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Original Article

Evaluation of Antihyperlipidemic Activity of Leaf Extracts of *Rivea hypocrateriformis* (Desr.) in High-Fat Diet-Induced Hyperlipidemia Rat Model with Focus on Gene Expression

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Abstract

Background: Hyperlipidemia, characterized by a persistent elevation of lipid levels in the bloodstream, serves as a major indicator of cardiovascular risk. Although statin drugs are effective in managing this condition, their use is often accompanied with adverse side effects and drug reactions. **Aim and Objective:** In light of these limitations, the exploration of herbal and natural remedies for the treatment of hyperlipidemia has gained momentum. This study investigates the antihyperlipidemic potential of *Rivea hypocrateriformis* (Desr.) in experimental rats subjected to a high-fat diet (HFD)-induced hyperlipidemia model. **Methodology:** The study examines the potency of hypocrateriformis chloroform extract (HCE) and hypocrateriformis ethanol extract (HEE) of *R. hypocrateriformis* on the HFD-associated hyperlipidemia in two doses, 100 and 200 mg/kg b.w. The effect of extracts on the changes in body weight, lipid indices and levels, and antioxidant enzymes was measured using standard procedures. **Results:** HFD-induced hyperlipidemia groups exhibit significant enhancement in all measured parameters among the drug-treated groups. Higher dose normalized the values significantly compared to the standard drug, atorvastatin (20 mg/kg). The results of the antioxidant enzymes showed a significant lowering of oxidative-free radicals and improving antioxidant defense in extract-treated groups. In comparison, HEE showed a significant activity compared to HCE at both doses. Histopathology analysis showed decrease in the size of adipocytes in adipose tissue. This suggests that HEE and HCE may have an inhibitory effect on adipogenesis and could potentially reduce the accumulation of fat in adipose tissue. **Conclusion:** The current study demonstrated that extracts of *R. hypocrateriformis* show antihyperlipidemic activity along with known antioxidant and inflammatory mechanisms, opening the scope for future research in developing lead molecules for treating the diseases effectively. Thus, the plant presents itself as a promising candidate which demands future research to improve cardiovascular health and overall life style of the patients.

Keywords: antihyperlipidemic, antioxidant enzymes, cardiovascular health, high-fat diet

Introduction

Hyperlipidemia is a chronic genetic or acquired condition characterized by chronic elevation in the lipid levels in the blood. The prevalence of hyperlipidemia is common in patients with coronary artery disease (CAD) and other diseases.[1] The results of some studies show that over 50% of adults have higher low-density lipoprotein (LDL) levels and out of them only 35% can manage the elevated levels which indicate that the disease is untreated. In countries

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with low obesity rates and consumption of less fatty food, the prevalence of elevated LDL and very low-density lipoproteins (VLDL), which is indicative of hyperlipidemia and CAD, is relatively lower compared to developed countries like America and Europe.[2] Thus, in patients with hyperlipidemia, it is a polygenic phenomenon that is influenced and triggered by the intake of saturated fatty acids and dietary cholesterol, leading to obesity.[3] In order to lower the risk of Cardiovascular Disease (CVD) and hyperlipidemia, treatments targeting the elevated LDL were proven to be effective, but there is no clinical evidence of this treatment benefiting the hypertriglyceridemia and lowering high-density lipoproteins (HDL).[4]

Rivea hypocrateriformis (Desr.), (Convolvulaceae), commonly known as elephant creeper plant, is a native plant of Southeast Asian countries like India and Australia. The plant is traditionally used to treat gastro intestinal and respiratory disorders.[5] Reports also show the use of *R. hypocrateriformis* plant for fever, snake bites, piles, skin disorders, rheumatic pain, and urinogenital disorders. *R. hypocrateriformis* was proven to contain glycosides, alkaloids, xanthanes, stilbenes, and flavonoids. Scientific studies have demonstrated that *R. hypocrateriformis* possesses diverse pharmacological properties, including antioxidant, anti-inflammatory, antibacterial, anticancer, immunomodulatory, and anti-urolithiatic activities. The rationale for the current research arises from the limitations associated with existing treatments for hyperlipidemia.

Statin drugs and other antihyperlipidemic agents are effective in lowering LDL levels, but they are frequently associated with significant side effects and adverse reactions, such as muscle pain, liver damage, and increased risk of diabetes.[5,6] These limitations necessitate the search for safer, more comprehensive alternatives to manage hyperlipidemia effectively. The plant *R. hypocrateriformis*, known for its diverse pharmacological activities, has not been extensively studied for its lipid-lowering effects in scientific research. This study aims to validate traditional claims and evaluate the plant's effectiveness in mitigating high-fat diet (HFD)-induced hyperlipidemia in experimental rats, thereby bridging the gap between traditional knowledge and contemporary pharmacological practices.

Materials and Methods

Preparation from extract

The leaves of *R. hypocrateriformis* was collected from Tirunelveli District, Tamil Nadu, India and identified by Dr. V. Chelladurai, Research Officer, at Central Council for Research in Ayurveda and Siddha, Palayamkottai (No: XCH40380). These leaves were shade dried for 7 days until they became colorless. A Soxhlet apparatus was used to extract 100 g of the powdered leaves with chloroform and ethanol. The resulting solutions were filtered and concentrated to form thick pastes, designated as hypocrateriformis chloroform extract (HCE) and hypocrateriformis ethanol extract (HEE). The extraction process yielded 15% for HCE and 23.46% for HEE. To prevent contamination, the extracts were stored in airtight containers at room temperature.[7,8]

Experimental animals

Sprague Dawley (SD) rats weighing 180–220 g were collected in Bengaluru from a reputable breeder. Their cages are stainless steel with food (Nutrivet Pvt. Ltd) and water ad labitum available free of charge. Cages were rotated between light and dark for 12 hours at 23 °C with 55% relative humidity. Institutional Animal Ethics Committee (IAEC) approval number KLRCP/IAEC/009/2021-22 was obtained for all the experiments on animals. During the experiment, a cage card system was employed to code the animals, which were fasted for 12 hours prior to the procedure, without access to food or water.[9,10]

Acute toxicity studies

As part of the Organisation for Economic Co-operation and Development (OECD) 423 (Acute Toxic Class Method), HCE and HEE extracts were evaluated for acute toxicity. The extracts were administered orally as a solution using 0.5% carboxy methyl cellulose solution to the overnight fasted rats to achieve doses of 2000 mg/kg. Following extract administration, the animal's body weight was assessed, and they were then given food and water. The rats were meticulously monitored throughout the study for signs of toxicity, with assessments covering their respiratory, circulatory, and nervous systems, as well as all physical attributes, including the skin, hair, eyes, and mucous membranes. Following the administration of the extracts,[11,12] each behavior pattern was observed at specific intervals as follows: 3, 6, 9, and 14 days. These were noted in addition to the usual signs of toxicity, including comas, diarrhea, trembling, and convulsions.

Antihyperlipidemia Activity of HCE and HEE

Extract preparation

As a drug administration medium, a 5% gum acacia suspension (GAS) was prepared by dissolving gum acacia in warm distilled water, followed by filtration. The extracts of HCE and HEE were separately dissolved in the gum acacia to prepare a 30% suspension. Additionally, a 2% solution of atorvastatin was formulated in the same acacia suspension. All solutions were filtered to ensure clarity and uniformity before being stored for later use.[13]

Preparation of HFD

The HFD was prepared using 35% beef tallow, 20% casein, 15% corn starch, 20% sucrose, 5% corn oil, and 5% vitamin and mineral mix. These ingredients were mixed and homogenized in warm water to form a dough, which was made into pellets and dried in a hot air oven. Fresh batches of

feed were prepared weekly to ensure quality and consistency.[14,15]

Administration of drugs in HFD-induced obesity method

Animals were divided randomly in groups with six rats in each cage as follows:

Group 1: Normal control group: GAS-1.5 mL/kg

Group 2: HFD group: HFD in GAS

Group 3: HFD + HCE (100 mg/kg)

Group 4: HFD + HEE (100 mg/kg)

Group 5: HFD + HCE (200 mg/kg)

Group 6: HFD + HEE (200 mg/kg)

Group 7: Standard group: HFD + atorvastatin (20 mg/kg).

The experiments were conducted for 28 days, and the initial body weights of the animals were noted. After that, the drugs were administered using a feeding syringe via the oral route.[16,17] The body weights were noted at regular intervals of 7, 14, 21, and 28 days.

Estimation of lipid indices and parameters

Blood was drawn from the retro-orbital plexus under anesthesia to estimate the final lipid parameters, followed by serum separation. To measure fat indices, including body mass index (BMI) and the Lee index, the naso-anal length of each rat was recorded.[18,19]

$$\text{Lee index} = \frac{\sqrt[3]{\text{Body weight (g)}}}{\text{Nose-to-anus length (cm)}} \text{X } 1000$$

$$\text{BMI} = \frac{\text{Body weight}}{\text{Length}^2}$$

A total cholesterol (TC) level, triglycerides (TG), LDL, VLDL, and high-density lipoprotein level were determined in serum samples isolated from experimental animals. Standard laboratory procedures were used to determine biochemical parameters using commercially available diagnostic kits. Lipid data were also utilized to calculate biochemical parameters, including the atherogenicity index, using standard formulas.[20]

Using the separated serum, biochemical measurements were carried out on enzymes such as superoxide dismutase (SOD), catalase (CAT), lipid peroxidase, glutathione reductase, and glutathione peroxidases (GPx). Biochemical parameters were estimated using commercially available kits, following standard procedures as outlined in the referenced protocols.[21-23]

RTPCR analysis

The liver homogenate was centrifuged and subjected to Reverse Transcriptase Polymerase Chain Reaction (RTPCR) analysis to estimate the expression of actin, adenosine monophosphate-activated protein kinase (AMPK), 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA), fetal alcohol syndrome (FAS), and LDL-receptor (LDL-R) using agarose gel electrophoresis.[24,25]

Histopathological studies

For histological analysis, liver tissues from experimental rats were removed and stored in 10% formalin solution. An established method for histology involved cleaning, segmenting, coloring using haematoxylin and eosin (H and E), and reviewing samples under the microscope for any morphological alterations.

Statistical analysis

We analyzed the data, and the coefficient of variance was determined using one-way analysis of variance followed by Dunnett multiple comparison test in Graph Pad Prism version 5.0. The results are presented as means and standard errors of the means (mean ± SEM). Statistical significance was considered at $P < 0.001$.

Results

Acute toxicity studies

The acute oral toxicity of leaf extracts (HCE and HEE) was investigated based on OECD 423 guidelines for various doses available 100–2000 mg/kg body weight. There has been no change in any of those behaviors or features in either extract until the 14th day of the study. None of the above-stated features were altered by either extract. The rats administered with HCE snoozed excessively compared to those given HEE, which was not significant, but still noteworthy. A normal motor activity was also indicated by the symptoms. Accordingly, test animals showed no toxicity for either extract when administered at 2000 mg/kg to any of them. It was found that the rats neither showed any signs of disease or abnormal behavior, nor did they show any mortality. As a result, we calculated that the extracts have an effective dose of 1/20th the minimum dose and 1/10th the biggest dose.

Effect of extracts of *R. hypocrateriformis* leaves on body weights of rats in HFD-induced hyperlipidemia

In the investigation of the antihyperlipidemic activity of extracts from *R. hypocrateriformis* leaves, the impact body size of rats with HFD-induced hyperlipidemia was assessed over a period of 28 days. The standard group exhibited a gradual increase in body weight, while the HFD group displayed a notable weight gain, emphasizing the effects of the diet. The groups treated with HCE and HEE extracts at both 100 and 200 mg/kg demonstrated a dose-dependent reduction in body weight relative to the HFD group [Table 1]. Particularly noteworthy were the significant reductions observed on the 21st and 28th days in both extract groups at 200 mg/kg ($P < 0.001$). The standard treatment group, serving as a reference, also exhibited

Table 1: Effect of extracts on the body weight of rats in HFD-induced hyperlipidemia

Group	Body weight (g)				
	Initial	7th day	14th day	21st day	28th day
Control	211.54±1.76	215.66±3.55	224.16±1.6	232.5±3.83	244.66±2.87
HFD	210.66±1.5	224.1±1.67	244.2±2.19	273.5±4.84	337.66±7.73
HEC 100 mg/kg	211.12±3.01	222.41±3.52	232.59±2.1	242.61±3.62*	256.25±8.9*
HEC 200 mg/kg	214.65±1.62	214.49±1.85	227.12±2.61	240.8±3.3*	249.81±5.1**
HEE 100 mg/kg	211.16±3.06	222.5±3.72	232.66±2.5	242.66±3.72*	256.33±7.52*
HEE 200 mg/kg	214.66±1.63	214.5±1.87	227.16±2.63	240.83±3.31*	249.83±5.19**
Standard	211.66±1.96	218.2±4.77	232.66±3.66	241.66±6.83*	252.33±2.42**

The values are expressed mean ± SEM ($n = 6$); *$P < 0.05$; **$P < 0.001$ significant compared to HFD-induced group.

significant decreases in body weight on the 21st and 28th days compared to the HFD group ($P < 0.001$). These findings suggest a pronounced antihyperlipidemic effect of the extracts, especially at the higher dose, as evidenced by the substantial attenuation of body weight gain in the rat model of hyperlipidemia.

Effect of extracts on obesity indicators

The study assessed the effect of HCE and HEE on obesity indices, specifically the Lee index and BMI, in rats subjected to an HFD. The control group [Table 2] showed a Lee index of 43.35 ± 1.72 and a BMI of 1.17 ± 0.09. In contrast, the HFD group demonstrated a significant increase in both indices, with a Lee index of 49.78 ± 3.87 and a BMI of 1.73 ± 0.28, indicating the development of obesity. Treatment with HCE at a dose of 100 mg/kg resulted in a Lee index of 47.25 ± 2.35 and a BMI of 1.61 ± 0.34, indicating a moderate reduction in obesity markers compared to the HFD group. However, the higher dose of HCE (200 mg/kg) showed more pronounced effects, reducing the Lee index to 43.54 ± 1.63 and significantly lowering the BMI to 1.2 ± 0.09 (*$P < 0.05$), suggesting a protective effect against obesity. Similarly, treatment with HEE at 100 mg/kg lowered the Lee index to 46.05 ± 2.15 and the BMI to 1.48 ± 0.45. The higher dose of HEE (200 mg/kg) further reduced these indices, with a Lee index of 44.76 ± 2.48 and a significantly lower BMI of 1.26 ± 0.14 (*$P < 0.05$), indicating a substantial protective effect against the HFD-induced obesity. The standard treatment group also showed reduced obesity indices, with a Lee index of 45.21 ± 1.94 and a BMI of 1.29 ± 0.12, both of which were significantly reduced compared to the HFD group (*$P < 0.05$). Both HCE and HEE, particularly at the higher doses, effectively mitigated the increase in obesity indices caused by HFD, demonstrating their potential as protective agents against diet-induced obesity.

Effect of extracts on regulating the lipid levels in HFD-induced hyperlipidemia

The study examined the effect of HCE and HEE on lipid levels in rats with HFD-induced hyperlipidemia. The control group exhibited normal lipid levels, with TC at 105.58 ± 2.64 mg/dL, TG at 81.54 ± 2.39 mg/dL, HDL at 43.38 ± 0.88 mg/dL, and TP at 8.48 ± 0.32 mg/dL [Table 3]. However, the HFD group showed a significant increase in TC (182.63 ± 4.5 mg/dL) and TG (172.39 ± 3.31 mg/dL), along with a decrease in HDL (24.93 ± 2.66 mg/dL) and TP (2.81 ± 0.19 mg/dL), indicating the development of hyperlipidemia. Treatment with HCE at 100 mg/kg resulted in a reduction of TC (155.32 ± 5.26 mg/dL) and TG (126.85 ± 4.25 mg/dL), with a moderate increase in HDL (29.36 ± 2.16 mg/dL) and TP (3.98 ± 0.37 mg/dL). The higher dose of HCE (200 mg/kg) showed a more significant improvement, with TC reduced to 130.5 ± 2.73 mg/dL, TG to 92.59 ± 2.19 mg/dL, HDL increased to 37.56 ± 1.7 mg/dL, and TP elevated to 6.33 ± 0.68 mg/dL, all showing statistically significant changes compared to the HFD group (**$P < 0.001$). Similarly, the HEE 100 mg/kg treatment group showed significant improvements in lipid levels, with TC at 142.39 ± 4.22 mg/dL, TG at 114.57 ± 3.16 mg/dL, HDL at 31.08 ± 1.77 mg/dL, and TP at 5.34 ± 0.46 mg/dL (*$P < 0.05$, **$P < 0.001$). The higher dose of HEE (200 mg/kg) further reduced TC to 115.23 ± 1.51 mg/dL and TG to 85.59 ± 1.48 mg/dL, while HDL increased to 43.88 ± 1.61 mg/dL and TP reached 7.94 ± 0.97 mg/dL, showing significant improvements compared to the HFD group (**$P < 0.001$). The standard treatment group also demonstrated significant improvements, with TC at 117.67 ± 1.99 mg/dL, TG at 87.29 ± 1.16 mg/dL, HDL at 43.55 ± 1.36 mg/dL, and TP at 6.4 ± 0.24 mg/dL, all significantly better than the HFD group (**$P < 0.001$). Both HCE and HEE extracts, particularly at the higher

Table 2: Effect of extracts on the obesity indices

Group	Lee index	BMI
Control	43.35 ± 1.72	1.17 ± 0.09
HFD	49.78 ± 3.87	1.73 ± 0.28
HEC 100 mg/kg	47.25 ± 2.35	1.61 ± 0.34
HEC 200 mg/kg	43.54 ± 1.63	1.2 ± 0.09*
HEE 100 mg/kg	46.05 ± 2.15	1.48 ± 0.45
HEE 200 mg/kg	44.76 ± 2.48	1.26 ± 0.14*
Standard	45.21 ± 1.94	1.29 ± 0.12*

The values are expressed mean ± SEM ($n = 6$). *$P < 0.05$; significant compared to HFD-induced group.

Table 3: Effect of extracts on the lipid levels in HFD-induced hyperlipidemia

Group	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	TP (mg/dL)
Control	105.58 ± 2.64	81.54 ± 2.39	43.38 ± 0.88	8.48 ± 0.32
HFD	182.63 ± 4.5	172.39 ± 3.31	24.93 ± 2.66	2.81 ± 0.19
HEC 100 mg/kg	155.32 ± 5.26	126.85 ± 4.25	29.36 ± 2.16	3.98 ± 0.37
HEC 200 mg/kg	130.5 ± 2.73**	92.59 ± 2.19**	37.56 ± 1.7**	6.33 ± 0.68**
HEE 100 mg/kg	142.39 ± 4.22*	114.57 ± 3.16*	31.08 ± 1.77*	5.34 ± 0.46**
HEE 200 mg/kg	115.23 ± 1.51**	85.59 ± 1.48**	43.88 ± 1.61**	7.94 ± 0.97**
Standard	117.67 ± 1.99**	87.29 ± 1.16**	43.55 ± 1.36**	6.4 ± 0.24**

The values are expressed mean ± SEM ($n = 6$); *$P < 0.05$; **$P < 0.001$ significant compared to HFD-induced group.

Table 4: Effect of extracts on the lipid parameters in HFD-induced hyperlipidemia

Group	LDL (mg/dL)	VLDL (mg/dL)	Atherogenicity index
Control	73.56 ± 0.89	32.89 ± 1.32	2.43 ± 0.08
HFD	148.01 ± 4.18	75.38 ± 3.25	7.39 ± 0.78
HEC 100 mg/kg	105.25 ± 2.16*	61.05 ± 3.62*	3.66 ± 0.54*
HEC 200 mg/kg	84.18 ± 1.05**	45.28 ± 2.37**	3.47 ± 0.11**
HEE 100 mg/kg	93.57 ± 3.12*	55.28 ± 5.24*	3.59 ± 0.18*
HEE 200 mg/kg	73.73 ± 2.07**	31.1 ± 1.85**	2.62 ± 0.08**
Standard	74.52 ± 1.32**	32.27 ± 1.14**	2.7 ± 0.11**

The values are expressed mean ± SEM ($n = 6$); *$P < 0.05$; **$P < 0.001$ significant compared to HFD-induced group.

doses, significantly ameliorated the lipid profile abnormalities induced by the HFD, demonstrating their potential in managing hyperlipidemia.

Effect of extracts on the lipid parameters in HFD-induced hyperlipidemia

The study evaluated the impact of HCE and HEE on lipid parameters, including LDL, VLDL, and the Atherogenicity index, in rats with HFD-induced hyperlipidemia. The control group exhibited normal lipid parameters, with LDL at 73.56 ± 0.89 mg/dL, VLDL at 32.89 ± 1.32 mg/dL, and an Atherogenicity index of 2.43 ± 0.08 [Table 4]. In contrast, the HFD group showed significantly elevated lipid levels, with LDL increasing to 148.01 ± 4.18 mg/dL, VLDL to 75.38 ± 3.25 mg/dL, and the Atherogenicity index rising to 7.39 ± 0.78, indicating a heightened risk of cardiovascular disease. Treatment with HCE at 100 mg/kg significantly reduced LDL to 105.25 ± 2.16 mg/dL, VLDL to 61.05 ± 3.62 mg/dL, and the Atherogenicity index to 3.66 ± 0.54 (*$P < 0.05$). The higher dose of HCE (200 mg/kg) demonstrated even more significant improvements, with LDL reduced to 84.18 ± 1.05 mg/dL, VLDL to 45.28 ± 2.37 mg/dL, and the Atherogenicity index to 3.47 ± 0.11 (**$P < 0.001$). Similarly, treatment with HEE at 100 mg/kg resulted in reductions in LDL (93.57 ± 3.12 mg/dL), VLDL (55.28 ± 5.24 mg/dL), and the Atherogenicity index (3.59 ± 0.18), with statistically significant improvements compared to the HFD group (*$P < 0.05$). The higher dose of HEE (200 mg/kg) further improved these parameters, reducing LDL to 73.73 ± 2.07 mg/dL, VLDL to 31.1 ± 1.85 mg/dL, and the Atherogenicity index to 2.62 ± 0.08 (**$P < 0.001$). The standard treatment group also showed substantial reductions in lipid levels, with LDL at 74.52 ± 1.32 mg/dL, VLDL at 32.27 ± 1.14 mg/dL, and the Atherogenicity index at 2.7 ± 0.11, all significantly improved compared to the HFD group (**$P < 0.001$). Both HCE and HEE, particularly at the higher doses, significantly improved lipid parameters and reduced the Atherogenicity index, highlighting their potential as protective agents against

Table 5: Antioxidant activity of extracts in HFD-induced hyperlipidemia

Group	SOD (U/mg)	CAT (μM H_2O_2/mg)	GPx (μg/mg)	GSH (μg/mg)	MDA (nM/mg)
Control	9.2 ± 0.28	94.56 ± 0.8	9.8 ± 0.42	56.2 ± 2.69	6.4 ± 0.71
HFD	4.61 ± 0.18	42.57 ± 1.61	5.42 ± 0.39	21.68 ± 0.91	12.49 ± 0.27
HEC 100 mg/kg	5.69 ± 0.37	49.22 ± 3.27	6.88 ± 0.55	28.54 ± 2.64	8.67 ± 0.41
HEC 200 mg/kg	7.4 ± 0.36**	52.33 ± 2.08	7.59 ± 0.41*	39.47 ± 2.12**	6.87 ± 0.3**
HEE 100 mg/kg	6.95 ± 0.18*	61.28 ± 4.16*	7.16 ± 0.71*	41.07 ± 3.17***	6.22 ± 0.27**
HEE 200 mg/kg	8.6 ± 0.54**	82.37 ± 2.91**	8.54 ± 0.55**	53.55 ± 1.66**	5.17 ± 0.32**
Standard	6.82 ± 0.34**	87.05 ± 1.6**	9.83 ± 0.35**	54.56 ± 2.26**	4.43 ± 0.65**

The values are expressed mean ± SEM ($n = 6$); *$P < 0.05$; **$P < 0.001$ significant compared to HFD-induced group

Table 6: Percentage of expression of various genes due to treatment with extracts

Group	β-Actin	AMPK-α	FAS	HMG-CoA	LDL-R
Control	99.38 ± 2.43	31.26 ± 2.65	34.16 ± 1.55	31.28 ± 2.07	36.52 ± 2.44
HFD	98.45 ± 3.21	30.57 ± 2.85	64.57 ± 3.41	63.27 ± 2.17	29.67 ± 1.98
HEC 200mg/kg	99.49 ± 2.96	34.07 ± 1.96	47.44 ± 2.47*	41.08 ± 2.75*	35.33 ± 1.76
HEE 200mg/kg	98.56 ± 2.33	39.75 ± 1.57*	38.68 ± 2.47**	34.77 ± 2.19**	46.28 ± 2.07*
Standard	99.07 ± 3.08	35.22 ± 2.14**	45.68 ± 2.58**	33.78 ± 2.55**	42.11 ± 2.02*

The values were expressed as mean ± SEM, $n = 3$ $^{**}P < 0.001$; $^{*}P < 0.05$ significant compared to HFD group.

HFD-induced hyperlipidemia and associated cardiovascular risks.

Effect of extracts on the antioxidant profile of the HFD-induced hyperlipidemia in rats

The antioxidant activity of HCE and HEE was evaluated in rats with HFD-induced hyperlipidemia by measuring key antioxidant markers such as SOD, CAT, GPx, reduced glutathione (GSH), and malondialdehyde (MDA). The control group exhibited normal antioxidant levels with SOD at 9.2 ± 0.28 U/mg, CAT at 94.56 ± 0.8 μM H_2O_2/mg, GPx at 9.8 ± 0.42 μg/mg, GSH at 56.2 ± 2.69 μg/mg, and MDA at 6.4 ± 0.71 nM/mg [Table 5]. In contrast, the HFD group showed a significant decline in antioxidant activity with SOD reduced to 4.61 ± 0.18 U/mg, CAT to 42.57 ± 1.61 μM H2O2/mg, GPx to 5.42 ± 0.39 μg/mg, and GSH to 21.68 ± 0.91 μg/mg, while MDA levels increased to 12.49 ± 0.27 nM/mg, indicating oxidative stress.

Treatment with HCE at 100 mg/kg showed a moderate improvement in antioxidant levels, with SOD increasing to 5.69 ± 0.37 U/mg, CAT to 49.22 ± 3.27 μM H2O2/mg, GPx to 6.88 ± 0.55 μg/mg, GSH to 28.54 ± 2.64 μg/mg, and MDA decreasing to 8.67 ± 0.41 nM/mg. The higher dose of HCE (200 mg/kg) further enhanced antioxidant activity, with significant improvements in SOD (7.4 ± 0.36 U/mg, $^{**}P < 0.001$), GPx (7.59 ± 0.41 μg/mg, $^{*}P < 0.05$), GSH (39.47 ± 2.12 μg/mg, $^{**}P < 0.001$), and MDA (6.87 ± 0.3 nM/mg, $^{**}P < 0.001$) compared to the HFD group. The HEE treatment groups demonstrated more pronounced antioxidant effects. The 100 mg/kg dose resulted in significant increases in SOD (6.95 ± 0.18 U/mg, $^{*}P < 0.05$), CAT (61.28 ± 4.16 μM H2O2/mg, $^{*}P < 0.05$), GPx (7.16 ± 0.71 μg/mg, $^{*}P < 0.05$), GSH (41.07 ± 3.17 μg/mg, $^{***}P < 0.001$), and a reduction in MDA (6.22 ± 0.27 nM/mg, $^{**}P < 0.001$). The higher dose of HEE (200 mg/kg) further elevated antioxidant levels, with SOD at 8.6 ± 0.54 U/mg, CAT at 82.37 ± 2.91 μM H2O2/mg, GPx at 8.54 ± 0.55 μg/mg, GSH at 53.55 ± 1.66 μg/mg, and MDA significantly reduced to 5.17 ± 0.32 nM/mg, all showing $^{**}P < 0.001$ significance compared to the HFD group. The standard treatment group also showed significant improvements in antioxidant activity, with SOD at 6.82 ± 0.34 U/mg, CAT at 87.05 ± 1.6 μM H2O2/mg, GPx at 9.83 ± 0.35 μg/mg, GSH at 54.56 ± 2.26 μg/mg, and MDA reduced to 4.43 ± 0.65 nM/mg, all $^{**}P < 0.001$ compared to the HFD group. In summary, both HCE and HEE, particularly at higher doses, significantly improved antioxidant enzyme levels and reduced oxidative stress markers in rats with HFD-induced hyperlipidemia, demonstrating their potential as effective antioxidant agents.

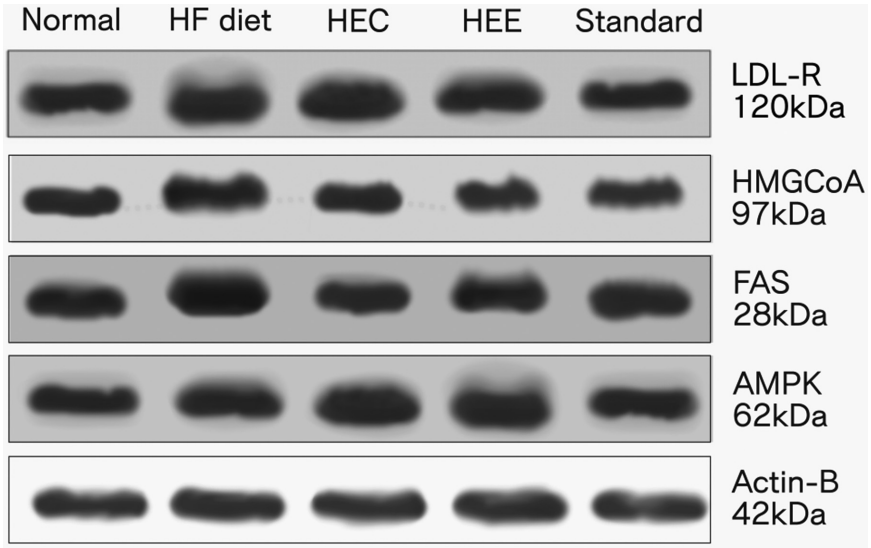

Figure 1: Expression of various genes in the presence of extracts of *Rivea hypocrateriformis* (Desr.) leaves.

Effect of extract on the adipogenic gene expression

Table 6 presents the percentage expression of various genes in response to treatment with *R. hypocrateriformis* leaf extracts in a HFD-induced hyperlipidemia rat model. The expression levels of key genes related to lipid metabolism were assessed, and the results demonstrated [Figure 1] significant modulations in gene expression compared to the HFD group. Particularly noteworthy are the effects of the HCE and HEE extracts, both showing a marked impact on the expression of AMP-activated protein kinase alpha (AMPK-α), LDL-R, FAS, and HMG-CoA reductase. At 200 mg/kg, HCE significantly down regulates FAS (47.44 ± 2.47) and HMG-CoA (41.08 ± 2.75), while HEE shows significant up-regulation of AMPK-α (39.75±1.57), LDL-R (46.28 ± 2.07) and down-regulation of FAS (35.68±2.47), and HMG-CoA (34.77±2.19). The standard treatment group also exhibits significant modulations in AMPK-α, LDL-R, FAS, and HMG-CoA gene expressions, emphasizing the potential of the extracts and the standard treatment in regulating key genes involved in lipid metabolism.

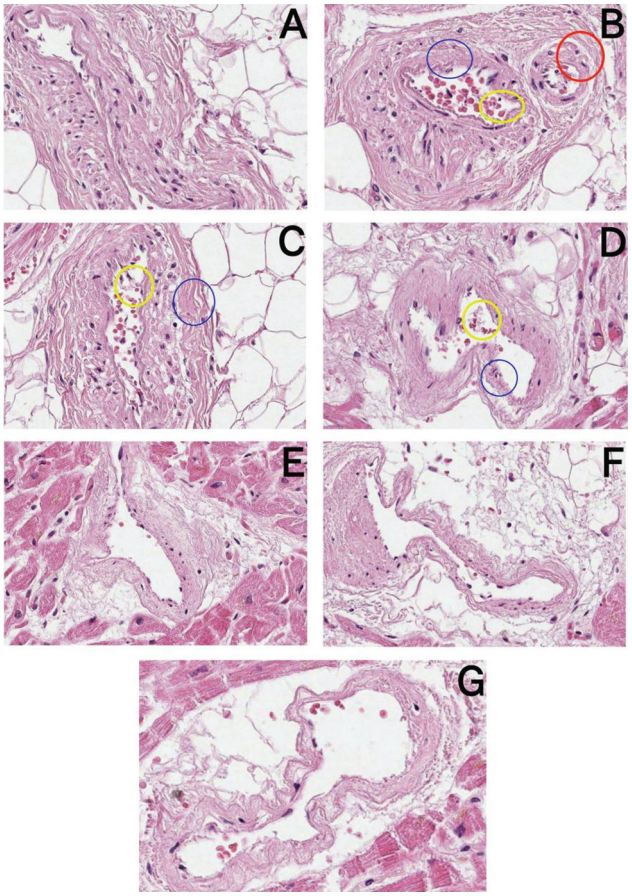

Figure 2: Histopathology of arteries of HFD-induced hyperlipidemia. A. Normal group; B. HFD-induced group; C. HEC 100 mg/kg; D. HEC 200 mg/kg; E. HEE 100 mg/kg; F. HEE 200 mg/kg; G. Standard group (Endothelial thickening marked in blue circle; lipid accumulation marked in red circle; atheromatous plaques marked in yellow circle).

Histopathology studies

Effect of extracts on the arteries of HFD-induced hyperlipidemia

The histopathological examination of arterial tissues from different experimental groups [Figure 2], particularly in the context of HFD-induced hyperlipidemia, unveiled crucial insights into the effects of HCE and HEE extracts on vascular health. The HFD group exhibited characteristic alterations, including endothelial thickening, lipid accumulation within the vessel walls, and increased deposition of atheromatous plaques, indicative of atherosclerosis onset. These changes suggested a compromised vascular integrity due to the HFD.

However, treatments with HCE and HEE extracts portrayed promising outcomes. Specifically, arteries from groups treated with both extracts exhibited notably reduced lipid accumulation and atheromatous plaque formation compared to the HFD group. These observations hinted at the potential of HCE and HEE extracts in mitigating the progression of atherosclerosis induced by the HFD. Additionally, signs of improved endothelial health and reduced vessel wall thickening were noted, implying potential vascular protective effects.

Effect of extracts on the liver tissue of HFD-induced hyperlipidemia

The histopathological assessment of liver tissues within the context of HFD-induced hyperlipidemia and subsequent treatment with HCE and HEE extracts revealed pivotal insights into their effects on hepatic health. The HFD group exhibited characteristic features including hepatic steatosis, ballooning of hepatocytes, and signs of inflammation, indicative of liver injury and lipid accumulation associated with hyperlipidemia [Figure 3]. In contrast, liver tissues from groups treated with HCE and HEE extracts displayed remarkable alterations. Particularly, reduced hepatic steatosis and decreased hepatocyte ballooning were observed in these treated groups compared to the HFD group. Furthermore, signs of diminished inflammatory changes and improved hepatic architecture were evident, suggesting potential hepatoprotective effects of HCE and HEE extracts against hyperlipidemia-induced liver damage.

These histopathological findings underscore the potential therapeutic effects of both HCE and HEE extracts in ameliorating vascular and hepatic abnormalities induced by the HFD. The observed reduction in atherosclerotic changes in arteries and mitigation of liver injury suggest promising avenues for these extracts in managing hyperlipidemia-associated complications.

Discussion

The study data suggest that both HCE and HEE extracts, at varying doses, exhibited potential in mitigating the weight

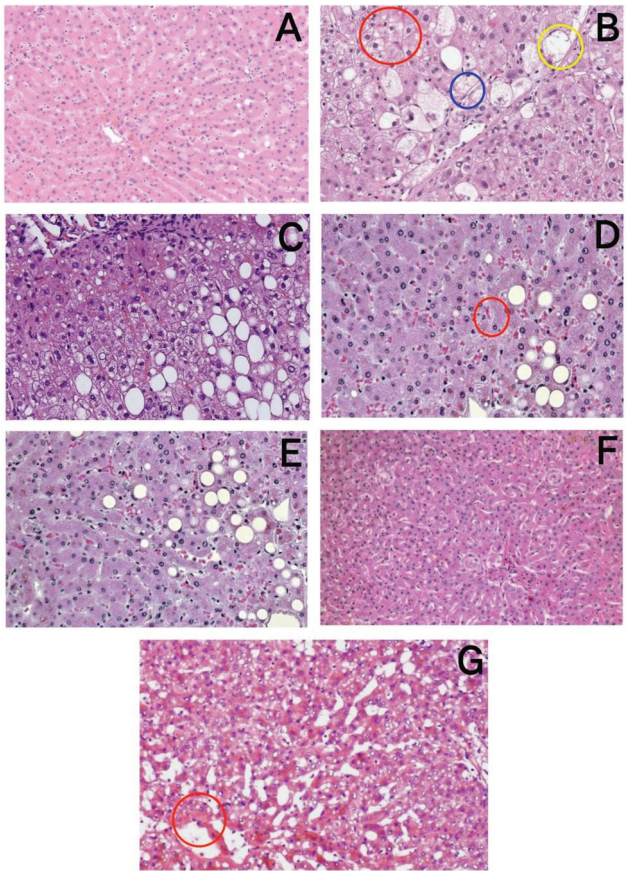

Figure 3: Histopathology of liver of HFD-induced hyperlipidemia. A. Normal group; B. HFD-induced group; C. HEC 100mg/kg; D. HEC 200mg/kg; E. HEE 100mg/kg; F. HEE 200mg/kg; G. Standard group (lipid accumulation marked in blue circle; inflammation marked in red circle; ballooning marked in yellow circle).

gain induced by the HFD in rats. This trend is especially prominent in the latter part of the study (from the 14th day onward), where the treated groups showed significantly lower weight gain compared to the HFD group. Additionally, the effects observed in the standard drug group align with the trends seen in the groups treated with the extracts, indicating comparable or potentially similar efficacy in reducing weight gain induced by the HFD. These findings indicate the potential of both HE and HEE extracts in moderating weight gain associated with the HFD.

The results suggest a trend toward attenuating obesity-related indices by both HCE and HEE extracts, especially at higher doses. Notably, HCE at 200 mg/kg demonstrated the most promising effects in bringing the obesity indices closer to the control group levels. Similarly, HEE exhibited trends towards normalizing these indices, indicating its potential in managing obesity induced by the HFD.

The lipid analysis data highlight the notable lipid-regulating effects of both HCE and HEE extracts in ameliorating the imbalanced lipid profile induced by the HFD. Particularly, HEE at higher doses demonstrated superior efficacy in reducing TC and TG levels, elevating HDL levels, and restoring TP levels compared to both the HFD-induced group and the standard drug. These findings underscore the potential of HCE and especially HEE extracts as effective natural remedies for managing hyperlipidemia and restoring lipid and protein homeostasis. HEE, particularly at higher doses, demonstrated superior efficacy in reducing LDL and VLDL levels and mitigating the atherogenicity index compared to both the HFD-induced group and the standard drug. These findings highlight the potential of HCE and especially HEE extracts as effective natural remedies for managing hyperlipidemia and potentially reducing the risk of atherosclerosis.

The study underscores the potential of HCE and particularly HEE extracts in ameliorating oxidative stress induced by the HFD. HEE, especially at higher doses, exhibited superior antioxidant effects compared to both the HFD-induced group and the standard drug, demonstrating enhanced scavenging of free radicals, restoration of antioxidant enzyme activities, preservation of GSH levels, and mitigation of lipid peroxidation. These findings signify the promising antioxidant activity of HEE, suggesting its potential as a natural therapeutic agent in combating oxidative stress associated with hyperlipidemia.

The investigation into the antihyperlipidemic potential of chloroform and ethanol extracts from the HCE and HEE revealed compelling insights into gene expression related to lipid metabolism. The study, utilizing RTPCR analysis on liver tissue homogenate, encompassed the following distinct groups: control, HFD induced, as well as those treated with HCE, HEE extracts, and a standard reference. The binding of excess of lipoproteins in the blood to the LDL-R on the cell membranes is an essential step for cholesterol homeostasis. This bound lipoprotiens will then be transported inside the cell and converted into cholesterol that is used up by the cell. This depletion of the intracellular cholesterol will activate the Sterol Regulatory Element-Binding Protein 2 (SREBP-2) transcription and triggers further LDL-R expression. Hence, absence or under expression of LDL-R hinders the lipoprotein clearance from blood stream leading to hyperlipidemia.[26] Our research results suggest that there is an increase in the expression of LDL-R which is suppressed by the HFD induction and overload of cholesterol. This increase in expression of LDL-R is evident with the lowering of LDL and VLDL in the blood in extract and standard treated groups.

Moreover, the investigation delved into the expression of FAS, a crucial enzyme involved in fatty acid synthesis,[27] and HMG-CoA, a key enzyme in cholesterol biosynthesis.[28] The HFD group exhibited marked increases in both FAS and HMG-CoA expression, indicative of enhanced lipid synthesis pathways. However, treatment with HCE and particularly HEE extracts showcased remarkable alterations in FAS gene expression. Both extracts notably suppressed FAS, showcasing their potential in inhibiting fatty acid synthesis

pathways. Similarly, they reduced HMG-CoA expression, hinting at their efficacy in curtailing cholesterol synthesis pathways.[29,30]

Notably, the AMPK-α gene expression levels, responsible for regulating cellular energy balance, showcased intriguing patterns. While the HFD group displayed a subtle decrease in AMPK-α expression compared to the control, both HCE and HEE treatments demonstrated similar patterns, but surpassing standard drug slightly. This suggests an activation of AMPK-α by the extracts, potentially implicating their role in modulating lipid metabolism pathways.

These findings indicate the promising antihyperlipidemic properties of both HCE and HEE extracts, demonstrating their ability to modulate key genes involved in lipid metabolism. The observed suppression of FAS and HMG-CoA expression suggests a potential mechanism for their therapeutic action against lipid disorders. Especially, it is evident from the results that inhibition of *FAS* gene is better than atorvastatin, which is typical for any HMG-CoA inhibitor.[31,32] Also, enhancing expression of LDL receptor is considered as a strong mechanism behind lowering of blood LDL levels. Thus, *FAS* gene inhibition along with LDL receptor expression can be attributed significantly for the activity of extract better than the standard drug, atorvastatin. Overall, the effect of the extract on all the genes might be due to the presence of various chemical constituents in the extracts showing synergistic activity overall contributing to the antihyperlipidemic activity in HFD-induced animals.

Conclusion

The current investigation emphasizes the antihyperlipidemic potential of *R. hypocrateriformis* extracts in combating HFD-induced hyperlipidemia and countering antioxidant defenses focusing on the activity of extracts toward altering the expression of hyperlipidemia related *FAS* and *LDL-R* genes. The extracts pose themselves as a promising natural therapy, showcasing a multimechanistic effect on hyperlipidemia by influencing lipid metabolism by expressing *LDL-R* gene and suppressing *FAS* gene along with antioxidant activities. Opportunities for future exploration lie in the identification of specific bioactive compounds, a deeper understanding of underlying molecular mechanisms, and the initiation of clinical trials to validate these findings in human contexts. *R. hypocrateriformis* emerges as a compelling candidate for drug development and nutraceutical research based on lead molecules, holding the promise of advancing hyperlipidemia management and contributing to enhanced cardiovascular health.

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Conflicts of interest

There are no conflicts of interest.

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