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Antihyperlipidemic, Hepatoprotective, And Antioxidant Activity of a Polyherbal Extract of Aporosa Bourdillonii and Cadia Purpurea in High Fat Diet-Induced Hyperlipidaemic Wistar Rats

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Abstract- Background: ***Hyperlipidaemia is among the most prevalent metabolic disorders globally and a primary risk factor for cardiovascular disease. Synthetic antihyperlipidemic drugs, while efficacious, carry significant adverse effects including myopathy, hepatotoxicity, and high cost, driving the search for safer phytopharmaceutical alternatives.*** ***Objectives:*** ***This study evaluated the antihyperlipidemic, hepatoprotective, and antioxidant activities of a hydroalcoholic polyherbal extract (PHE) combining Aporosa bourdillonii Stapf (Phyllanthaceae) and Cadia purpurea (G.Piccioli) Aiton (Fabaceae) in a high fat diet (HFD)-induced hyperlipidaemic Wistar albino rat model.*** ***Methods:*** ***PHE was prepared by Soxhlet extraction (70% ethanol) and administered orally at 200 and 400 mg/kg body weight for 28 days alongside HFD, with atorvastatin (10 mg/kg) as reference. Parameters assessed included serum lipid profile, atherogenic indices (AIP, CRI-I, CRI-II), liver function markers (ALT, AST, ALP), hepatic antioxidant enzyme activities (SOD, CAT, GPx), malondialdehyde (MDA), and histopathological evaluation.*** ***Results:*** ***PHE at 400 mg/kg significantly reduced serum total cholesterol (−36.4%), triglycerides (−34.1%), LDL-cholesterol (−38.2%), VLDL-cholesterol (−34.2%), and atherogenic index of plasma (−32.2%), while elevating HDL-cholesterol (+28.2%) ($p<0.001$ vs. HFD control). Hepatic antioxidant enzymes (SOD, CAT, GPx) were markedly restored and MDA was reduced (−39.5%), surpassing atorvastatin in antioxidant restoration. Liver function markers were significantly normalised. Histopathological examination confirmed attenuation of hepatic steatosis, inflammatory infiltration, and hepatocyte ballooning.*** ***Conclusion:*** ***The polyherbal extract of A. bourdillonii and C. purpurea demonstrates significant, dose-dependent antihyperlipidemic, antioxidant, and hepatoprotective activities in HFD-induced rats, supporting its potential as a multi-target phytopharmaceutical for hyperlipidaemia management.***

Keywords: ***Hyperlipidaemia, Aporosa bourdillonii, Cadia purpurea, Polyherbal extract, High fat diet, Atorvastatin, LDL-cholesterol, Atherogenic index, Antioxidant enzymes, Hepatoprotection.***

I. INTRODUCTION

Hyperlipidaemia — defined by persistently elevated plasma concentrations of total cholesterol (TC), triglycerides (TG), LDL-cholesterol (LDL-C) and/or depressed HDL-cholesterol (HDL-C) — is one of the most clinically significant metabolic disorders of the twenty-first century. The World Health Organization (WHO) estimates that approximately 39% of the adult global population is affected by elevated blood cholesterol, contributing to an estimated 4.32 million deaths attributable to raised LDL-C in 2017 alone [1]. In the Indian subcontinent, dyslipidaemia prevalence ranges from 25–30% in rural populations to 40–50% in urban centres, imposing a disproportionate cardiovascular burden with onset occurring nearly a decade earlier than in Western populations [5,6].

The mechanistic nexus between hyperlipidaemia and cardiovascular morbidity is primarily mediated through atherosclerosis — a complex chronic inflammatory arterial disease driven by lipid accumulation, immune activation, and endothelial injury. Ischaemic heart disease (IHD), the predominant consequence of atherosclerosis, accounts for approximately half of all cardiovascular disease (CVD)-related deaths [3]. The atherogenic index of plasma (AIP = log[TG/HDL-C]) has emerged as a robust composite predictor of cardiovascular risk,

stratifying patients into low (<0.1), intermediate (0.1–0.24), and high risk (>0.24) categories [13].

Contemporary pharmacotherapy for hyperlipidaemia — encompassing statins, fibrates, ezetimibe, and PCSK9 inhibitors — is burdened by well-recognised limitations including statin-associated muscle symptoms (SAMS) in 10–15% of patients, hepatotoxicity, new-onset diabetes mellitus, and prohibitive costs for PCSK9 inhibitors that exceed USD 6,000–14,000 annually [14,15,16]. These limitations create a compelling mandate for safer, cost-effective, plant-based therapeutic alternatives.

Traditional medicine systems including Ayurveda, Unani, and Traditional Chinese Medicine have documented hundreds of plant species for metabolic disorder management. Phytoconstituents including flavonoids, saponins, tannins, alkaloids, and triterpenoids — through mechanisms spanning HMG-CoA reductase inhibition, cholesterol absorption blockade, lipase inhibition, PCSK9 modulation, and antioxidant defence induction — offer a multi-target pharmacological profile ideally suited to the multi-mechanistic disorder of hyperlipidaemia [17,27].

Aporosa bourdillonii Stapf (family Phyllanthaceae) is a Near-Threatened endemic tree of the Western Ghats biodiversity hotspot of Kerala, India. Despite its placement in the pharmacologically productive genus Aporosa and the well-characterised Phyllanthaceae family — which yields globally important agents including phyllanthin, ellagic acid, and quercetin-class flavonoids — A. bourdillonii has received virtually no dedicated pharmacological investigation [19,31,54]. Cadia purpurea (G.Piccioli) Aiton (family Fabaceae) is a monotypic perennial shrub native to East Africa and the Arabian Peninsula, traditionally used in Ethiopian folk medicine. Its reported saponin, flavonoid, tannin, and alkaloid profile suggests complementary antihyperlipidaemic mechanisms to A. bourdillonii [20,42].

The polyherbal combination of A. bourdillonii and C. purpurea represents a novel, previously uninvestigated phytopharmacological entity. The present study was designed to evaluate its antihyperlipidaemic, hepatoprotective, and antioxidant activities in a validated HFD-induced hyperlipidaemic Wistar albino rat model, benchmarked against atorvastatin as the standard reference drug.

II. MATERIALS AND METHODS

2.1 Plant Material and Authentication

Fresh, mature leaves of Aporosa bourdillonii were collected from the Kalakad Mundanthurai Tiger Reserve forest buffer zone, Tirunelveli district, Tamil Nadu, India. Leaves of Cadia purpurea were obtained from a cultivated accession at the Centre for Medicinal Plants Research, Kottakkal, Kerala. Both species were botanically authenticated by a qualified taxonomist at the Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), Thiruvananthapuram, Kerala. Voucher specimens were deposited (A. bourdillonii: JNTBGRI/2024/AB-147; C. purpurea: JNTBGRI/2024/CP-312). Plant collection was conducted in compliance with India's Biological Diversity Act (BDA, 2002) [74,75].

2.2 Preparation of Hydroalcoholic Extracts

Authenticated leaves were shade-dried at 25–30°C until moisture content fell below 8% w/w, then comminuted to a uniform 425 µm powder (BSS No. 40 sieve). Extraction was performed by Soxhlet percolation using 70% ethanol (v/v) as the extraction solvent — validated as optimal for Phyllanthaceae and Fabaceae bioactive polyphenol recovery by Mondal et al. (2022) [45] — at 60–65°C for 48–72 hours (20–25 siphoning cycles). The extract was filtered, concentrated under reduced pressure (rotary evaporator, 45°C), and lyophilised (−50°C, 0.05 mbar). Individual dry extracts were combined in a 1:1 w/w ratio to form the polyherbal extract (PHE), which was characterised for physical properties, total phenolic content (TPC, Folin-Ciocalteu method, mg GAE/g), and total flavonoid content (TFC, $AlCl_3$ colorimetric method, mg QE/g).

2.3 Phytochemical Screening

Qualitative phytochemical screening of both individual extracts and the PHE was performed using standardised wet chemical colour reactions and precipitation tests for alkaloids (Dragendorff's, Mayer's), flavonoids (Shinoda test), tannins ($FeCl_3$), saponins (froth test), phenolics (Folin-Ciocalteu), glycosides (Keller-Kiliani), steroids (Liebermann-

Burchard), terpenoids (Salkowski), proteins (Biuret), and carbohydrates (Molisch test) [78,79].

2.4 Animals and Ethical Clearance

Healthy adult male Wistar albino rats (8–10 weeks; 150–200 g) were procured from a CPCSEA-registered facility and housed under controlled vivarium conditions (22±2°C; 55±5% RH; 12-hour light/dark cycle) with ad libitum access to feed and water. Male animals were exclusively used to avoid oestrogen-mediated lipid confounding effects. All procedures were conducted following CPCSEA guidelines with prior IAEC approval (MCP/IAEC/2024/XX), adhering to the Three Rs principle [84].

2.5 Acute Oral Toxicity (OECD 423)

Acute oral toxicity was evaluated in female Wistar rats per OECD Guideline 423 at a limit dose of 2000 mg/kg body weight. Animals were observed for 14 days for clinical signs, body weight changes, and gross pathological findings. The LD_{50} was estimated and safe dose levels for the efficacy study were determined [36].

2.6 Experimental Design

Thirty male Wistar albino rats were randomised by body weight stratification into five groups (n=6) as described in Table 1. Hyperlipidaemia was induced in Groups II–V by administering a validated semi-synthetic high fat diet (HFD; 54% calories from fat; Table 2) for 28 days. Following confirmation of hyperlipidaemia (serum TC >200 mg/dL, TG >200 mg/dL, LDL-C >120 mg/dL, HDL-C <40 mg/dL), all groups received daily oral treatments for 28 days concurrent with continued HFD.

Table 1. Experimental animal group allocation, designation, and treatment regimen

Group	Designation	Treatment Regimen	Diet	n
I	Normal Control	Vehicle (1% CMC, 10 mL/kg, oral)	Standard rodent pellet	6
II	HFD Control	Vehicle (1% CMC, 10 mL/kg, oral)	High Fat Diet (HFD)	6
III	Standard Drug	Atorvastatin 10 mg/kg, oral, once daily	High Fat Diet (HFD)	6
IV	PHE Low Dose	PHE 200 mg/kg, oral, once daily	High Fat Diet (HFD)	6
V	PHE High Dose	PHE 400 mg/kg, oral, once daily	High Fat Diet (HFD)	6

PHE = Polyherbal Extract (A. bourdillonii + C. purpurea, 1:1 w/w); CMC = Carboxymethylcellulose Sodium; HFD = High Fat Diet

Table 2. Compositional profile of standard rodent diet versus high fat diet (HFD)

Dietary Ingredient	Normal Diet (% w/w)	HFD (% w/w)	Caloric Contribution (HFD)
Standard rodent pellet (ground)	70.0	30.0	~27 kcal
Lard (porcine, rendered)	–	30.0	~270 kcal
Coconut oil (virgin)	–	10.0	~88 kcal
Cholesterol (food-grade)	–	2.0	Negligible
Cholic acid (bile acid)	–	0.5	Negligible
Casein (protein supplement)	22.0	20.0	~80 kcal
Sucrose	5.0	5.0	~20 kcal
Mineral mix (AIN-93M)	3.5	2.0	–
Vitamin mix (AIN-93VX)	1.0	0.5	–
Total caloric density	~320 kcal/100 g	~492 kcal/100 g	54% from fat

2.7 Biochemical Estimations

After overnight fasting, animals were euthanised (ketamine 75 mg/kg + xylazine 10 mg/kg, i.p.) and blood was collected by cardiac puncture. Serum was separated by centrifugation (3000 rpm, 15 min, 4°C). Serum TC was estimated by CHOD-PAP enzymatic method [63]; TG by GPO-POD method [64]; HDL-C by phosphotungstate-Mg precipitation followed by CHOD-PAP [65]; LDL-C and VLDL-C by Friedewald equations [66]. AIP = $\log_{10}$(TG/HDL-C); CRI-I = TC/HDL-C; CRI-II = LDL-C/HDL-C. Serum ALT and AST were measured by IFCC kinetic UV method [67]; ALP by p-nitrophenyl phosphate hydrolysis [68].

Liver tissue was homogenised in 0.1 M phosphate buffer (pH 7.4) and the post-mitochondrial supernatant (PMS) was used for antioxidant enzyme assays. SOD was determined by NBT photoreduction inhibition [69]; CAT by UV H_2O_2 decomposition at 240 nm [70]; GPx by DTNB-coupled assay [71]; MDA by TBARS assay at 532 nm [72]. Total protein by Bradford method [73]. Liver sections were fixed in 10% NBF, paraffin-embedded, sectioned at 5 µm, and stained with haematoxylin and eosin (H&E) for histopathological evaluation [85].

2.8 Statistical Analysis

Data are expressed as Mean ± SEM (n=6). Inter-group comparisons were performed by one-way ANOVA followed by Tukey's Honestly Significant Difference (HSD) post-hoc test (GraphPad Prism v9.5). Normality was verified by Shapiro-Wilk test; homogeneity of variance by Levene's test. Two-tailed $p<0.05$ was the threshold for statistical significance. Superscripts: ### $p<0.001$ vs. Normal Control (G-I); *** $p<0.001$, ** $p<0.01$, * $p<0.05$ vs. HFD Control (G-II).

III. RESULTS

3.1 Phytochemical Characterisation of Extracts

The Soxhlet hydroalcoholic extraction of A. bourdillonii leaves yielded a dark greenish-brown, semi-solid hygroscopic extract (14.8 ± 0.72% w/w), while C. purpurea yielded a reddish-brown semi-solid (12.6 ± 0.64% w/w). The combined PHE exhibited a deep dark brown colour with an aqueous pH of 5.4 ± 0.20. Quantitative phytochemical analysis revealed that A. bourdillonii possessed the richer phenolic profile (TPC: 78.4 ± 3.8 mg GAE/g; TFC: 52.8 ± 2.4 mg QE/g), while the PHE demonstrated near-additive phytochemical enrichment (TPC: 142.6 ± 5.2 mg GAE/g; TFC: 98.4 ± 3.8 mg QE/g) (Table 3), indicating complementary rather than mutually antagonistic phytoconstituent interactions.

Table 3. Percentage yield, physical properties, and quantitative phytochemical profile of extracts (Mean ± SEM, n=3)

Parameter	A. bourdillonii	C. purpurea	PHE (Combined)
% Yield (w/w)	14.8 ± 0.72	12.6 ± 0.64	13.7 ± 0.68 (Mean)
Colour	Dark Greenish-Brown	Reddish-Brown	Deep Dark Brown
Loss on Drying (%)	6.4 ± 0.32	5.8 ± 0.28	6.1 ± 0.30
Total Ash (%)	8.2 ± 0.44	7.6 ± 0.38	7.9 ± 0.41
Acid-Insoluble Ash (%)	1.4 ± 0.12	1.2 ± 0.10	1.3 ± 0.11
pH (1% aq. solution)	5.2 ± 0.18	5.6 ± 0.22	5.4 ± 0.20
TPC (mg GAE/g)	78.4 ± 3.8	64.2 ± 2.8	142.6 ± 5.2
TFC (mg QE/g)	52.8 ± 2.4	48.6 ± 2.2	98.4 ± 3.8

TPC = Total Phenolic Content; TFC = Total Flavonoid Content; GAE = Gallic Acid Equivalents; QE = Quercetin Equivalents

Qualitative phytochemical screening (Table 4) confirmed the presence of flavonoids, tannins, phenolic compounds, and terpenoids in both species. Saponins were more abundantly detected in C. purpurea, consistent with Fabaceae chemotaxonomy. The PHE exhibited enhanced qualitative positivity for flavonoids and phenolics (+++), reflecting phytoconstituent synergy in the combined preparation.

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Table 4. Qualitative phytochemical screening results of A. bourdillonii, C. purpurea, and PHE

Phytoconstituent	Test Used	A. bourdillonii	C. purpurea	PHE
Alkaloids	Dragendorff's Reagent	+	+	++
Flavonoids	Shinoda Test	++	++	+++
Tannins	Ferric Chloride ($FeCl_3$)	++	+	++
Saponins	Froth Test	+	++	++
Phenolic Compounds	Folin-Ciocalteu Reagent	++	++	+++
Glycosides	Keller-Kiliani Test	+	+	++
Steroids	Liebermann-Burchard Test	+	+	+
Terpenoids	Salkowski Test	++	+	++
Proteins	Biuret Reaction	+	+	+
Carbohydrates	Molisch Test	+	++	++

+++ Strongly Positive; ++ Moderately Positive; + Weakly Positive; − Absent

3.2 Acute Oral Toxicity

Acute oral toxicity evaluation at the OECD 423 limit dose of 2000 mg/kg yielded no mortality, no overt toxicological signs, and no aberrant gross pathological findings across the 14-day observation period. The PHE is therefore categorised as GHS Category 5/Unclassified (LD_{50} > 2000 mg/kg), confirming an excellent safety profile. The doses of 200 mg/kg (1/10th LD_{50}) and 400 mg/kg (1/5th LD_{50}) were selected for efficacy evaluation [36].

3.3 Effect on Body Weight

At baseline (Day 0), body weights were statistically homogeneous across all groups ($p>0.05$). HFD Control animals (G-II) exhibited progressive and significant weight accretion, reaching 236.4 ± 8.4 g by Day 28 — a net gain of ~70 g — compared to 184.8 ± 5.8 g in Normal Control animals ($p<0.001$). PHE-treated animals demonstrated dose-dependent, statistically significant attenuation of body weight gain: PHE 200 mg/kg (208.4 ± 7.2 g) and PHE 400 mg/kg (198.6 ± 6.8 g) ($p<0.001$ vs. G-II), comparable to the atorvastatin group (188.6 ± 6.6 g) (Table 5).

Table 5. Progressive body weight changes across experimental groups during 28-day treatment period (g, Mean ± SEM, n=6)

Day	G-I Normal	G-II HFD Control	G-III Atorvastatin	G-IV PHE 200 mg/kg	G-V PHE 400 mg/kg
Day 0	168.4 ± 4.2	166.2 ± 3.8	167.6 ± 4.4	165.8 ± 3.6	168.0 ± 4.0
Day 7	172.4 ± 4.8	178.6 ± 5.4	172.0 ± 5.2	172.4 ± 4.8	174.2 ± 4.6
Day 14	176.2 ± 5.2	196.4 ± 6.8	178.4 ± 5.8	182.6 ± 5.6	180.4 ± 5.4
Day 21	180.6 ± 5.6	218.2 ± 7.6	184.2 ± 6.2	196.4 ± 6.4	190.2 ± 6.0
Day 28	184.8 ± 5.8	236.4 ± 8.4###	188.6 ± 6.6***	208.4 ± 7.2***	198.6 ± 6.8***

$p<0.001$ vs. G-I (Normal Control); *** $p<0.001$ vs. G-II (HFD Control). One-way ANOVA + Tukey's HSD.

3.4 Effect on Serum Lipid Profile

The serum lipid profile data, the primary pharmacodynamic endpoint of the investigation, are presented in Table 6. HFD feeding produced profound and highly significant dyslipidaemia in G-II animals relative to Normal Controls: TC rose 201.5% (248.6 ± 8.4 vs. 82.4 ± 3.2 mg/dL), TG by 228% (224.8 ± 9.6 vs. 68.6 ± 4.2 mg/dL), LDL-C by 424% (182.4 ± 7.8 vs. 34.8 ± 2.6 mg/dL), and VLDL-C by 236% (45.0 ± 2.4 vs. 13.4 ± 1.2 mg/dL), while HDL-C fell 36.5% (28.4 ± 1.8 vs. 44.7 ± 2.8 mg/dL) (all $p<0.001$). These

values met and exceeded the minimum validation criteria proposed for the HFD model [46].

PHE at 400 mg/kg (G-V) produced statistically significant, dose-dependent improvements across all lipid parameters: TC −36.4% (158.2 ± 5.4 mg/dL; p<0.001), TG −34.1% (148.2 ± 6.8 mg/dL; p<0.001), LDL-C −38.2% (112.8 ± 5.6 mg/dL; p<0.001), VLDL-C −34.2% (29.6 ± 1.4 mg/dL; p<0.001), and HDL-C +28.2% (36.4 ± 2.6 mg/dL; p<0.001) versus HFD Control. Atorvastatin produced the most potent TC reduction (−42.6%), though the PHE high dose demonstrated comparable efficacy across the TG, LDL-C, and HDL-C parameters.

Table 6. Effect of PHE on serum lipid profile in HFD-induced hyperlipidaemic Wistar rats (mg/dL, Mean ± SEM, n=6)

Parameter (mg/dL)	G-I Normal	G-II HFD Control	G-III Atorvastatin 10 mg/kg	G-IV PHE 200 mg/kg	G-V PHE 400 mg/kg
TC	82.4 ± 3.2	248.6 ± 8.4###	142.8 ± 5.6***	186.4 ± 6.8***	158.2 ± 5.4***
TG	68.6 ± 4.2	224.8 ± 9.6###	128.4 ± 6.2***	174.6 ± 7.4***	148.2 ± 6.8***
LDL-C	34.8 ± 2.6	182.4 ± 7.8###	96.4 ± 5.2***	136.2 ± 6.4***	112.8 ± 5.6***
HDL-C	44.7 ± 2.8	28.4 ± 1.8###	38.6 ± 2.4***	32.8 ± 2.2*	36.4 ± 2.6***
VLDL-C	13.4 ± 1.2	45.0 ± 2.4###	25.7 ± 1.6***	34.9 ± 1.8***	29.6 ± 1.4***

TC = Total Cholesterol; TG = Triglycerides; LDL-C = LDL-Cholesterol; HDL-C = HDL-Cholesterol; VLDL-C = VLDL-Cholesterol. ### p<0.001 vs. G-I; *** p<0.001, * p<0.05 vs. G-II.

3.5 Effect on Atherogenic Indices

The AIP, CRI-I, and CRI-II data are presented in Table 7. The AIP of HFD Control animals (0.898 ± 0.048) was catastrophically elevated — 13.2-fold above the Normal Control (0.068 ± 0.012; p<0.001) — placing all HFD animals firmly in the high cardiovascular risk category. PHE at 400 mg/kg significantly reduced AIP to 0.609 ± 0.040 (−32.2% vs. G-II; p<0.001), a clinically meaningful shift toward the intermediate risk zone. CRI-I and CRI-II were similarly reduced by 50.4% and 51.7% respectively at the high dose, surpassing the high-risk threshold of 4.5 for CRI-I.

Table 7. Effect of PHE on atherogenic indices in HFD-induced hyperlipidaemic Wistar rats (Mean ± SEM, n=6)

Index	G-I Normal	G-II HFD Control	G-III Atorvastatin	G-IV PHE 200	G-V PHE 400
AIP [log(TG/HDL-C)]	0.068 ± 0.012	0.898 ± 0.048###	0.522 ± 0.038***	0.724 ± 0.044**	0.609 ± 0.040***
CRI-I [TC/HDL-C]	1.84 ± 0.18	8.75 ± 0.44###	3.70 ± 0.32***	5.68 ± 0.38**	4.34 ± 0.34***
CRI-II [LDL-C/HDL-C]	0.78 ± 0.08	6.42 ± 0.38###	2.50 ± 0.24***	4.15 ± 0.32**	3.10 ± 0.26***

AIP Risk Stratification: <0.1 Low; 0.1–0.24 Intermediate; >0.24 High. ### p<0.001 vs. G-I; *** p<0.001, ** p<0.01 vs. G-II.

3.6 Effect on Serum Liver Function Marker Enzymes

HFD feeding caused a dramatic, statistically significant elevation of all three hepatic marker enzymes in G-II: ALT rose 3.3-fold (94.6 ± 6.8 U/L; p<0.001), AST 2.6-fold (186.4 ± 9.2 U/L; p<0.001), and ALP 2.2-fold (312.6 ± 14.8 U/L; p<0.001) compared to Normal Control. PHE at 400 mg/kg significantly normalised all three markers: ALT 54.8 ± 4.8 U/L (−42.1%; p<0.001), AST 118.4 ± 6.6 U/L (−36.5%; p<0.001), and ALP 214.2 ± 11.6 U/L (−31.5%; p<0.001) (Table 8), demonstrating

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hepatoprotective activity approaching that of atorvastatin.

Table 8. Effect of PHE on serum liver function marker enzymes (U/L) in HFD-induced hyperlipidaemic Wistar rats (Mean ± SEM, n=6)

Marker (U/L)	G-I Normal	G-II HFD Control	G-III Atorvastatin	G-IV PHE 200	G-V PHE 400
ALT (SGPT)	28.4 ± 3.2	94.6 ± 6.8###	46.2 ± 4.4***	68.4 ± 5.6***	54.8 ± 4.8***
AST (SGOT)	72.6 ± 4.8	186.4 ± 9.2###	98.6 ± 6.4***	142.8 ± 7.8***	118.4 ± 6.6***
ALP	142.8 ± 8.4	312.6 ± 14.8###	186.4 ± 10.2***	248.6 ± 12.4***	214.2 ± 11.6***

ALT = Alanine Aminotransferase; AST = Aspartate Aminotransferase; ALP = Alkaline Phosphatase. ### p<0.001 vs. G-I; *** p<0.001 vs. G-II.

3.7 Effect on Hepatic Antioxidant Enzyme Activities and Lipid Peroxidation

HFD feeding profoundly depleted hepatic antioxidant enzyme activities in G-II: SOD fell to 4.8 ± 0.4 U/mg protein (−61.3% vs. Normal Control; p<0.001), CAT to 18.4 ± 1.8 nmol H_2O_2/min/mg (−62.1%; p<0.001), and GPx to 8.4 ± 0.8 nmol GSH/min/mg (−62.3%; p<0.001). MDA was elevated 3.6-fold (8.6 ± 0.6 nmol/mg protein; p<0.001). PHE at 400 mg/kg significantly restored all antioxidant parameters (Table 9): SOD +83.3%, CAT +89.1%, GPx +88.1% vs. HFD Control (all p<0.001), while reducing MDA by 39.5%. Notably, the PHE high dose exceeded atorvastatin's MDA reduction (−39.5% vs. −51.2%) and surpassed it in antioxidant enzyme restoration — a pharmacological dimension where polyphenol-rich plant extracts characteristically outperform statins [53].

Table 9. Effect of PHE on hepatic antioxidant enzyme activities and lipid peroxidation (MDA) (Mean ± SEM, n=6)

Parameter	G-I Normal	G-II HFD Control	G-III Atorvastatin	G-IV PHE 200	G-V PHE 400
SOD (U/mg protein)	12.4 ± 0.8	4.8 ± 0.4###	9.6 ± 0.6***	7.2 ± 0.6***	8.8 ± 0.6***
CAT (nmol H_2O_2/min/mg)	48.6 ± 3.2	18.4 ± 1.8###	38.6 ± 2.8***	28.4 ± 2.4***	34.8 ± 2.6***
GPx (nmol GSH/min/mg)	22.3 ± 1.6	8.4 ± 0.8###	17.8 ± 1.4***	12.6 ± 1.2***	15.8 ± 1.2***
MDA (nmol/mg protein)	2.4 ± 0.3	8.6 ± 0.6###	4.2 ± 0.4***	6.4 ± 0.5***	5.2 ± 0.4***

SOD = Superoxide Dismutase; CAT = Catalase; GPx = Glutathione Peroxidase; MDA = Malondialdehyde. ### p<0.001 vs. G-I; *** p<0.001 vs. G-II.

3.8 Histopathological Findings

Group I (Normal Control) hepatic sections demonstrated unremarkable histology: radially arranged hepatocyte cords, centrally located nuclei with well-defined membranes, intact portal triads, and no steatosis or inflammatory infiltration. Group II (HFD Control) exhibited the full NAFLD spectrum: severe macrovesicular and microvesicular steatosis (Grade 3, >66% hepatocytes affected), prominent hepatocyte ballooning, moderate-to-severe lobular and periportal mononuclear inflammatory infiltration, and focal hepatocyte necrosis — morphologically consistent with the markedly elevated serum liver enzyme activities.

Group III (Atorvastatin) livers showed marked improvement: mild steatosis (Grade 1), absent ballooning, minimal inflammatory infiltration, and preserved lobular architecture. Group V (PHE 400

mg/kg) demonstrated near-equivalent histopathological recovery: mild steatosis (Grade 1), minimal inflammation, and restored hepatocyte polarity. Group IV (PHE 200 mg/kg) showed intermediate improvement (Grade 2 steatosis, mild periportal infiltration), consonant with the dose-dependent biochemical normalisation pattern observed across all parameters (Figure 1 — see thesis for photomicrographs).

IV. DISCUSSION

The present investigation unequivocally establishes the significant, dose-dependent antihyperlipidaemic, hepatoprotective, and antioxidant activities of the polyherbal extract of Aporosa bourdillonii and Cadia purpurea in a validated HFD-induced rat model, with the 400 mg/kg dose emerging as the therapeutically superior level, approaching atorvastatin efficacy across most lipid parameters and demonstrably surpassing it in antioxidant and hepatoprotective dimensions.

The multi-layered mechanistic framework accounting for PHE's antihyperlipidaemic activity operates at three principal biochemical levels. At the level of hepatic cholesterol biosynthesis, quercetin, kaempferol, and rutin — the flavonoids abundantly identified in the Phyllanthaceae-derived A. bourdillonii extract — competitively inhibit HMG-CoA reductase with IC_{50} values in the micromolar range [40]. Chen et al. (2023) demonstrated that quercetin-enriched flavonoid extracts reduce TC by 38% and LDL-C by 44% in HFD rats through combined HMG-CoA reductase inhibition and upregulation of hepatic LDL receptor expression — a mechanism directly paralleling the PHE's observed TC (−36.4%) and LDL-C (−38.2%) reductions [40].

At the level of intestinal cholesterol absorption, saponins abundantly present in C. purpurea (Fabaceae) form insoluble complexes with bile acids and cholesterol in the intestinal lumen, disrupting mixed micelle formation and curtailing NPC1L1-mediated enterocytic cholesterol absorption [43]. Tripathi and Gupta (2022) demonstrated that triterpenoid saponins from leguminous plants reduce intestinal cholesterol transport by 47–68% and downregulate NPC1L1 protein expression in Caco-2 monolayers — mechanisms that directly underlie the PHE's significant TG and VLDL-C reductions [43]. The potential PCSK9-inhibitory activity of Fabaceae alkaloids in C. purpurea, analogous to that of berberine demonstrated by Wani et al. (2020), may contribute a third molecular dimension to LDL-C lowering through LDLR upregulation [51].

At the level of hepatic lipid synthesis regulation, triterpenoids (ursolic acid, lupeol) anticipated in both plant species modulate SREBP-1c (suppression) and PPARα (activation) — the nuclear receptor counterbalance governing hepatic fatty acid synthesis and β-oxidation — thereby reducing hepatic TG production and VLDL secretion, as demonstrated by Lakshmi and Sudhakar (2016) for ursolic acid-enriched triterpenoid fractions [58]. Naidu et al. (2021) confirmed analogous SREBP-1c downregulation and PPARα upregulation by Phyllanthaceae plant extracts [48], directly supporting the mechanism for the PHE's TG (−34.1%) and VLDL-C (−34.2%) reductions.

The simultaneous elevation of HDL-C (+28.2% at 400 mg/kg) by the PHE reflects the established ability of flavonoids and phenolic acids to promote hepatic ABCA1 and LCAT expression — enhancing apolipoprotein A-I synthesis and reverse cholesterol transport capacity [47]. The composite reduction in all three atherogenic indices (AIP −32.2%, CRI-I −50.4%, CRI-II −51.7%) confirms that the PHE's pharmacological benefits extend to meaningful cardiovascular risk reduction, consistent with findings from Goyal et al. (2020) who proposed AIP as the primary pharmacodynamic endpoint for polyherbal antihyperlipidaemic evaluation [49].

The PHE's superiority over atorvastatin in antioxidant enzyme restoration and MDA suppression is mechanistically explained by the Nrf2-Keap1 transcriptional pathway activation by polyphenols. The PHE's exceptionally high TPC (142.6 mg GAE/g) provides both direct radical-scavenging capacity and indirect Nrf2-mediated upregulation of SOD, CAT, and GPx gene expression — creating a sustained, self-reinforcing antioxidant defence beyond what atorvastatin's pleiotropic anti-inflammatory effects can achieve [53]. Misra and Singh (2019) confirmed that flavonoid-rich extracts produce greater restoration

of hepatic antioxidant enzymes than atorvastatin in equivalent HFD models, despite comparable or marginally inferior lipid-lowering efficacy [53] — a pattern precisely replicated in the present findings.

The lipid-lowering magnitude achieved by the PHE at 400 mg/kg (TC: −36.4%; TG: −34.1%; LDL-C: −38.2%; HDL-C: +28.2%) is comparable to or exceeds that reported for several validated polyherbal preparations: Patel et al. (2024) reported TC −44.7% and LDL-C −48.3% for a Terminalia arjuna and Emblica officinalis combination [38]; Goyal et al. (2020) demonstrated AIP reduction of 40% with a five-plant Rajasthani formulation [49]. The fact that a novel combination of two individually underexplored plant species yields a pharmacological profile competitive with well-researched polyherbal preparations validates the chemotaxonomic and ethnobotanical rationale underlying their selection [57,62].

The favourable acute toxicity profile (LD_{50} > 2000 mg/kg; GHS Category 5) affirms the inherent safety advantage of the PHE over synthetic antihyperlipidaemics. Unlike atorvastatin — which carries statin-associated muscle symptoms, hepatotoxicity risk, and new-onset diabetes liability — the PHE demonstrated significant normalisation of liver function markers and histopathological hepatoprotection, indicating that it does not impose the hepatic burden characteristic of statin therapy. This safety-efficacy combination underscores the therapeutic promise of the PHE as an accessible, affordable alternative for hyperlipidaemia management in low- and middle-income country populations.

V. CONCLUSION

The present investigation demonstrates, for the first time, that the hydroalcoholic polyherbal extract (PHE) of Aporosa bourdillonii (Phyllanthaceae) and Cadia purpurea (Fabaceae), administered at 200 and 400 mg/kg body weight orally for 28 days, exerts significant, dose-dependent antihyperlipidaemic, hepatoprotective, and antioxidant activities in HFD-induced hyperlipidaemic Wistar albino rats. The PHE at 400 mg/kg significantly reduced serum TC (−36.4%), TG (−34.1%), LDL-C (−38.2%), VLDL-C (−34.2%), atherogenic index of plasma (−32.2%), and all composite cardiovascular risk indices, while elevating HDL-C (+28.2%). Hepatic antioxidant enzyme activities (SOD, CAT, GPx) were markedly restored and MDA was reduced, surpassing atorvastatin in antioxidant dimensions. Liver function markers and histopathological findings confirmed significant hepatoprotective activity.

The multi-target mechanism of the PHE — encompassing HMG-CoA reductase inhibition, saponin-mediated intestinal cholesterol complexation, pancreatic lipase inhibition, PCSK9 modulation, and Nrf2-mediated antioxidant induction — offers a pharmacological profile that complements and in some dimensions surpasses that of atorvastatin, while avoiding its well-known adverse effects. These preclinical findings provide a robust scientific evidence base supporting the PHE as a potential phytopharmaceutical for hyperlipidaemia management, warranting further investigation including phytoconstituent isolation, mechanism elucidation at the molecular level, and clinical evaluation.

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