Original Research Article

Red Rice DE 40 Alleviates High-fat Diet-induced Hyperlipemia by Lowering Lipid Profiles in Rats

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Abstract

Background: Hyperlipidaemia is characterised by an abnormal increase in one or more lipids in plasma, and is a major contributor to obesity, coronary heart disease, myocardial infarction, and stroke if they are not treated promptly.
Objectives: In this study, we intended to discover the therapeutic actions of Red Rice DE 40 (RR DE 40) on high-fat diet (HFD)-induced rats.
Materials and Methods: The experimental rats were fed with HFD for 90 days to initiate the hyperlipidaemia and subsequently treated with 250 and 500 mg/kg of RR DE 40 from 60th to 90 days. The body weight of the rats was measured regularly. After the scarification, the blood, liver, and adipose tissue specimens were collected for biochemical assays. The liver and adipose tissues were weighed properly. The levels of haematological indices were counted using a haemocytometer. The levels of biochemical markers and liver marker enzymes were assessed using standard methods. The levels of lipid profiles were determined by standard methods. The excised liver and adipose tissues were analysed microscopically by histopathological analysis.
Results: RR DE 40 treatment effectively decreased the body weight gain in the HFD-induced rats. The liver and adipose tissue weight was also reduced by the RR DE 40 treatment. The changes in the haematological parameters such as RBC, WBC, Hb, monocytes, lymphocytes, and polymorphs were modulated by the RR DE 40 treatment on EG-induced rats. The levels of total protein, albumin, glucose, and urea and activities of SGOT and SGPT were substantially decreased by the RR DE 40 treatment. The RR DE 40 treatment also diminished the status of TC, TG, LDL, and VLDL in the HFD-treated rats. The findings of histopathological analysis also witnessed the therapeutic effects of RR DE 40.
Conclusion: The findings of this work highlighted that RR DE 40 is effective in HFD-induced hyperlipidaemia, probably due to its lipid-lowering potential. Hence, it may be used for the treatment of hyperlipidaemia-associated diseases.

Keywords

Hyperlipidaemia, triglycerides, adipose tissues, obesity, high-fat diet, RR DE 40

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Introduction

Hyperlipidaemia, which manifests as hypercholesterolemia, hypertriglyceridemia, or both, refers to a condition when the metabolism or functioning is abnormal of fat.[1] The accumulation of fat may be sped up by several metabolic mechanisms. Dyslipidaemia is also a significant factor in the development of fatty liver, conditions linked to the heart and brain, excessive blood sugar, and hypertension.[2] Chronic metabolic diseases may develop as a result of a high-energy, high-fat diet (HFD) being consumed regularly because it may cause the immune system to become harmfully activated.[3,4] Over the

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past years, obesity prevalence has drastically increased in more than 70 countries, which is creating a serious global public health issue.[5,6]

The abnormalities in plasma lipids profiles that characterise obese people's fat metabolism are the major symptoms. Hyperlipidaemia and obesity are closely related. Patients who are obese have higher blood triglyceride levels. The increase in fasting and postprandial insulin in obese people may be the cause of the rise in fat synthesis and decline in fat breakdown. Obesity, diabetes, and hypertension are all closely associated with dyslipidaemia, which could be reasons for the high death rate from cardiovascular illnesses in both humans and animals.[7,8]

Hyperlipidaemia is characterised by an abnormal rise in one or more lipids, primarily triglycerides (TG) and total cholesterol (TC) in plasma, which is a major contributor to obesity.[9] Patients will get major illnesses such as coronary heart disease, myocardial infarction, and stroke if they are not treated in a timely manner. As a result, lowering blood cholesterol levels is the best strategy to protect against developing cardiovascular and cerebrovascular disorders.[10] Therefore, the aforementioned modifications in combination with decreasing HDL levels, eventually lead to hyperlipidaemia, which then starts the development of advanced cardiac pathological diseases. Consuming a high-cholesterol diet is considered to be a significant risk factor for hyperlipidaemia since nutrition has a significant role in managing cholesterol balance.[11] In addition, an HFD promotes lipid build-up in adipose tissues.[12,13]

In hypercholesterolemic patients, the serum cholesterol level must be decreased in order to lower the incidence of CVD. Statins are the most well-known treatments for hypercholesteremia. However, myalgia, physical heaviness, fatigue, low energy, and hyperglycaemia are some of the side effects of statins that have been described.[14] Therefore, dietary approaches that lessen hyperlipidaemia, decrease levels of free fatty acids, and prevent the production of lipids in the liver and the storage of fat have caught the attention of researchers.[15,16] Finding a new class of lipid-lowering medications is of significant clinical importance. Researchers discovered that natural sources had no toxicity or very little toxicity, making it a possible therapy option for hyperlipidaemia.[17] Over the past decades, several research on the use of medicinal plants as an alternative strategy have demonstrated their potential and effectiveness to produce trustworthy data, highlighting the use of plant-derived medicines as important pharmacological resources.[18,19] Natural remedies have the benefits of being easily accessible, more usable, having minimal side effects, and being economically cost-effective.

Due to their potential as a safer, well-tolerated treatment approach with low side effects compared to synthetic medications, the use of herbal products and medicinal plants to treat these many pathological disorders is emerging as a new trend in recent years.[20] As the WHO estimated that the population trusts the use of herbal medicine to cure a variety of pathological illnesses and diseases, a tremendously expanding interest has been displayed by researchers in nutraceuticals. To treat illnesses and disease prevention, a rising number of people today want to use pharmaceuticals that are safe, nontoxic, and naturally sourced.[21] Foods that are easily accessible and heavy in sugar, processed carbs, and saturated fat are replacing fruits, vegetables, and whole grains. The treatment of obesity appears to be improved by increasing dietary fibre intake. Typically, fibre-rich foods are filling without being high in calories.[22,23] Red rice, scientifically known as *Oryza sativa* L., belongs to the Poaceae family, which is commonly referred to as the grass family. Red rice is a prominent staple meal globally, especially in Asian nations. The popularity of pigmented rice has increased mostly because of its nutritious and health-promoting properties. Red rice, a type of pigmented rice, is a primary provider of proanthocyanidins, which possess powerful antioxidant, anti-inflammatory, anticancer, and immunomodulatory properties.[24,25] Red rice naturally contains proanthocyanidins in its pericarps, which possess strong antioxidant properties and provide health benefits to humans.[26,27] Consequently, in this work, we investigated the antihyperlipidemic activity of Red Rice DE 40 (RR DE 40) in HFD-induced rats.

Materials and Methods

Animals

For the current anti-hyperlipidaemic study, male C57BL/6 mice weighing 20–25 g were employed. The animals were kept in sanitary polypropylene cages with a 12-hour light/dark series. Standard pelleted food and unlimited access to clean water were provided for the animals. After receiving approval from the institutional ethics committee and following the guidelines, all procedures involving animals were carried out.

Study Procedure for Acute Toxicity

According to the recommendations of the OECD, an acute oral toxicity study was conducted. A single dose of RR DE 40 was administered using a tuberculin syringe. Three hours before treatment, animals are fasted by withholding food for 3 hours but not water. Animals were weighed after the fasting period, and oral doses of the test drug of 500, 1000, and 2000 mg/kg were given. Food was withheld from mice for two hours following the RR DE 40 treatment. For a total of 14 days, each animal is monitored individually at least once during the first 30 minutes, several times over the first 24 hours, with extra focus on the first four hours.

Induction of Hyperlipidaemia

In mice, hyperlipidaemia was produced by feeding them an HFD for 60 days. To verify the development of

hyperlipidaemia, blood samples were taken from mice's retro-orbital sinuses at the end of the 60th day while they were lightly sedated with ether. When the clear serum was ready, it was maintained in frozen containers after being separated at 2500 rpm for 10 minutes. On the 60th day, the blood lipid levels, including TG, TC, HDL, and LDL, were assessed to ensure that the hyperlipidaemia animal was successful. Based on the elevation of the cholesterol level, the rats were then separated into five groups of six animals, and they underwent the following treatment.

Group I: Regular diet (90 days)
Group II: HFD (90 days)
Group III: HFD (90 days) + Standard drug Rosuvastatin (4 mg/kg for 30 days)
Group IV: HFD (90 days) + 250 mg/kg of RR DE 40 (30 days)
Group V: HFD (90 diets) + 500 mg/kg of RR DE 40 (30 days)

A known amount of fresh food was replenished every day, and mice's body weight was measured and recorded each week to determine the body weight of the animals. Throughout the course of the study, daily observations of the animal's general health and behaviour were made. To provide the animals with the greatest comfort possible, the litter in the cage was changed twice a week. On the 90th day, animals were starved during the night. All of the animals were slaughtered the following day while being lightly sedated with ether. Mice's retro-orbital sinus was employed to collect blood. After being separated at 2500 rpm for 10 minutes, the clear serum was stored in frozen containers. The liver and adipose tissues were excised and weighed carefully.

Measurement of Biochemical Profiles

As per the manufacturer's protocols, the status of total protein, albumin, urea, glucose, and albumin in the serum of experimental rats was measured using marker-specific ELISA kits (Thermo Fisher Scientific, USA).

Estimation of Haematological Parameters

Enumeration of RBCs

Well-mixed blood was filled up to the 0.5 mark, and RBC diluting liquid was drawn up to mark II using an RBC pipette from a haemocytometer. The fluid-blood combination was agitated and then poured into the counting chamber. For two minutes, the cells were permitted to rest at the bottom of the compartment. RBCs were consistently counted in the larger corner squares using a 45× magnification.

Enumeration of WBC

Well-mixed blood was filled up to the 0.5 mark on a haemocytometer's white blood cell pipette, and WBC-diluting solution was drawn up to mark II. After being shaken, the fluid-blood mixture was poured into the counting chamber. The cells were given two minutes to sink at the bottom. The WBCs were consistently counted using a 10× magnification.

Differential Leucocyte Count

Under oil immersion, a blood film stained with Leishman's stain was studied to determine the various WBC subtypes. The distribution of these cells' percentages was then calculated. Blood samples that were anticoagulant were converted into smears and stained with Leishman's stain. The slides were kept so that the number of neutrophils and lymphocytes per 100 cells could be counted.

Estimation of Haemoglobin

Add 0.1 N HCl using a pipette until the haemoglobinometer reaches the lowest mark. The Sahli's pipette held 20 µl of blood, which was pulled up to that level. carefully adjusted the blood column to prevent bubbles. used a dry piece of cloth to wipe away the extra blood that had collected on the pipette's sides. Blood was blown into the graduated tube containing the acid solution, and the pipette was thoroughly washed. After mixing the reaction, let the mixture rest at room temperature for 10 minutes. By carefully adding a few drops of water and blending the reaction mixture, the solution was diluted with distilled water until the colour matched that of the comparator. The lower meniscus of the fluid was observed, and the reading in g/100 ml was recorded.

Estimation of Serum Lipid Parameters

Estimation of TC

To deproteinise the serum (0.1 ml), ferric chloride-acetic acid solution (9.9 ml) was mixed. The mixture was centrifuged for 15 minutes at 3000 rpm. Five millilitres of supernatant were taken, and 3.0 millilitres of sulfuric acid were mixed. This mixture was then left at 37°C for 20 minutes. The pink hue that formed was read at 540 nm. Additionally, a set of standards were carried out similarly.

Estimation of TG

A total of 400 mg of alumina and 4.0 ml of isopropanol were added to 0.1 ml of serum, which was placed in a centrifuge tube with a glass stopper. After shaking vigorously for 10 minutes, the tubes were sealed tightly. A supernatant volume of 2 ml was pipetted into clean, dry test tubes after the tubes had been centrifuged at 3000 rpm for 15 minutes. This was given 0.6 ml of alcohol-based KOH and maintained at 70°C for 15 min before being cooled to 37°C. Then, for 30 minutes at 50°C, the mixture was incubated with 0.5 ml of the acetyl-acetone reagent and 1.0 ml of the meta-periodate reagent. Triolein was utilised in place of serum in a manner comparable to standard. The reagent blank was used as a reference while the colour formed was read at 405 nm.

HDL, LDL, and VLDL Cholesterols

The 500 ml of cholesterol precipitating reagent (from the assay kit) was combined with 200 ml of serum and thoroughly mixed in a 1.5 ml centrifuge tube. The aforementioned solution was centrifuged at 4000 rpm for 10 minutes. 500 ml of reagent 1 (from the cholesterol kit) was taken. 100 l of the supernatant from the previously centrifuged solution was added. After thoroughly blending, incubate for 15 minutes at 37°C. Finally, read out the test sample.

Histological Examinations

Immediately after being removed, the liver and adipose tissue were torn out, excised, and rinsed in a chilly saline solution. The liver and pancreas were partially fixed in a 10% formalin fixative solution, dehydrated in alcohol, and then embedded in paraffin. The use of rotating microtome tissues was sliced with a thickness of 4–5 µm. For the purpose of identifying histopathological alterations, the sections were stained with the H&E dye and monitored beneath the microscope.

Statistical Analysis

GraphPad Prism software was utilised to analyse the outcomes from each assay, and the data are illustrated as a mean ± SD of triplicates. One-way ANOVA and the DMRT assay were used to examine the values, and the significance level was established at $p < .05$.

Results

Effect of RR DE 40 on the Acute Toxicity Study in the Experimental Rats

The administration of RR DE 40 to Wister rats did not result in drug-related toxicity or mortality up to a dosage of 2000 mg/kg, according to an acute toxicity study. Hence, it was clear that the RR DE 40 is safer for experimental animal usage without possessing toxicity.

Effect of RR DE 40 on the Lipid Profiles in the Experimental Rats

Table 1 demonstrates levels of lipid profiles such as TC, TG, and HDL levels in the serum of control and RR DE 40 treated experimental rats. The elevated status of TC, TG, and HDL was observed in the serum of HFD-induced rats on the 30th, 60th, and 90th day when compared to the control. However, the 250 and 500 mg/kg of RR DE 40 treatment appreciably diminished the status of cholesterols, TG, and HDL in the HFD-treated rats, which is similar to the findings of standard drug rosuvastatin treatment (Table 1). The rosuvastatin treatment also diminished the TC, TG, and HDL in the serum of experimental rats.

Effect of RR DE 40 on the LDL, VLDL, and Atherogenic Index in the Experimental Rats

The LDL, VLDL, and atherogenic index on the serum of both control and experimental rats were assessed, and outcomes

Table 1: Effect of RR DE 40 on the Lipid Profiles in the Experimental Rats.

	Initial Lipid Profile			Lipid Profile After 60th Day with HFD			Lipid Profile After 90th Day with HFD		
Groups	**TC**	**TG**	**HDL**	**TC**	**TG**	**HDL**	**TC**	**TG**	**HDL**
Control	57 ± 5.50	110.5 ± 5.48	47.5 ± 0.20	54.67 ± 2.40	98.5 ± 2.59	48.5 ± 0.86	56 ± 1.15	104.5 ± 26.21	46 ± 1.15
ONLY HFD	53 ± 2.08#	157 ± 0.57#	24 ± 2.16#	409.7 ± 26.77#	150 ± 1.15#	72.5 ± 1.44#	403.7 ± 13.79#	171.4 ± 20.48ns	56 ± 1.15#
HFD + Rosuvastatin	49.67 ± 2.72*	94.5 ± 0.28*	47 ± 1.63*	185 ± 3.21ns	131.5 ± 0.28*	45.33 ± 5.33*	109.8 ± 4.09*	112 ± 3.50*	40.67 ± 9.68*
HFD + RRD 40 (250 mg/kg)	54.33 ± 0.88*	120 ± 6.92*	38 ± 1.22*	222 ± 6.11*	159 ± 0.57*	67.5 ± 0.28*	147.6 ± 4.87ns	130.1 ± 4.21*	33.67 ± 1.76*
HFD + RRD 40 (500 mg/kg)	53.67 ± 2.60*	93 ± 3.46ns	47 ± 0.40*	183.7 ± 3.93*	110 ± 6.92*	52.5 ± 8.37*	119.6 ± 1.61*	97.37 ± 13.17*	31.33 ± 2.40*

Note: The values are given as the mean ± SD of triplicate assays. Using the GraphPad Prism software, all of the data were statistically analysed using one-way ANOVA and Dunnett's post hoc analysis. An asterisk '#' denotes that the values are statistically significant at $p < .01$ from the control group; '*' denotes that the values are significant at $p < .05$ from the HFD group; ns: denotes that the values are not significant.

Table 2: Effect of RR DE 40 on the LDL, VLDL, and Atherogenic Index in the Experimental Rats.

	Initial Lipid Profile		Lipid Profile After 60th Day with HFD		Lipid Profile After 90th Day with HFD		Atherogenic	% Lipid
Groups	LDL	VLDL	LDL	VLDL	LDL	VLDL	Index	Lowering
Control	12.08 ± 0.05	21.65 ± 0.25	13.4 ± 0.32	19.69 ± 0.008	10.82 ± 0.49	20.55 ± 0.19	2.757 ± 0.06	-
ONLY HFD	52.86 ± 3.47#	31.75 ± 0.20#	307.2 ± 0.34#	29.89 ± 0.06#	312.3 ± 0.64#	34.55 ± 0.15#	5.183 ± 0.04#	168.12%
HFD + Rosuvastatin	16.24 ± 0.36*	18.9 ± 0.12*	80.19 ± 32.91*	26.31 ± 0.005ns	46.32 ± 0.23*	22.42 ± 0.01*	2.6 ± 0.11ns	143.92%
HFD + RRD 40 250 mg/kg	22.57 ± 0.32*	23.95 ± 0.02ns	123.8 ± 0.47*	29.96 ± 0.09*	70.55 ± 0.32*	28.06 ± 0.09*	3.067 ± 0.02*	154.70%
HFD + RRD 40 500 mg/kg	12.11 ± 0.23*	18.65 ± 0.02*	109.5 ± 0.54*	21.95 ± 0.03*	69.14 ± 0.45ns	19.39 ± 0.04*	2.75 ± 0.07*	149.88%

Notes: The values are given as the mean ± SD of triplicate assays. Using the GraphPad Prism software, all of the data were statistically analysed using one-way ANOVA and Dunnett's post hoc analysis. An asterisk '#' denotes that the values are statistically significant at $p < .01$ from the control group; '*' denotes that the values are significant at $p < .05$ from the HFD group; ns: denotes that the values are not significant.

Table 3: Effect of RR DE 40 on the Bodyweight Changes in the Experimental Rats.

	Control	ONLY HFD	HFD + Rosuvastatin	HFD + RRD 40 250 mg/kg	HFD + RRD 40 500 mg/kg
Initial body weight	22.83 ± 0.6009	22.83 ± 0.654#	22.17 ± 0.6009*	22.33 ± 0.5578*	21.67 ± 0.6667*
Final body weight	26 ± 0.3651	39.17 ± 1.869#	24 ± 4.872ns	28.67 ± 6.037ns	25.33 ± 5.258*

Notes: The values are given as the mean ± SD of triplicate assays. Using the GraphPad Prism software, all of the data were statistically analysed using one-way ANOVA and Dunnett's post hoc analysis. An asterisk '#' denotes that the values are statistically significant at $p < .01$ from the control group; '*' denotes that the values are significant at $p < .05$ from the HFD group; ns: denotes that the values are not significant.

Table 4: Effect of RR DE 40 on the Organ Weight Index in the Experimental Rats.

	Control	ONLY HFD	HFD + Rosuvastatin	HFD + RRD 40 250 mg/kg	HFD + RRD 40 500 mg/kg
Liver weight	3.6 ± 0.162	4.97 ± 0.087#	3.88 ± 0.0230*	3.85 ± 0.557*	3.663 ± 0.263ns
Adipose tissue	0.178 ± 0.0034	0.298 ± 0.0069#	0.19 ± 0.0011*	0.19 ± 0.0034*	0.177 ± 0.0017*

Notes: The values are given as the mean ± SD of triplicate assays. Using the GraphPad Prism software, all of the data were statistically analysed using one-way ANOVA and Dunnett's post hoc analysis. Note: An asterisk '#' denotes that the values are statistically significant at $p < .01$ from the control group; '*' denotes that the values are significant at $p < .05$ from the HFD group; ns: denotes that the values are not significant.

were revealed in Table 2. The HFD-diet-treated rats demonstrated increased LDL, VLDL, and atherogenic index in the serum on the 30th, 60th, and 90th day. Interestingly, the treatment with the 250 and 500 mg/kg of RR DE 40 treatment effectively suppressed the LDL, VLDL, and atherogenic index in the serum of HFD-induced rats (Table 2). The rosuvastatin treatment also reduced these levels.

Effect of RR DE 40 on the Bodyweight Changes in The Experimental Rats

Table 3 reveals the effect of RR DE 40 on the changes in bodyweight of the experimental rats. The results considered bodyweight gain in the HFD-induced rats when compared to the control. Interestingly, the treatment with the 250 and 500 mg/kg of RR DE 40 effectively decreased the bodyweight in the HFD-treated rats, which is similar to the standard drug rosuvastatin treatment. The rosuvastatin also considerably reduced the body weight gain in the HFD-treated rats (Table 3).

Effect of RR DE 40 on The Organ Weight Index in The Experimental Rats

The effects of RR DE 40 treatment on the organ weight index (liver and adipose tissues) were examined and findings were revealed in Table 4. The HFD-treated rats demonstrated a

Table 5: Effect of RR DE 40 on the Haematological Parameters in the Experimental Rats.

	Control	ONLY HFD	HFD + Rosuvastatin	HFD + RRD 40 250 mg/kg	HFD + RRD 40 500 mg/kg
RBC ($\times 10^6$/µl)	5.007 ± 0.1068	5.06 ± 0.1637#	5.03 ± 0.08145*	5.1 ± 0.2937ns	5.013 ± 0.2107*
WBC ($\times 10^3$/µl)	10.1 ± 0.8963	8.767 ± 0.491#	9.6 ± 1.762ns	8.667 ± 0.348*	8.467 ± 0.8212ns
HB (g/dl)	12.73 ± 0.669	13.07 ± 0.3333#	13.13 ± 1.312*	13.37 ± 1.452*	13.53 ± 0.8686*
Polymorphs (%)	7.667 ± 0.6667	4.667 ± 1.453#	8 ± 2.309*	5 ± 1.155ns	4.667 ± 2.186*
Lymphocytes (%)	87.33 ± 2.667	88 ± 1.155#	87.33 ± 2.333*	86.33 ± 3.844*	89.33 ± 3.712*
Monocytes (%)	3.333 ± 1.333	4.333 ± 0.6667#	2.667 ± 0.6667ns	6.333 ± 2.186*	4 ± 0.5774*

Notes: The values are given as the mean ± SD of triplicate assays. Using the GraphPad Prism software, all of the data were statistically analysed using one-way ANOVA and Dunnett's post hoc analysis. An asterisk '#' denotes that the values are statistically significant at $p < .01$ from the control group; '*' denotes that the values are significant at $p < .05$ from the HFD group; ns: denotes that the values are not significant.

Table 6: Effect of RR DE 40 on the Biochemical Parameters in the Experimental Rats.

	Control	ONLY HFD	HFD + Rosuvastatin	HFD + RRD 40 250 mg/kg	HFD + RRD 40 500 mg/kg
Total protein (mg/dl)	2.533 ± 0.480	8.8 ± 0.916#	2.067 ± 0.592*	6.1 ± 0.208**	2.933 ± 0.638ns
SGPT (U/L)	31 ± 4.583	64.67 ± 4.667#	39.33 ± 1.764*	63.67 ± 3.18*	46.67 ± 3.528*
SGOT (U/L)	37.67 ± 1.202	91 ± 3.786#	36 ± 2.309*	70.67 ± 1.764*	40.33 ± 2.028ns
Urea (mg/dl)	8.167 ± 1.565	20.3 ± 5.029#	7.4 ± 1.193*	18.7 ± 6.317*	11.37 ± 1.602*
GLUCOSE (mg/dl)	144.2 ± 21.24	369.4 ± 40.44#	109.7 ± 0.696ns	175.6 ± 0.7506ns	143.9 ± 1.849ns
ALBUMIN (mg/dl)	3.167 ± 0.233	7.667 ± 0.448#	2.9 ± 0.379*	7.1 ± 0.643*	3.2 ± 0.115ns

Notes: The values are given as the mean ± SD of triplicate assays. Using the GraphPad Prism software, all of the data were statistically analysed using one-way ANOVA and Dunnett's post hoc analysis. An asterisk '#' denotes that the values are statistically significant at $p < .01$ from the control group; '*' denotes that the values are significant at $p < .05$ from the HFD group; ns: denotes that the values are not significant.

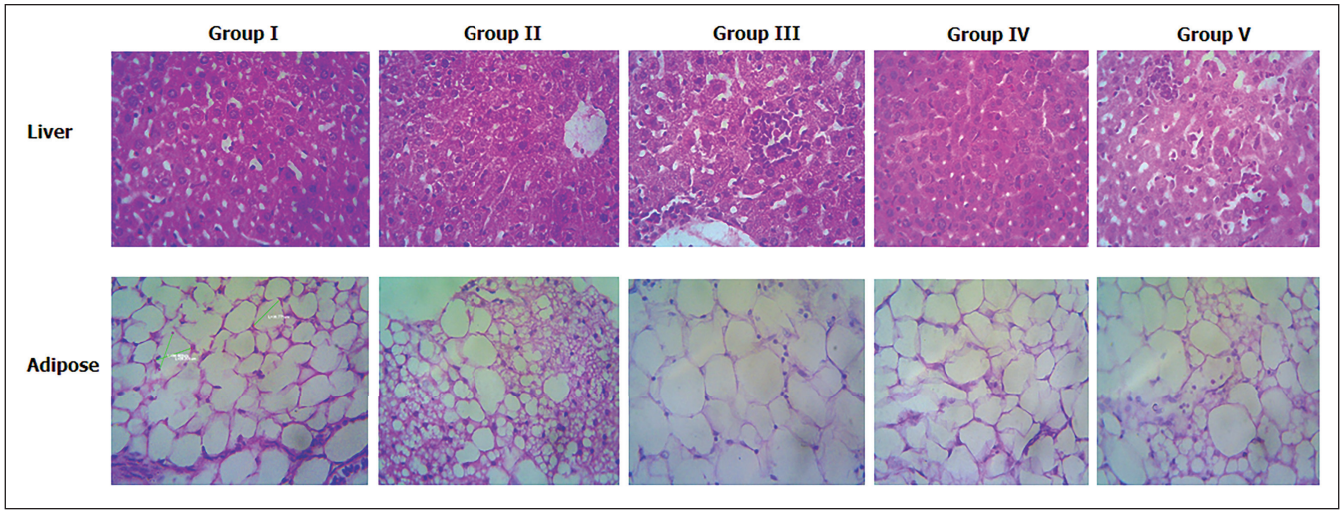

Figure 1. Effect of RR DE 40 on the Liver Histopathology of Experimental Rats.

Notes: Group I: The control animals showed normal histomorphology. Group II: The HFD-induced rats demonstrated the swollen and scattered loosely arranged with white vacuole in liver tissues and epididymal fat cell hypertrophy in adipose tissues. Group III: Standard drug rosuvastatin effectively ameliorated the histological changes in both liver and adipose tissues. Group IV & V: The 250 and 500 mg/kg of RR DE 40 treated rats demonstrated the hepatocytes with near-normal morphology decreased white vacuoles in liver tissues and decreased the epididymal fat cell hypertrophy in adipose tissues of HFD-induced rats.

significant increase in both liver and adipose tissues. However, these increments were effectively reversed by the RR DE 40 treatment. The treatment with 250 and 500 mg/kg of RR DE 40 appreciably reduced both liver and adipose tissue weight in the HFD-treated rats. The standard drug rosuvastatin also significantly decreased the liver and adipose tissue weights in the HFD-treated rats (Table 4).

Effect of RR DE 40 on the Haematological Parameters in the Experimental Rats

Table 5 indicates the effects of RR DE 40 treatment on the changes in haematological parameters such as RBC, WBC, Hb, lymphocytes, monocytes, and polymorphs in the serum of experimental rats. Our findings revealed that the HFD diet considerably polymorphs and WBC count and did not show major changes in the RBC, Hb, lymphocytes, and monocytes. The treatment with 250 and 500 mg/kg of RR DE 40 effectively modulated the HFD-induced changes in the haematological parameters of experimental rats (Table 5).

Effect of RR DE 40 on the Biochemical Parameters in the Experimental Rats

The levels of total protein, urea, glucose, and albumin and the activities of liver marker enzymes such as SGPT and SGOT were examined in the serum, and findings were demonstrated in Table 6. The increased levels of total protein, urea, glucose, and albumin and the increased activities of SGPT and SGOT were noted in the serum of HFD-induced rats. However, the treatment with 250 and 500 mg/kg of RR DE 40 effectively decreased the levels of total protein, urea, glucose, and albumin and the activities of SGPT and SGOT in the HFD-induced rats (Table 6). The rosuvastatin treatment also effectively decreased these parameters.

Effect of RR DE 40 on the Liver Histopathology of Experimental Rats

Figure 1 indicates the findings of histopathological analysis of the liver and adipose tissues of experimental rats. The liver and adipose tissues of control rats revealed a normal morphology without fatty degeneration. However, the HFD-induced rats demonstrated the swollen and scattered loosely arranged white vacuoles by different lipid droplets. Interestingly, the 250 and 500 mg/kg of RR DE 40 treated rats demonstrated hepatocytes with near-normal morphology and decreased white vacuoles (Figure 1). The RR DE 40 treatment also effectively decreased epididymal fat cell hypertrophy, which is triggered by HFD (Figure 1). All these outcomes reveal that RR DE 40 treatment may decrease the lipids of the liver by diminishing lipid accumulation and inhibiting fatty liver development to a certain extent.

Discussion

Obesity is caused by an energy imbalance—a higher ratio of calories consumed to calories expended—and is linked to metabolic illnesses such as dyslipidaemia, atherosclerosis, and type 2 diabetes. An intriguing strategy in ethno-pharmacological research is the examination of herbal remedies with the potential for lowering blood sugar, blood pressure, and cholesterol.

A growing number of people are becoming obese as a result of unhealthy diets and insufficient exercise. TG and TC are typically markedly elevated in persons with obesity. Regulation of lipid metabolism can lower the risk of lipid disorders, obesity, cardiovascular disease, and subsequent pathological complications. Nutritional and pharmaceutical preparations that target these physiological conditions, which include dyslipidaemia, elevated plasma TG level, LDL and VLDL, and a low level of HDL, can regulate lipid metabolism.[28] To prevent and treat cardiovascular and cerebrovascular disorders as well as lower social pressure, it is crucial to improve hyperlipidaemia.

Currently, the majority of lipid-lowering medications used in clinical therapy have various negative effects in humans. To achieve the lipid-lowering action and reduce the negative effects of lipid-lowering medications, it is required to investigate innovative natural substances. Previous studies demonstrated that several foods and natural substances could lower plasma lipid levels without causing any immediately noticeable negative effects.[29,30] At the moment, hyperlipidaemia is primarily treated with nutrition therapy. Mostly, low-fat and low-sugar items make up the diet. When it is false, it can be adequately supplemented with lipid-lowering medications. Natural products have a distinct and significant role in the direction and reference of novel drug development because some lipid-lowering medications might result in adverse effects such as muscular myopathy, liver malfunction, hyperglycaemia, excessive uric acid or gout, and upper gastrointestinal pain. Multiple studies have shown that natural products can decrease blood lipids as naturally active components.[31] The goal of the current investigation was to determine RR DE 40's antihyperlipidemic potential in mice fed with HFD.

The aberrant metabolism of blood lipids that results from an HFD will also contribute to weight gain, visceral obesity, and other symptoms. Hyperlipidaemia could arise in serious situations. Elevated LDL-cholesterol, TG, and fasting blood sugar, as well as a reduction in HDL-cholesterol, were linked to an excess of body weight.[32] Thus, elevated blood lipid levels are frequently present in obese people. Natural medications and high-efficiency health care products composed of plant functional elements have great biological activity thanks to ongoing medical research and innovation,[33] which can reach high efficacy in treating diseases but also has adverse effects on the human body.[34] Here, we noted that the RR DE 40 treatment effectively decreased the body weight of the

HFD-induced rats, which is similar to the results of rosuvastatin.

Biochemical markers can also change as a result of obesity due to HFD. WBCs affect the immune system, and RBCs oxygenate body tissue, maintain necessary blood pressure, create viscosity, and transport carbon dioxide to the lungs. All need to be in specific ranges in the body.[35,36] An essential part of obesity-related disorders is played by chronic inflammation around adipocytes.[37] It is possible that obese patients have raised WBC counts because inflammation causes an increase in WBC count. Adipose tissue secretes IL-6 involved in WBC proliferation and differentiation as well as bone marrow granulopoiesis, which provides a possible explanation for the elevated WBC count in obese people.[38] In earlier research, there was a link between the higher WBC count and poor glucose tolerance as well as carotid atherosclerosis. Despite high WBC levels that were still within the normal range, Tong et al.[39] found that patients with high WBC counts had poor metabolic profiles. Additionally, according to various research, the RBC count and Hb level are both strongly linked to high blood pressure and MS, respectively.[40,41] Because of the low Hb level that results from anaemia, there are both hemodynamic and non-hemodynamic compensation mechanisms. Acute anaemia causes transitory clinical and hemodynamic alterations, while chronic anaemia causes left ventricular hypertrophy and gradual heart enlargement.[42] In this study, our outcomes demonstrated that the RR DE 40 treatment substantially modulated the HFD-induced alterations in the haematological parameters such as RBC, WBC, lymphocytes, monocytes, and polymorphs in the HFD-induced rats.

An increase in TG, cholesterol, or other lipids is a symptom of hyperlipidaemia. Atherosclerosis and other cardiovascular conditions are linked to hyperlipidaemia.[43] The most often reported health consequence worldwide is diabetes, followed by CVDs. They are significantly connected with hyperlipidaemia, which is one of the main risk factors for CVDs and is indicated by an increase in the blood lipid profile indices of TG, LDL, TC, and VLDL. In the affected patients, hyperlipidaemia is directly associated with a noticeable metabolic imbalance.[44] In addition, hyperlipidaemia leads to the oxidative alteration of LDL, which triggers the inflammatory response that is mediated by inflammatory cytokines and activates chemoattractant monocytes, leading to atherosclerosis development and other associated cardiac disorders.[45,46] TG are also directly linked to the development of coronary heart problems.[47] In order to identify hyperlipidaemia clinically, four plasma lipid indices must be found; hyperlipidaemia is typically accompanied by an increase in TC, TG, LDL, and a reduction in HDL levels.[48,49] Our results proved that the RR DE 40 treatment considerably decreased the levels of cholesterol, TG, LDL, VLDL, and HDL in the serum of HFD-induced rats. These outcomes evidenced the antihyperlipidemic potential of the RR DE 40.

The body's energy metabolism takes place mostly in the liver. Due to changes in the integrity and permeability of the plasma membrane, various enzymes, including AST and ALT can be released from damaged hepatic cells and enter the extracellular fluid.[50] For the clinical diagnosis of hyperlipidaemia, the activity of the ALT and AST enzymes is frequently employed.[51] As shown by liver tests, including ALT and AST in mice fed with the HFD are increased than in healthy animals These are liver-specific enzymes that offer a thorough understanding of how the liver is working; they rise in situations involving hepatic damage or dysfunction.[52] The degree of liver abnormalities is known to connect with the activity of ALT and AST strongly.[53,54] The ratio of AST/ALT is frequently utilised in clinical settings to represent liver cell damage. Our findings demonstrated that activities of AST and ALT in HFD-induced rats were significantly greater than those in healthy mice, suggesting that feeding HFD could have a negative impact on normal rats' liver function and seriously harm their liver cells, which is also evidenced by the histopathological analysis of liver tissues. Significantly reduced serum ALT and AST activities were seen in RR DE 40-treated rats, demonstrating the ability of RR DE 40 to preserve liver function.

Conclusion

In conclusion, the current results suggest that RR DE 40 is effective in HFD-induced hyperlipidaemia in rats. RR DE 40 treatment demonstrated ameliorative properties against HFD-induced hyperlipidaemia in rats by decreasing cholesterol, TG, LDL, VLDL, atherogenic index, urea, glucose, albumin, total protein, SGOT, and SGPT levels. Furthermore, the RR DE 40 treatment substantially attenuated the HFD-induced alterations and fat depositions in the liver and adipose tissues and upheld the histoarchitectures. The fundamental ameliorative mechanisms of RR DE 40 may be regulated by its lipid-lowering properties. Furthermore, further study is still required in the future to verify the precise therapeutic mechanism of RR DE 40.

Abbreviations

TG: Triglycerides; **TC:** Total cholesterol; **HFD:** High-fat diet; **CVD:** Cardiovascular disease; **OECD:** Organisation for Economic Co-operation and Development; **HDL:** High-density lipoprotein; **LDL:** Low-density lipoprotein.

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Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Ethical Approval

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