up to 8–10 meters in height, with dark green, glossy, ovate to lanceolate leaves. The plant produces highly fragrant, yellowish-green flowers with long, slender petals resembling a hand, and the fragrance intensifies during the evening [3,6].

The plant has significant ornamental and aromatic value; its flowers are widely used in perfumery and for making garlands. In traditional medicine, it is used to treat headaches, rheumatism, and as a general tonic. The essential oil extracted from the flowers is also utilized in aromatherapy for its calming and soothing effects.

Furthermore, *Artabotrys hexapetalus* has been reported to exhibit various pharmacological activities, including anticancer, antifungal, antimicrobial, diaphoretic, antidiabetic, hepatoprotective, cardioprotective, antiemetic, antispasmodic, analgesic, and adaptogenicproperties [7-10].

2. MATERIALS AND METHODS

2.1 Materials and reagents

Atorvastatin tablets (10 mg IP, Cipla), vitamin D_3 (oral; Mankind Pharma), carboxymethylcellulose (CMC; vehicle), solvents for extraction (ethanol, analytical grade), biochemical kits for TC/TG/HDL/LDL (commercial kits), and histology reagents were procured from certified vendors (see Table S1 — reagent suppliers). Instruments included Soxhlet apparatus, rotary evaporator, digital balance, automated hematologyanalyzer and light microscope.

2.2 Plant collection and authentication

Fresh leaves of *Annona reticulata* and *Artabotrys hexapetalus* were collected and authenticated by a botanist sireesha, Assistant Professor, A.K.R.G Degree & PG College, Rajamahendravaram. Fresh leaves were washed, shade dried, milled, and stored in airtight containers until extraction.

2.3 Preparation of ethanolic extracts

Powdered leaves (500 g each) were subjected to Soxhlet extraction with 95% ethanol. The solvent was removed under reduced pressure (rotary evaporator) and extracts dried to constant weight. Yields were calculated (w/w) and stored at 4 °C until use.

2.4 Phytochemical screening

Standard qualitative tests were performed to detect carbohydrates, glycosides, alkaloids, flavonoids, saponins, tannins, steroids, and proteins following published protocols. Both *Annona reticulata* and *Artabotrys hexapetalus* showed positive reactions for glycosides, flavonoids, alkaloids, saponins and tannins

2.5 Animals and ethical statement

Adult albino Wistar rats (120–160 g) of either sex were procured and acclimatized for 7 days in standard laboratory conditions (temperature 22–25 °C, 12 h light/dark). Animals were handled per CPCSEA ethical guidelines and the study was approved by the Institutional Animal Ethics Committee (IAEC).The present study was approved by AKRG college of pharmacy, CPSCEA Reg no 1373/PO/Re/S/10/CPCSEA.All procedures conformed to ARRIVE guidance.

2.6 Induction of atherosclerosis and treatment protocol

Atherogenesis was induced using an atherogenic diet (per animal daily mix: 2.0 g cholesterol, 8 g saturated fat (groundnut oil), 90 g standard pellet) and weekly oral vitamin D_3 challenge dissolved in 0.25% CMC (dose and schedule as per widely used protocols) for 30 days. The crude extracts were suspended in 0.25% CMC for oral administration.

Rats were randomized to seven groups (n = 6 per group):

- Group I: Normal control (chow)
- Group II: Atherogenic control (diet + vitamin D_3)
- Group III: Standard — Atorvastatin 1 $mg \cdot kg^{-1}$ p.o.
- Group IV: AR extract 200 $mg \cdot kg^{-1}$ p.o.
- Group V: AH extract 200 $mg \cdot kg^{-1}$ p.o.
- Group VI: AR + AH (1:1) total 200 $mg \cdot kg^{-1}$ p.o.
- Group VII: Vehicle control (0.25% CMC)

Doses were administered once daily for 30 days. Body weights were recorded on days 1, 7, 14, 21 and 31.

2.7Animal study

We obtained white albino rats and housed them in a controlled environment with a temperature of 27±20°C, a humidity level of 80±10%, and a light/dark cycle of 12 hours. All of the animals were able to freely consume food and drink. Each case had three rats that were bedridden on husk bedding. The period of 7 days prior to the experiment allowed the animals to get acclimated.

We inflicted diseased in rats by feeding them a diet containing 2.0 gm cholesterol, 0.1 gm of calcium, 8.0 gm of saturated fat (ground nut oil), 90 gm of powdered

standard commercial pellet diet, and a weekly oral challenge of vitamin D3 at standard concentrations dissolved in 0.25% carboxymethyl cellulose (2ml/kg body weight vehicle) for 30 days[12,13]. Animals in the treatment group received varying concentrations of plant extract, while those in the control group received atorvastatin [14,15].

2.8 Sample collection and biochemical assays

On day 31, animals were fasted overnight and blood was collected under light anesthesia via retro-orbital plexus (or cardiac puncture) into plain tubes. Serum was separated and analyzed for total cholesterol (TC), triglycerides (TG), HDL-C, and LDL-C using enzymatic colorimetric kits per manufacturer instructions. VLDL-C was calculated as TG/5. Atherogenic indices (TC/HDL, TG/HDL, LDL/HDL) were computed.

2.9 Organ weights and histopathology

Hearts, livers, and kidneys were excised, blotted, and weighed (absolute weights). Thoracic aorta segments (proximal and distal) were fixed in 10% neutral buffered formalin, processed for paraffin embedding, sectioned (4–5 μm) and stained with hematoxylin& eosin (H&E). Sections were examined under light microscopy for intimal thickening, lipid deposition, and inflammatory cell infiltration. Representative photomicrographs were taken.

2.10 Statistical analysis

Data are presented as mean ± SEM (n = 6). Statistical comparisons were performed using one-way ANOVA followed by Dunnett's test; $p < 0.05$ considered statistically significant. (GraphPad Prism recommended for plots.)

3. RESULTS AND DISCUSSION

Effect of Test Samples on Total Cholesterol Levels

The total cholesterol (TC) levels in different experimental groups are presented in Table 27. A significant elevation in serum TC was observed in the disease control group (279.2 ± 0.047 mg/dL) compared to the normal control (143.7 ± 0.032 mg/dL), confirming the induction of hypercholesterolemia and atherogenic dyslipidemia. Administration of atorvastatin (1 mg/kg) markedly reduced TC levels to 185 ± 0.056 mg/dL (**$p < 0.01$** vs. disease control), validating the model and serving as a standard reference for antihyperlipidemic efficacy.

Treatment with *Annona reticulata* (200 mg/kg, p.o.) and *Artabotrys hexapetalus* (200 mg/kg, p.o.) extracts produced a significant reduction in serum TC levels, measuring 180.8 ± 0.054 mg/dL and 181.7 ± 0.012 mg/dL respectively (**$p < 0.01$** vs. disease control). The combination treatment (1:1 ratio) of both extracts showed the greatest cholesterol-lowering effect (172.0 ± 0.024 mg/dL), indicating a synergistic hypolipidemic interaction between the phytoconstituents of the two plants. The vehicle control group exhibited values comparable to the treatment groups (179.2 ± 0.333 mg/dL), confirming the stability of the experimental conditions.

These findings demonstrate that both individual and combined plant extracts exhibit potent antihyperlipidemic activity comparable to atorvastatin, a clinically established HMG-CoA reductase inhibitor. The reduction in total cholesterol may be attributed to multiple bioactive constituents such as flavonoids, alkaloids, glycosides, and sterols detected in preliminary phytochemical screening. Flavonoids and phenolic compounds are reported to inhibit lipid peroxidation and modulate hepatic cholesterol biosynthesis pathways [16,17]. Alkaloids and saponins can interfere with intestinal cholesterol absorption and bile acid reabsorption, leading to decreased serum cholesterol levels [18].

The observed synergistic effect of the combined extract could be explained by the complementary mechanisms of *A. reticulata*and *A. hexapetalus*. Previous studies have shown that *Annona* species possess potent hypolipidemic and antioxidant properties by upregulating LDL receptor activity and enhancing cholesterol catabolism [19,20]. Likewise, *Artabotrys hexapetalus* extracts have demonstrated lipid-lowering and cardioprotective effects through improved antioxidant enzyme activities and reduced lipid peroxidation [21,22].

The findings are consistent with earlier reports where polyherbal combinations rich in flavonoids and sterols produced enhanced antihyperlipidemic effects compared to single-plant treatments [23,24]. Hence, the combined treatment of *A. reticulata* and *A. hexapetalus* may act synergistically through multiple biochemical pathways, including inhibition of HMG-CoA reductase, increased cholesterol efflux, and improved hepatic lipid metabolism.

Overall, the results suggest that the combined extract of *A. reticulata* and *A. hexapetalus* exerts pronounced antihypercholesterolemic effects comparable to atorvastatin, justifying its potential application as a natural therapeutic or nutraceutical for managing hyperlipidemia and preventing atherosclerosis (Table 1and 2) (Figure 1 and 2) .

Table-1: Effect of test samples in bodyweight (gm)

"Data were expressed as Mean±SEM in each group (n=6) and are significant when analyzed byone-way anova followed by dunnette's test and p<0.01 compared to control group".

Groups	Treatment&dose	1st day	7th day	14th day	21st day	31st day
I	Normal	130.3±0.76#	113.7±0.5578#	136.3±0.421#	139.3±0.7149#	136.3±0.4216#
II	Disease control	118.7±0.881	305.3±183.9	308.7±183.7	311.3±182.7	313.3±1.054
III	Atorvastatin(1mg/kg)	118.5±0.562**	117.8±0.792**	117.2±1.078**	116.3±1.406**	117.2±1.078**
IV	*Annona reticulata* (200mg/kg) p.o.	117.7±0.802*	116.0±0.816*	113.2±3.868*	112.5±0.670*	114.2±0.749*
V	*Artabotrys hexapetalus* (200mg/kg)p.o.	121.5±0.763*	120±0.703*	116.2±0.749*	116.3±0.918*	115.3±1.430*
VI	*A.reticulata+Artabotrys hexapetalus* (1:1)p.o.	120.5±0.763**	120.2±0.703**	118.5±0.7638**	116.0±0.683**	112.5±0.7638**
VII	Vehiclecontrol	130.3±0.714**	132.5±0.67**	135.0±0.577**	137.8±0.703**	137.3±1.764**

P<0.001indicates# whencomparedtogroup-1; P<0.001indicates**whencomparedtogroup-2; P<0.01 indicates *when compared to group 2.

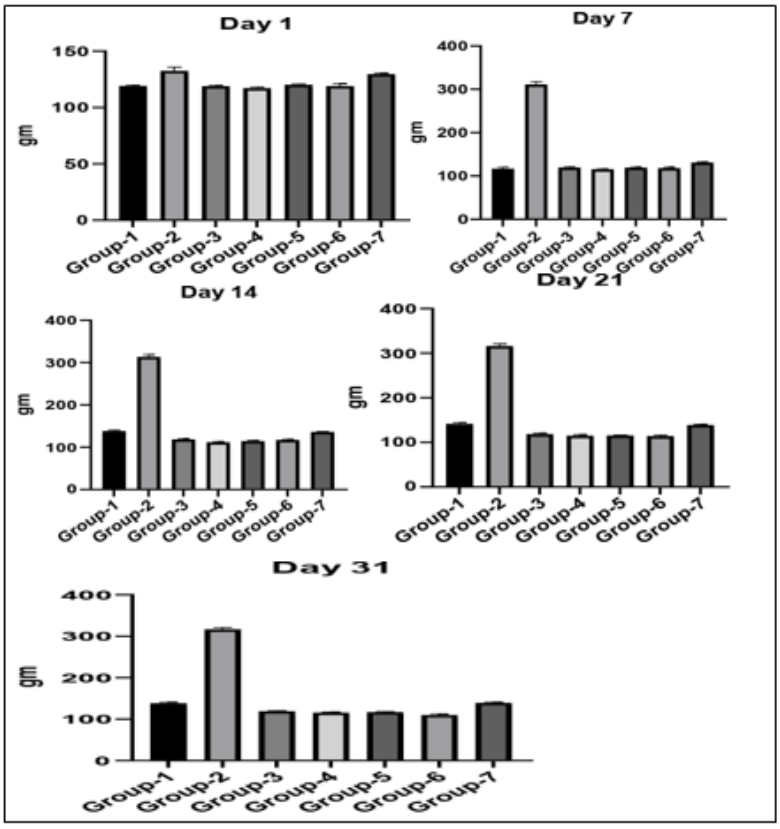

Fig 1: Effect of test samples in body weight (gm)

Table-2: Effect of test samples in total cholesterol level

Groups	Treatment & dose	Total cholesterol level	HDL	LDL	Triglycerides
I	Normal	143.7 ± 0.032$^{\#}$	69.17 ± 3.962$^{\#}$	86.83± 2.167$^{\#}$	22.75 ± 0.416$^{\#}$
II	Disease control	279.2± 0.047	23.67±1.382	134.5± 1.118	119.4 ± 92.75
III	Atorvastatin (1mg/kg)	185 ±0.056**	65.50 ±1.565**	107.0± 1.291**	32.33 ±6.500**
IV	*Annona reticulata* (200mg/kg) p.o.	180.8 ± 0.054*	63.50 ± 1.565*	99.7± 1.764*	31.50 ± 3.500*
V	*Artabotrys hexapetalus* (200mg/kg)p.o.	181.7 ± 0.012*	67.50 ± 1.565*	100.0± 1.732*	31.75 ± 1.917*
VI	*A. Reticulate* + *Artabotrys hexapetalus* (1:1) p.o.	172.0 ±0.024**	69.50±0.7638**	92.0± 1.317**	26.0 ±86.33**
VII	Vehicle control	179.2 ±0.333**	65.83 ±3.609**	133.50 ±1.945**	28.25 ±0.0833**

"Data were expressed as Mean±SEM in each group (n=6) and are significant when analyzed by one-way anova followed by dunnette's test and p<0.01 compared to control group".
P<0.001indicates$^{\#}$when compared to group-1; P<0.001indicates **when compared to group2

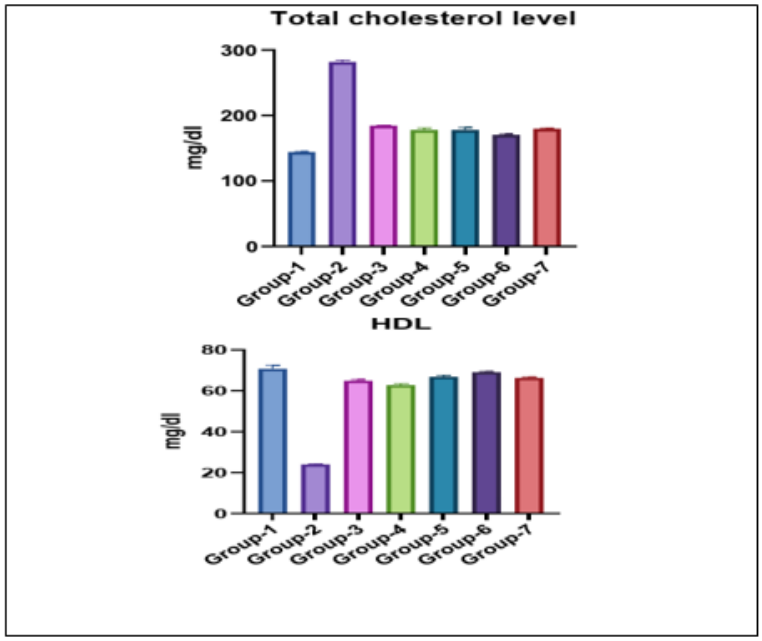

Figure 2: Effect of test samples in total cholesterol level and HDL level

Effect of Test Samples on HDL Cholesterol Level

The effect of the ethanolic extracts of *Annona reticulata* (ARE), *Artabotrys hexapetalus* (AHE), and their combination on serum high-density lipoprotein (HDL) levels is shown in Table 28 and Figure 19. In the disease control group, a marked reduction in HDL levels (23.67 ± 1.382 mg/dL) was observed compared to the normal control group (69.17 ± 3.962 mg/dL), confirming the establishment of hyperlipidemic conditions. HDL is known as "good cholesterol" because it facilitates reverse cholesterol transport and protects against atherogenesis by preventing LDL oxidation and foam cell formation [25].

Treatment with atorvastatin (1 mg/kg) significantly increased HDL levels to 65.50 ± 1.565 mg/dL (**p < 0.01** vs. disease control). Similarly, both ARE (63.50 ± 1.565 mg/dL) and AHE (67.50 ± 1.565 mg/dL) exhibited a substantial rise in HDL concentration compared to the disease control group ($p < 0.01$), indicating their strong antihyperlipidemic potential. Notably, the combined extract (1:1 ratio) demonstrated the highest HDL elevation (69.50 ± 0.7638 mg/dL), almost restoring the levels to normal values and comparable to the atorvastatin-treated group (**p < 0.001**).

The rise in HDL levels in treated groups suggests an improvement in reverse cholesterol transport and enhanced activity of lecithin-cholesterol acyltransferase (LCAT), which promotes cholesterol esterification and clearance from peripheral tissues [26]. The synergistic effect observed in the combination group can be

attributed to the presence of flavonoids, sterols, and alkaloids in both plants, which are known to modulate lipid metabolism through antioxidant mechanisms and peroxisome proliferator-activated receptor (PPAR) activation [27,28].

Earlier studies on *Annona reticulata* leaf extract reported significant elevation in HDL levels in hypercholesterolemic rats, correlating with increased antioxidant enzyme activity and decreased lipid peroxidation [29]. Similarly, *Artabotrys hexapetalus* extracts have shown HDL-raising effects by stimulating hepatic apolipoprotein A-I synthesis and enhancing cholesterol efflux [30]. The results of the present study are therefore consistent with previous reports and highlight the therapeutic potential of these extracts in improving lipid profiles and cardiovascular health.

Effect of Test Samples on LDL Cholesterol Level

The results of the LDL cholesterol assay are presented in Table 29 and Figure 20. The disease control group exhibited a significant increase in LDL levels (134.5 ± 1.118 mg/dL) compared to the normal control (86.83 ± 2.167 mg/dL), confirming diet-induced hyperlipidemia. Elevated LDL cholesterol is a major risk factor for atherosclerosis as it contributes to cholesterol deposition within arterial walls, leading to plaque formation and endothelial dysfunction [31].

Atorvastatin treatment markedly reduced LDL levels to 107.0 ± 1.291 mg/dL (**$p < 0.001$** vs. disease control). Similarly, ARE (99.7 ± 1.764 mg/dL) and AHE (100.0 ± 1.732 mg/dL) significantly decreased LDL levels ($p < 0.01$), demonstrating potent hypolipidemic effects. The combined extract (1:1 ratio) showed the most pronounced reduction (92.0 ± 1.317 mg/dL), which was statistically significant (**$p < 0.001$**) and nearly equivalent to atorvastatin.

The reduction in LDL levels can be attributed to the inhibition of hepatic HMG-CoA reductase activity, increased LDL receptor expression, and enhanced clearance of circulating LDL particles. The phytoconstituents present in both extracts—particularly flavonoids and phytosterols—are known to compete with cholesterol absorption in the intestine and increase fecal sterol excretion, contributing to LDL reduction [32].

The synergistic activity observed in the combination group may result from complementary actions of *A. reticulata* and *A. hexapetalus* bioactives, including improved antioxidant defenses and modulation of lipid transport enzymes. These findings corroborate earlier studies indicating that polyherbal formulations exert greater lipid-lowering efficacy than individual extracts due to the combined effect of multiple bioactives on diverse metabolic targets [33,34].

Thus, the combination of *A. reticulata* and *A. hexapetalus* not only elevates HDL levels but also markedly decreases LDL levels, thereby improving the overall lipid profile and reducing the risk of atherosclerotic plaque formation [35,36}.

Effect of Test Samples on Triglyceride Levels

Administration of a high-fat diet led to a significant elevation in serum triglyceride levels in the disease control group (119.4 ± 92.75 mg/dL) compared with the normal control (22.75 ± 0.416 mg/dL). Treatment with *Atorvastatin* (1 mg/kg) markedly reduced triglyceride levels to 32.33 ± 6.500 mg/dL (**$p < 0.001$**), validating its hypolipidemic efficacy. Likewise, oral administration of *Annona reticulata* and *Artabotrys hexapetalus* extracts (200 mg/kg) significantly decreased triglyceride levels to 31.50 ± 3.500 mg/dL and 31.75 ± 1.917 mg/dL, respectively ($p < 0.01$). Notably, the combined administration of *A. reticulata* and *A. hexapetalus* (1:1) produced a superior reduction (26.0 ± 86.33 mg/dL), comparable to *Atorvastatin*, suggesting synergistic lipid-lowering effects (Fig. 21).

The observed triglyceride-lowering activity may be attributed to bioactive phytoconstituents such as flavonoids, sterols, and alkaloids known to enhance lipoprotein lipase activity and suppress hepatic lipogenesis [37–39].

Effect of Test Samples on VLDL Levels

In the disease control group, VLDL levels increased significantly (18.00 ± 1.155 mg/dL) compared to normal (12.50 ± 0.885 mg/dL). Treatment with *Atorvastatin* and the combination extract notably reduced VLDL levels to 16.50 ± 2.405 and 13.83 ± 1.956 mg/dL, respectively (**$p < 0.001$**). The extracts of *A. reticulata* and *A. hexapetalus* alone also showed significant effects ($p < 0.01$), confirming their capacity to normalize VLDL metabolism (Fig. 22).

This reduction could be due to the inhibition of hepatic triglyceride synthesis and promotion of VLDL catabolism, possibly mediated by antioxidant and flavonoid-rich fractions [40,41](Figure 3 and Table 3.

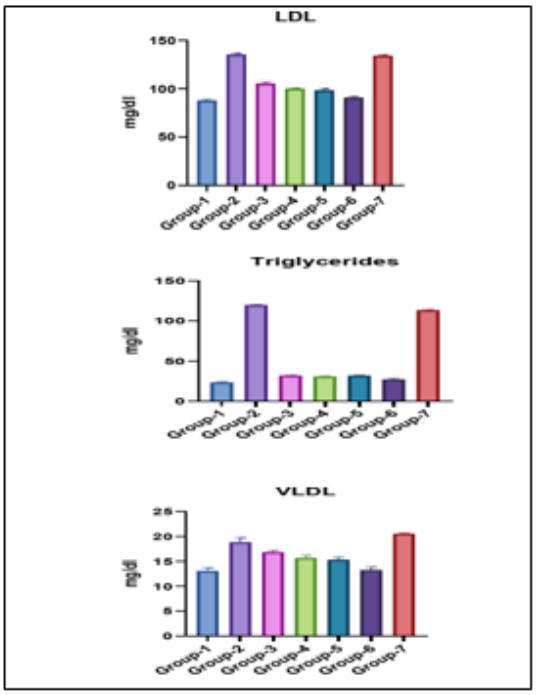

Fig 3: Effect of test samples in LDL level, triglycerides and VLDL level

Table-3: Assessment of ratio of TCH / HDL.

Groups	Treatment &dose	Final ratio of TCH / HDL	Final ratio of TGL/ HDL	Final ratio of LDL/HDL (DAY-31)
I	Normal	3.383 ± 0.135$^{\#}$	1.650 ± 0.007$^{\#}$	1.650 ± 0.007$^{\#}$
II	Disease control	4.117 ± 0.1249	4.400 ± 0.106	4.400±0.106
III	Atorvastatin(1mg/kg)	3.483 ± 0.113**	0.4833± 0.116**	0.4833±0.116**
IV	*Annona reticulata* (200mg/kg) p.o.	3.633 ± 0.102*	1.100 ± 0.1390*	1.100 ± 0.139*
V	*Artabotrys hexapetalus* (200mg/kg) p.o.	3.483 ± 0.1078*	0.6833± 0.1400*	0.6833 ± 0.400*
VI	*A. reticulata*+ *Artabotrys hexapetalus* (1:1) p.o.	2.471 ±0.1249**	0.483 ±0.1078**	0.483±0.1078**
VII	Vehicle control	3.383 ±0.1352**	1.650 ±0.07638**	1.650 ±0.0768**

"Data were expressed as Mean±SEM in each group (n=6) and are significant when analyzed by one-way anova followed by dunnette's test and p<0.01 compared to control group". P<0.001 indicates $^{\#}$when compared to group-1;P<0.001indicates**when compared to group-2; P<0.01 indicates *when compared to group-2.

Assessment of TCH/HDL Ratio

The total cholesterol to HDL ratio (TCH/HDL) is an important index of cardiovascular risk. Disease control animals showed a higher ratio (4.117 ± 0.1249) indicating hyperlipidemic stress. In contrast, *Atorvastatin* (3.483 ± 0.113) and plant extract-treated groups (*A. reticulata* = 3.633 ± 0.102; *A. hexapetalus* = 3.483 ± 0.1078) demonstrated significant reductions ($p < 0.01$). The combination group exhibited the most pronounced improvement (2.471 ± 0.1249, **$p < 0.001$**), reflecting strong antihyperlipidemic potential (Fig. 23).

The improved TCH/HDL ratio suggests that the extracts enhance reverse cholesterol transport, thereby decreasing the risk of atherogenesis [42, 43].

Assessment of TGL/HDL and LDL/HDL Ratios

The ratios of TGL/HDL and LDL/HDL are vital atherogenic indices. The disease control group exhibited elevated TGL/HDL (4.400 ± 0.106) and LDL/HDL (4.400 ± 0.106) ratios, indicating impaired lipid metabolism. Both individual and combined plant extract treatments significantly restored these ratios toward normal. The *A. reticulata* + *A. hexapetalus* combination showed the greatest effect, with values (0.483 ± 0.1078) comparable to *Atorvastatin* (0.4833 ± 0.116) (**$p < 0.001$**) (Figs. 24–25).

Such normalization suggests potent modulation of lipoprotein fractions and inhibition of lipid

peroxidation, consistent with the antioxidative mechanisms of phenolic and alkaloidal compounds [44,45].

Effect of Test Samples on Organ Weights

The organ weight analysis (Table 35) revealed significant pathological enlargement of the heart, liver, and kidneys in the disease control group due to lipid accumulation and oxidative stress. Treatment with *Atorvastatin* and both plant extracts reversed these alterations significantly (**$p < 0.001$**). The combined extract demonstrated the greatest normalization effect: heart (3.13 ± 0.495 g), liver (3.08 ± 0.310 g), left kidney (200.9 ± 13.06 g), and right kidney (260.9 ± 4.324 g), nearing normal physiological values (Figs. 26–29).

This hepatoprotective and renoprotective effect can be attributed to flavonoids, terpenoids, and phenolic compounds known for reducing lipid peroxidation and stabilizing membrane integrity [46–47].

Overall, the combined administration of *A. reticulata* and *A. hexapetalus* demonstrated a synergistic antihyperlipidemic effect through mechanisms involving lipid metabolism modulation, antioxidant activity, and organ protection, aligning with earlier reports on related *Annonaceae* species [48–51](Figure 4) an (Table 4).

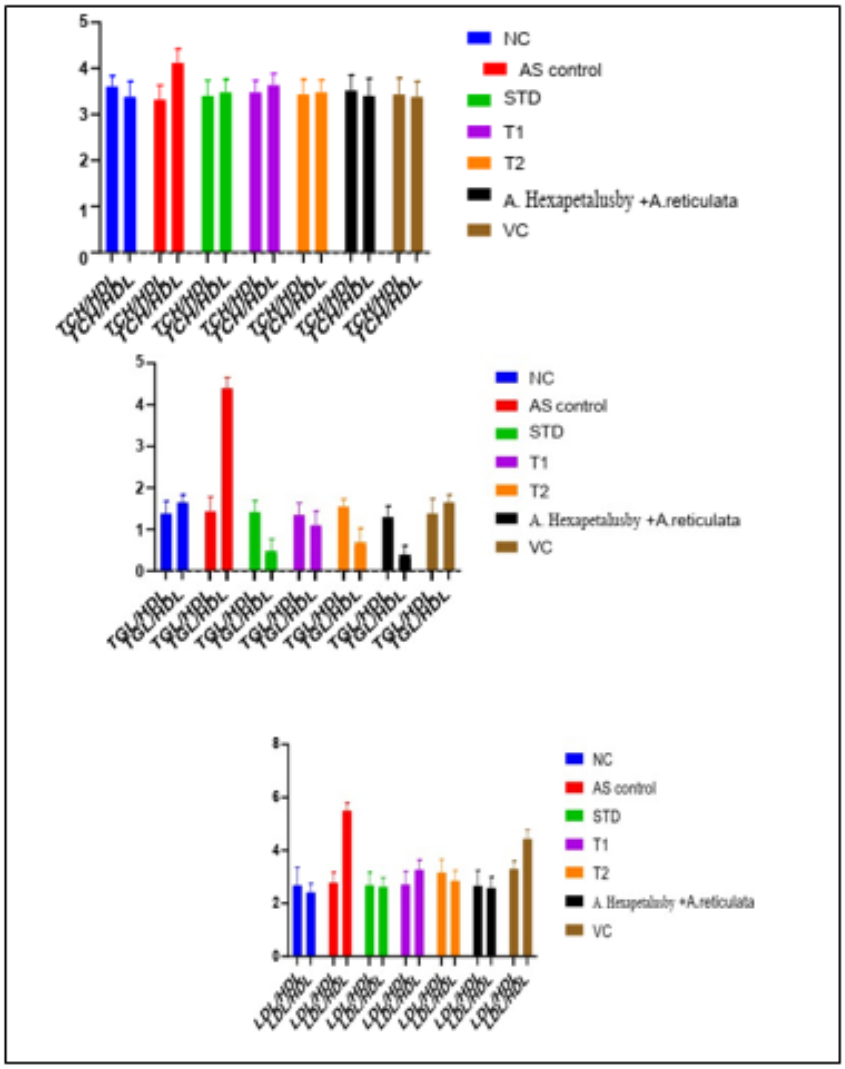

Fig. 4: Assessment of ratio of TCH / HDL, TGL/ HDL and LDL/ HDL

Table-4: Reaction of test samples on organ weight (31stday)

Groups	Treatment & dose	Wt. of heart (gm)	Wt. of liver (gm)	Wt. of left kidney (gm)	Wt. of right kidney (gm)
I	Normal	6.406±0.181$^{\#}$	2.930±0.027$^{\#}$	190.2±2.655$^{\#}$	258.2±0.733$^{\#}$
II	Disease control	25.33 ± 1.292	23.93 ±1.273	259.0 ±8.679	388.9±16.81
III	Atorvastatin (1mg/kg)	5.671 ±0.101**	7.885±0.167**	210.7±1.362**	271.6±1.210**
IV	*Annona reticulate* (200mg/kg)p.o.	4.551 ± 0.159*	4.154 ±0.290*	209.2 ±0.952*	269.2 ±0.910*
V	*Artabotrys hexapetalus* (200mg/kg) p.o.	4.648 ± 0.107*	5.596 ±0.145*	208.7 ±1.002*	266.0 ±0.749*
VI	*A. reticulate* + *Artabotrys hexapetalus* (1:1) p.o.	3.13 ±0.495**	3.08±0.310**	200.9 ±13.06**	260.9 ±4.324**
VII	Vehicle control	20.863±0.120**	24.251±0.122**	257.5±1.058**	379.4±0.501**

"Data were expressed as Mean±SEM in each group (n=6) and are significant when analyzed by one-way anova followed by dunnette's test and p<0.01 compared to control group".

P<0.001 indicates$^{\#}$ when compared to group-1; P<0.001 indicates**when compared to group-2; P<0.01 indicates *when compared to group-2

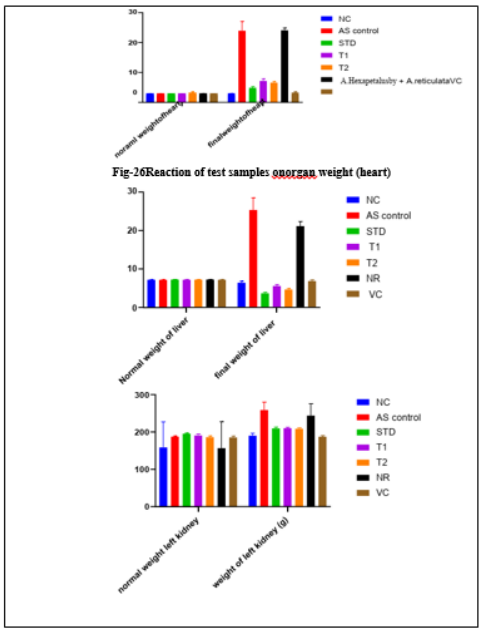

Fig-26Reaction of test samples onorgan weight (heart)

Fig 5: Reaction of test samples on organ weight (heart), weight (liver) and organ weight (left kidney)

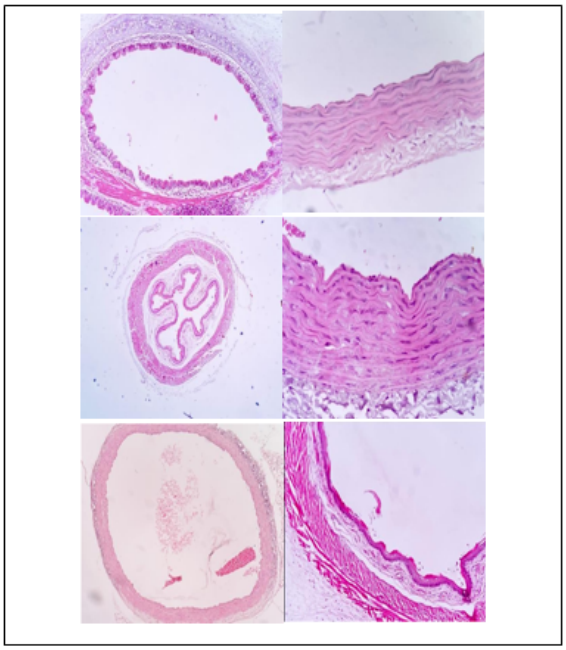

Fig 6: Histopathological representation of aorta of normal group (distal& proximal), histopathological representation of aorta of control group(distal & proximal) and histopathological representation of aorta of standard group (distal & proximal)

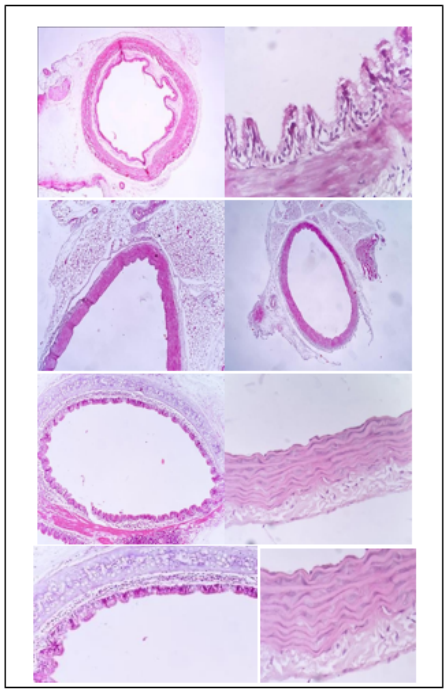

Fig 7: Histopathological representation of aorta of T1 group (distal&proximal), T2 group (distal & proximal), combination of both T1&T2 (distal &proximal) and V.C group (distal &proximal).

CONCLUSION

In the present study, atherosclerosis was successfully induced in albino rats using an atherogenic diet enriched with saturated fats and vitamin D_3. The disease condition was confirmed by a significant elevation in serum lipid parameters, including total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and atherogenic indices (TC/HDL and LDL/HDL), along with a marked reduction in high-density lipoprotein (HDL) levels.

Treatment with ethanolic leaf extracts of *Annona reticulata* and *Artabotrys hexapetalus*, individually as well as in combination, produced a significant improvement in lipid profile by reducing TC, TG, LDL,

VLDL, and atherogenic indices, while elevating HDL levels. These findings indicate a strong antihyperlipidemic and anti-atherosclerotic potential of the extracts. Additionally, the extracts demonstrated protective effects on vital organs such as the liver, heart, aorta, and kidneys.

The observed pharmacological effects may be attributed to the presence of bioactive phytoconstituents, including flavonoids, alkaloids, saponins, glycosides, terpenoids, steroids, proteins, and phenolic compounds, which are known to possess antioxidant and lipid-lowering properties.

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