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Rice bran oil reduces organ-specific fat deposition, attenuates hyperlipidaemia and abnormal liver function in Long Evans rats with high fat intake

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ABSTRACT

Introduction: High-calorie diets, particularly the quality of dietary fats, are regarded as an independent risk factor for developing obesity, hyperlipidaemia, and liver diseases. The present study examined the impact of rice bran oil (RBO) on organ-specific fat deposition, lipid profile, and liver function enzymes in Long Evans rats. **Methods:** Long Evans rats (n=24) were fed for six weeks with a controlled high-fat diet (HFD) to induce hyperlipidaemia and abnormal liver function. Rats were then divided into two groups: one group continued feeding on HFD, and the other group was fed with a RBO diet, replacing the fat source. After six weeks of feeding, six rats from each group were sacrificed and required analytical tests were performed. The remaining obese rats (n=12) were divided into continued HFD and RBO diet, and after sacrificing, essential analytical tests were done. **Results:** RBO feeding to hyperlipidaemic rats for six weeks significantly reduced brown adipose tissue, abdominal adipose tissue, epididymal adipose tissue, and liver fat compared to continuing HFD group (p<0.05). Similarly, serum levels of total cholesterol, triacylglycerides, and low-density lipoprotein cholesterol were all decreased, whereas high-density lipoprotein cholesterol increased in response to RBO compared to HFD (p<0.05). Additionally, rats fed with RBO showed reduced alanine aminotransferase, aspartate aminotransferase, and gamma-glutamyl transferase levels when compared with continuing HFD-fed rats (p<0.05). **Conclusion:** These findings suggest that RBO supports the reduction of fat storage from major fat depots, controls lipid profile, and restores healthy liver functions in rats.

Keywords: fat depots, high fat diet, hyperlipidaemia, liver enzymes, rice bran oil

INTRODUCTION

Overweight and obesity is a major risk factor for type 2 diabetes, cardiovascular diseases (CVD), some cancers, and overall mortality (Gray *et al.,* 2015). Dietary management has been recognised as a critical aspect of therapy for obesity since it can potentially improve adiposity and its related co-morbidities. Reducing saturated fat and cholesterol intakes through dietary interventions is an effective strategy in treating CVD

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and obesity-related disorders. Although extra energy consumed in any form of macronutrients can be converted and stored in the body as fat (Galgani & Ravussin, 2008), the amount and type of fat (saturated or unsaturated) consumed in the diet are significant contributors to the development of adiposity and hyperlipidaemia (Beulen *et al.,* 2018). Polyunsaturated fatty acids (PUFAs) are widely accepted as part of a healthy diet because of their beneficial effects on metabolism (Zarate *et al.,* 2017). Observational studies have suggested that consumption of PUFAs (omega-3 and omega-6 fatty acids) may reduce abdominal fat by decreasing fat cell size and contribute towards improving body composition by increasing metabolism and fat burning potential (Albar, 2022). Therefore, replacing saturated fat intake with PUFAs through dietary means can be a realistic approach to managing obesity and reducing hyperlipidaemia.

The coexistence of hyperlipidaemia and abnormal liver function are well-established risk factors for developing metabolic disorders, including CVD and diabetes (Chithra *et al.,* 2015). As a crucial metabolic organ, the liver plays a crucial role in lipid and lipoprotein metabolism, including the biosynthesis of cholesterol, fatty acids, apolipoproteins, and proteins involved in lipoprotein homeostasis. Proper liver function is essential for the regulation of these metabolic processes. Gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) are commonly used as markers of hepatic abnormality and are associated with numerous disease conditions, including non-alcoholic fatty liver disease (NAFLD) (Juo & Livingston, 2019). Recent studies have shown that a higher accumulation of adipose tissues in different fat depots can alter the endocrine and paracrine functions of these active organs. These changes can lead to insulin resistance, dysregulation of glucose and lipid metabolisms, coagulation, and inflammation, ultimately contributing to the progression of cardiometabolic diseases (Lim & Meigs, 2014).

Cooking oil is one of the vital contributors of fat to the diet. Rice bran oil (RBO) is a major source of PUFA, containing approximately 30% linoleic acid and 44% oleic acid with less (approximately 23%) saturated fatty acid. Apart from the better quality fatty acid profile, RBO also contains gamma-oryzanol, vitamin E, and phytosterols, which have higher antioxidant potentials compared to the other available cooking oils (Latha & Nasirullah, 2014). However, it is still uncertain whether RBO administration can be an effective strategy for preventing CVD and its associated co-morbidities. The present study aimed to elucidate the impact of RBO on lipid profiles, liver enzymes, and organ-specific fat deposition in obese Long Evans rats.

MATERIALS AND METHODS

Experimental animals

Twenty-four (12 males and 12 females) Long Evans (*Rattns norvigicus*) rats (4–5 weeks of age, body weight ~80 g) were obtained from the Animal House, International Center for Diarrheal Disease Research (ICDDR, B) Dhaka, Bangladesh. Rats were housed in a temperature-controlled laboratory room at 23±5°C with a 12:12 hour light–dark cycle and allowed to adapt to laboratory conditions for a week. All rats were maintained in the animal care facilities according to animal care and use guidelines. All experiments conducted in this study were approved by the Ethical Review Committee for the protection of human and animal subjects at the Department of Food Technology and Nutritional Science in Mawlana Bhashani Science and Technology

University, which gave full approval with the ethical approval number MBSTU/FTNS/42/2022/14.

Study design

The rats were randomly divided into two groups, namely control group (*n*=6) and high-fat diet (HFD) group (*n*=18), and were fed with a control diet and HFD, respectively, as described in Table 1. After six weeks of feeding, six rats from each of the control group and HFD group were sacrificed. In the next phase, according to Lee index (Bernardis, 1970), the obese rats from the HFD group (*n*=12) were further divided into two study groups. One group continued to receive HFD (continuing HFD group) and for the other group, dietary fat was replaced with RBO (RBO group). The rats from both groups were sacrificed after 6 weeks of feeding with respective diets. Figure 1 presents the design of the study and experimental protocols.

Preparation of control and experimental diets

Table 1 lists all the ingredients used to prepare the control and experimental diets. The ingredients were purchased from the local market and the diet was formulated following a standard protocol (Mridha *et al.,* 2010). RBO was purchased from a local market (Saffola Active, Marico Bangladesh Ltd, Dhaka, Bangladesh). According to the manufacturer's instructions, Saffola Active RBO contains saturated fatty acids 20%, monounsaturated fatty acids 39%, PUFA 40%, alpha-linolenic acids 2.4%, oryzanol 400 mg/100 g, vitamin E 25 mg/100 g, and energy 900 kcal/100 g, with no protein and carbohydrate. Rats had *ad libitum* access to water and diet.

Measurement of body weight

Body weight of experimental rats was recorded in triplicate at baseline

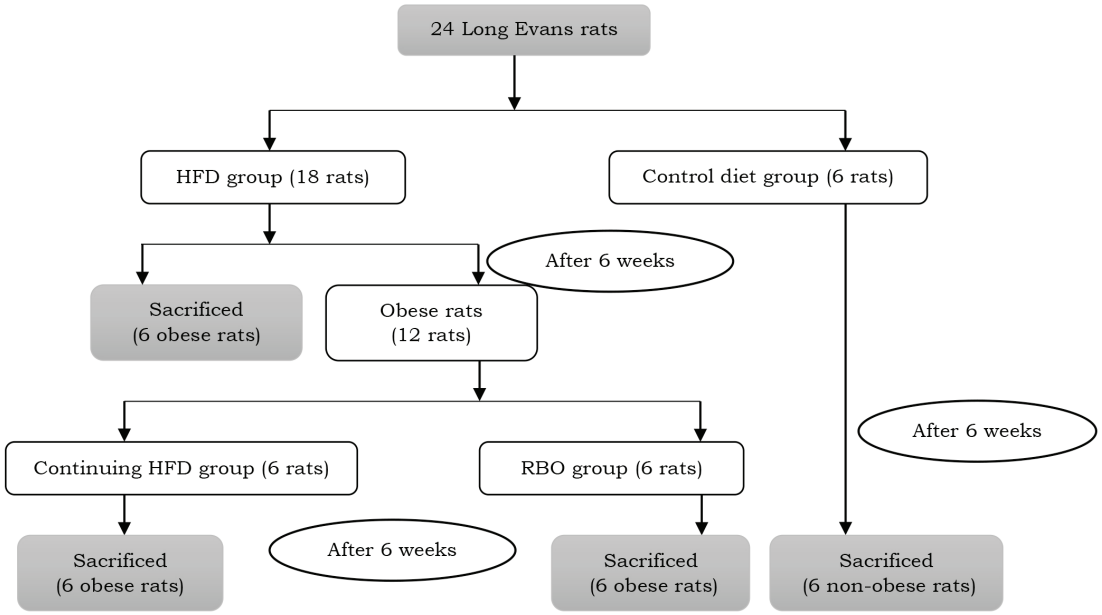

Figure 1. Flowchart of experimental protocols

Table 1. Composition of experimental diets (g/100 g) (Mridha *et al.,* 2010)

Ingredients	*Control diet (g)*	*High-fat diet (HFD) (g)*	*Rice bran oil (RBO) diet (g)*
Wheat	30.0	30.0	30.0
Wheat bran	25.0	25.0	25.0
Rice polish	25.0	25.0	25.0
Ghee	0.0	10.0	0.0
Egg yolk	0.0	5.0	0.0
Rice bran oil	0.0	0.0	15.0
Fish meal	7.0	7.0	7.0
Soybean cake	7.0	7.0	7.0
Vitamin GS	0.5	0.5	0.5
Soybean oil	1.5	1.5	1.5
Salt	1.5	1.5	1.5
Molasses	2.5	2.5	2.5

and weekly until the last day of the experiment. Average weight was used to calculate body weight gain per week.

Serum separation

At the end of each treatment period, rats were fasted for 12 hours and placed in metabolic cages. The rats were then insensible to an intraperitoneal injection of ketamin K (5 mg/100 g body weight; Abbott, IL, USA). Blood was sampled from the abdominal aorta into a sterile syringe and then into a tube, and allowed to clot at room temperature. The tubes were then centrifuged at 3000 rpm for 10 minutes, maintaining the temperature at 4°C (Clay-Adams Co. Inc. centrifuge, New York, USA). Serum was stored at -40°C until analysis.

Tissue isolation

Three regions of adipose tissue were carefully dissected, and interscapular brown adipose tissue (IBAT) was dissected as brown adipose tissue (BAT); dorsolumber, inguinal, and gluteal posterior subcutaneous depots were dissected as abdominal adipose tissue (AAT); and mediastinic, retroperitoneal, gonadal, and perirenal visceral depots were dissected as epididymal adipose tissue (EAT). For collecting IBAT, the rat was placed on its abdomen, with its head towards the investigator. To moisten the coat and avoid contaminating the samples with hair, the shoulder region was thoroughly rinsed with 70% ethanol. The skin was gripped with a tong and incised from the center of the head to the middle of the back revealing the butterfly shape of IBAT which was then carefully dissected as IBAT (Casteilla *et al.,* 2008). For collecting AAT, the rat was placed on its back with its tail towards the investigator. The abdomen was rinsed with ethanol, and the skin was widely incised. After removing the pad, the lymph nodes present among the fat were then discarded and dissected to harvest the AAT. After removing the AAT, the abdominal wall was opened to extract the genitals (ovaries or testes, according to sex) from the abdominal cavity. EAT was collected carefully by dissecting and gently pulling the fat tissue surrounding the gonadal tract and other tissues. All tissues were collected in warm saline, blotted, and weighed to the nearest milligram (Casteilla *et al.,* 2008).

Measurement of liver fat

Liver fat was determined following standard protocol (Domínguez-Avila *et al.,* 2015). Approximately 11 g of liver was sampled from each rat. The liver sample was then dried in an air oven

at 105°C for 24 hours. The dry weight was recorded and the percentage of water in the liver was calculated from the initial and final weights. The dried livers were crushed and inserted into pre-weighed cellulose thimbles and lipids were extracted for four hours in a Soxhlet apparatus using hexane as the solvent. Hexane was evaporated using a rotary evaporator and the flask was dried to a constant weight. The amount of lipids recovered was then calculated by measuring the difference in weight between the empty flask and its weight after the extraction was completed. This process was repeated three times and the mean value was recorded.

Biochemical measurements

Serum total cholesterol (TC), triacylglyceride (TAG), and high-density lipoprotein cholesterol (HDL-C), ALT,

Table 2. Obesity development phase (0–6 weeks)

A) Effect of high-fat diet (HFD) on body weight (Mean±SEM)

Group	*Initial body weight (g)*	*Final body weight (g)*	*p-value within the group*	*p-value between the groups*
Control diet group	59.0±4.4	207.0±14.0	<0.001	0.204
HFD group	55.0±3.3	231.0±9.5	<0.001	

B) Effect of high-fat diet (HFD) on the weight of different organs, lipid profile, and functional liver enzymes (Mean±SEM)

Parameter	*Control diet group*	*HFD group*	*p-value*
Liver (g)	10.1±0.8	10.2±0.7	0.924
Heart (g)	0.8±0.0	0.9±0.1	0.317
AAT (g)	1.6±0.2	3.8±0.2	<0.001*
EAT (g)	1.2±0.2	2.2±0.2	0.004*
IBAT (g)	0.3±0.1	0.6±0.0	0.001*
Liver fat (%)	2.2±0.1	2.6±0.1	0.010*
TC (mmol/L)	5.7±0.8	6.2±0.7	<0.001*
TAG (mmol/L)	6.4±1.2	6.8±0.8	0.003*
HDL-C (mmol/L)	2.4±0.6	1.8±0.7	<0.001*
LDL-C (mmol/L)	2.1±0.9	3.1±0.2	<0.001*
VLDL-C (mmol/L)	1.3±0.3	1.3±0.2	0.003*
TC/HDL-C	2.4±0.1	3.5±0.0	<0.001*
LDL-C/HDL-C	0.9±0.0	1.7±0.0	<0.001*
ALT (IU/L)	35.0±0.1	52.0±0.1	<0.001*
AST (IU/L)	117.0±0.1	142.0±0.1	<0.001*
ALP (IU/L)	196.0±0.0	202.0±0.1	<0.001*
GGT (IU/L)	5.8±0.1	6.9±0.1	<0.001*

AAT: abdominal adipose tissue; EAT: epididymal adipose tissue; IBAT: interscapular brown adipose tissue, TC: total cholesterol; TAG: triacylglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; VLDL-C: very low-density lipoprotein cholesterol; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase

Data are expressed as mean±SEM

*p<0.05 when comparing control diet to HFD

AST, alkaline phosphatase (ALP), and GGT were determined using standard clinical methods (enzyme colorimetric and enzyme kinetic methods) with a biochemical autoanalyser (Technicon Instruments Corporation, Tarrytown, NY, USA) using commercial kits (RANDOX kits; Randox Laboratories, Ltd., Antrim, UK). Low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) were determined using the Friedwald equations (Friedewald, Levy & Fredrickson, 1972), LDL-C (mg/dL) = TC (mg/dL) – HDL-C (mg/dL) – TAG (mg/dL)/5; VLDL = TAG/5.

Statistical analysis

Descriptive statistics were calculated for all variables by using the IBM SPSS Statistics for Windows version 25.0 (IBM Corp., Armonk, New York, NY, USA) and all values were expressed as mean±SEM. The significance of difference between the means of two groups was determined by independent sample Student's *t*-test. Differences were considered significant at $p<0.05$.

RESULTS

Obesity development phase (0–6 weeks)

Effect of HFD on body weight

Feeding the rats with HFD for six weeks resulted in increased body weight in both the control and HFD groups ($p>0.05$). However, it did not differ significantly between groups ($p<0.05$), as shown in Table 2A.

Effect of HFD on the weights of liver, heart, adipose tissue, and liver fat

HFD caused an insignificant ($p>0.05$) elevation in the weights of the liver and heart when compared to the control diet group in the first phase. The good physiological conditions had mildly increased the weight of the liver. Conversely, the weights of AAT, EAT, BAT, and liver fat increased significantly ($p<0.05$) than the control diet group, as presented in Table 2(B).

Hyperlipidaemic effect of HFD

Six-week feeding of HFD demonstrated a hyperlipidaemic effect in the HFD group and caused a significant elevation in serum TC, TAG, LDL-C, and VLDL cholesterol, but induced a significant reduction in serum HDL-C level compared to the control diet group ($p<0.05$). Lipid profile at week 6 is presented in Table 2(B).

Effect of HFD on functional liver enzymes

Besides developing hyperlipidaemia, HFD feeding for six weeks significantly increased the circulatory levels of functional liver enzymes (ALT, AST, ALP, and GGT) compared to the control group ($p<0.05$). Serum levels of liver enzymes at week 6 are presented in Table 2(B).

Experimental phase (6–12 weeks)

In the second phase, the remaining 12 rats from the HFD group (which became obese) were divided into two groups of similar average body weight; one group of rats continued the HFD (continuing HFD group), whereas the other group of rats were fed with a RBO diet (RBO group). After another six weeks of feeding with respective diets, all the rats were sacrificed and their serum, adipose tissue, and other organs were collected for analysis as described in the first phase.

Effect of RBO diet on body weight of obese rats

Both the HFD and RBO diet caused a continuous increase in body weight at the experimental phase (6–12 weeks) for six weeks in obese rats. This increase in body weight did not differ between groups, as shown in Table 3A.

Effect of RBO diet on the weights of liver, heart, adipose tissue, and liver fat

In the second phase (6–12 weeks), feeding of RBO diet in obese rats caused a non-significant reduction in the weights of the liver and heart compared to the continuing HFD group. Conversely, feeding on a diet with RBO caused a significant decrease (p<0.05) in the weights of AAT, EAT, IBAT, and liver fat compared to the continuing HFD group (Table 3B).

Hypolipidaemic effect of RBO diet on high fat-induced obese rats

A six-week feeding (6–12 weeks) of RBO diet on high fat-induced obese rats caused a significant reduction (p<0.05) in serum TC, TAG, LDL-C, and VLDL-C levels in the RBO diet group compared to the continuing HFD group. On the other hand, the RBO diet significantly (p<0.05) increased serum HDL-C in the RBO group in comparison to the continuing HFD group, as presented in Table 3B.

Table 3. Experimental phase (6–12 weeks)

A) Effect of rice bran oil (RBO) diet on body weight (Mean±SEM)

Group	*Initial body weight (g)*	*Final body weight (g)*	*p-value within the group*	*p-value between the group*
RBO diet group	230.0±7.4	326.0±17.6	0.003	0.744
Continuing high-fat diet group	218.0±11.2	335.0±18.9	<0.001	

B) Effects of continuing high-fat diet (HFD) and rice bran oil (RBO) diet on organ weight, lipid profile, and functional liver enzymes (Mean±SEM)

Parameters	*Continuing HFD group*	*RBO diet group*	*p-value*
Liver (g)	11.9±0.4	11.8±0.3	0.974
Heart (g)	1.0±0.1	0.9±0.0	0.531
AAT (g)	9.7±0.5	5.7±0.3	<0.001*
EAT (g)	7.1±0.6	5.1±0.2	0.008*
IBAT (g)	1.9±0.2	1.2±0.1	0.006*
Liver fat (%)	4.5±0.1	2.5±0.1	<0.001*
TC (mmol/L)	8.2±1.5	5.4±1.6	<0.001*
TAG (mmol/L)	8.8±9.9	5.1±1.4	<0.001*
HDL-C (mmol/L)	2.7±2.3	3.0±0.9	0.038*
LDL-C (mmol/L)	3.8±4.2	1.4±0.9	<0.001*
VLDL-C (mmol/L)	1.8±2.0	1.0±0.3	<0.001*
TC/HDL-C	3.1±0.1	1.8±0.0	<0.001*
LDL-C/HDL-C	1.4±0.1	0.5±0.1	<0.001*
ALT (IU/L)	56.0±0.0	39.0±0.0	<0.001*
AST (IU/L)	147.0±0.2	121.0±0.1	<0.001*
ALP (IU/L)	209.0±0.1	222.0±0.1	<0.001*
GGT (IU/L)	7.3±0.0	6.1±0.0	<0.001*

AAT: abdominal adipose tissue; EAT: epididymal adipose tissue; IBAT: interscapular brown adipose tissue, TC: total cholesterol; TAG: triacylglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; VLDL-C: very low-density lipoprotein cholesterol; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase

Data are expressed as mean±SEM

*p<0.05 when comparing continuing HFD to RBO diet

Effect of HFD on functional liver enzymes
Continuous feeding of HFD for another six weeks increased the circulatory levels of functional liver enzymes. However, rats fed with RBO diet resulted in reduced ALT, AST, and GGT levels, except ALP ($p<0.05$), compared to the continuing HFD rats. Serum levels of liver enzymes at week 12 are presented in Table 3B.

Efficiency of RBO diet on improving lipid profile of high fat-induced obese rats
RBO supplementation significantly improved the lipid profile of obese rats. RBO diet in comparison to the continuing HFD decreased 45.0% TC, 55.7% TG, and 18.8% LDL-C, and increased 76.3% HDL-C serum concentration, as presented in Table 4.

DISCUSSION

To conduct the present investigation, Long Evans rats were subjected to a controlled HFD for six weeks to promote adiposity and induce an imbalance in their lipid profile. Subsequently, obese and hyperlipidaemic rats were further fed with a diet supplemented with RBO for an additional six weeks and their effects on organ-specific fat deposition and regulation of hyperlipidaemia were compared with those rats that continued with HFD. Our results revealed that a RBO-supplemented diet for six weeks led to favourable outcomes, including maintenance of body weight, reduced organ-specific fat deposition, improved regulation of hyperlipidaemia, and restoration of liver function in obese rats.

In the present study, we observed an increase in food intake among all rats (control and HFD groups) during the initial six weeks of the study. Incorporation of high-fat increased the diet's palatability and calorie content, leading to altered body fat distribution and lipid profile in comparison to the control group. The present findings demonstrated a significantly higher AAT, EAT, IBAT, and hepatic fat, as well as an abnormal lipid profile with significantly higher TC, TAG, LDL-C, VLDL-C, and lower HDL-C in HFD rats compared to the control group. Although the HFD-fed rats showed a significantly greater fat mass than the control group, we observed a slightly greater body weight in HFD-fed rats, which may be attributed to the short-term feeding period. However, the achievement of targeted alterations in fat depots and lipid profiles in HFD rats provided a suitable pre-clinical model for evaluating the impact of RBO diet over HFD.

Energy-dense diets are considered a major factor in the development of obesity. Previous research supports that feeding animals with HFD results in a higher proportion of visceral adipose tissue compared to animals fed with a low-fat diet for the same period (Hariri & Thibault, 2010). During the experimental phase, feeding rats with fats from different sources (either RBO or continuing HFD) for six weeks resulted in a non-significant difference in body weight. Results related to the

Table 4. Efficiency of rice bran oil (RBO) diet over high-fat diet (HFD) in improving lipid profile

Group	*TC (%)*	*TAG (%)*	*LDL-C (%)*	*HDL-C (%)*
Continuing HFD to obese rats	33.3 ↑	30.3 ↑	23.6 ↑	50.0 ↑
RBO diet to obese rats	11.7 ↓	25.4 ↓	52.7 ↓	68.8 ↑

↑ indicates increase
↓ indicates decrease

impact of dietary fat content or total energy consumption on adiposity and body weight are inconsistent. In our study, the absence of a difference in body weight might be related to identical total energy consumption between the experimental groups. However, we have shown that feeding obese rats with RBO diet significantly reduced fat storage in AAT, EAT, and IBAT compared to HFD-fed rats. These outcomes align with a previous study that reported reduced epididymal fat pads in rat models after feeding with a diet supplemented with RBO compared to rats fed with a high-cholesterol control diet (Ha *et al.,* 2005). Contrary to our findings, another study demonstrated no significant changes in fat deposition in different fat depots when lean and obese rats were fed with high butter fat (Rolland *et al.,* 2002). Further reports suggested that mice fed with RBO prevented the growth of white adipose tissue and enhanced lipid metabolism (Al-Okbi *et al.,* 2014). Such observations correspond to the concept that although the energy density of a diet impacts body weight, it is the type and quality of fats in the diets that affect organ-specific fat deposition.

Accumulation of abdominal fat is associated with the development of insulin resistance, hyperlipidaemia, and metabolic syndrome in humans and rodent models (Wajchenberg *et al.,* 2002). In obese rats, surgical removal of EAT has been shown to enhance insulin action (Gabriely *et al.,* 2002). As reflected from the outcomes of our study, the reduction of fats from various fat depots in response to feeding on a RBO diet might play a protective role against the development of cardiometabolic diseases. Additionally, progressive hepatic fat accumulation is a risk factor for NAFLD, the leading cause of liver disease in the United States (Provencher, 2014), affecting 10%–35% of the adult population globally. In our study, feeding obese rats a RBO diet for six weeks significantly reduced liver fat content compared to the continuing HFD-fed rats, which is in line with a previous study (Al-Okbi *et al.,* 2014). Fat deposition in major fat depots, such as visceral fats, are shown to be linked with metabolic disorders and increased GGT and ALT levels (Liu *et al.,* 2013). Interestingly, our study demonstrated that feeding RBO resulted in enhanced serum levels of major functional liver enzymes (ALT, AST, and GGT) when compared with HFD-fed rats. These findings support the protective effect of RBO against the progression of fatty liver disease, which may be due to the presence of beneficial bioactive compounds.

In the present study, feeding obese rats with a diet containing RBO for six weeks demonstrated a robust hypolipidaemic effect by significantly reducing blood TC, TAG, and LDL-C levels compared to rats fed with HFD. Consistent with current findings, previous animal studies have reported similar reductions in serum cholesterol levels through RBO supplementation (Al-Okbi *et al.,* 2014). In a study comparing RBO to coconut oil, rats fed with RBO had a 35.5% reduction in serum cholesterol concentration (Reena & Lokesh, 2007). Furthermore, feeding rats with blended oils containing balanced fatty acids has been shown to lower serum and liver lipids. Another study reported significant reductions in serum TC, LDL-C, and TAG levels (23.8%, 32.4%, and 13.9%, respectively) in rats fed with coconut oil and RBO compared to rats given coconut oil alone (Reena & Lokesh, 2007). Although there is no significant difference in cholesterol-lowering potency between wheat bran oil and RBO, higher cholesterol-lowering potency was observed with RBO over high-cholesterol diets in rodents (Lei *et al.,* 2018). RBO is a rich source of bioactive compounds like γ-oryzanols,

tocopherols, phytosterols, tocotrienols, ferulic acid, and fatty acids (Latha & Nasirullah, 2014). Oleic acid and linoleic acid, which are the major fatty acids present in RBO, have been shown to play a crucial role in reducing blood cholesterol in both humans and rats (Barakat & Mahmoud, 2011). Tocotrienols have been shown to reduce cholesterol biosynthesis, whereas γ-oryzanols suppress cholesterol absorption, enhance faecal sterol excretion, and lower the levels of triglycerides and phospholipids, according to reports from animal and human studies (Chithra *et al.,* 2015).

Besides that, the RBO diet significantly increased serum HDL-C concentration in obese rats in comparison with continuing HFD-fed rats. These results are in agreement with previous animal studies that reported that HDL-C increased by 20% within 18 weeks in rats receiving RBO at the level of 20% in their diet in comparison to rats fed with peanut oil (Liang *et al.,* 2021). Numerous observational and intervention studies have shown the effect of HDL-C on reversing cardiovascular diseases (Siddiqi, Kiss & Rader, 2015). The ratios of TC/HDL-C and LDL/HDL-C are considered as more sensitive and specific indices of cardiovascular risk than total cholesterol alone. These ratios, known as atherogenic indices, are better predictors of cardiovascular risk than LDL cholesterol alone (Ridker *et al.,* 2005). Our results are consistent with previous studies that reported a significant reduction in the TC/HDL-C and LDL-C/HDL-C ratios of obese rats fed with RBO compared to those fed with continuing HFD (Chithra *et al.,* 2015). Based on our findings, we suggest that RBO may improve lipoprotein profile and reduce the risk of metabolic diseases and CVD in obese rats.

Some limitations in our study should be acknowledged. Firstly, we did not measure regular food intake, as rats had *ad libitum* access to food. This may have resulted in higher dietary energy intake for the HFD group compared to the RBO diet group, as reported in a previous study (Miras *et al.,* 2014). However, the lack of a significant difference in body weight between groups suggests the rats had similar food and energy intakes. Secondly, we did not analyse our findings by gender of the rats, which may affect the deposition of fat in specific organs (Hariri & Thibault, 2010). Finally, we did not assess the fatty acid composition of HFD or RBO diets. Moreover, the fatty acid profile of adipose tissues was not assessed, limiting our ability to describe the contribution of dietary fat composition to the deposition of fat in specific organs. To gain better understanding of the underlying mechanisms, these limitations should be addressed in future studies.

CONCLUSION

In conclusion, the study has shown that RBO induced a depot-dependent reduction in adiposity without changes in the body weight of rats. Similarly, these achieved improvements in reducing adiposity demonstrated a remarkable impact on regulating lipid profiles and restoring healthy liver functions in hyperlipidaemic rats. Although these findings are promising, it should be noted that the study was conducted on animals and may not necessarily extrapolate directly to humans. RBO's hypolipidaemic effect might have an effect on lowering cholesterol levels, regulating lipid profiles, and improving liver functions in people. Further studies are required to explore the effects of RBO on lipid metabolism and liver function in humans.

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Authors' contributions

Alam MJ, Hasan MK and Alim MA, responsible for methodology, clinical trial, rat management and feeding, formal analysis, data curation, writing original draft; Akter S, led the preparation of the draft and reviewed the manuscript; Linkon KMMR and Prodhan UK, accountable for supervision, conceptualisation, methodology, assisted in drafting, reviewing and editing.

Conflicts of interest

The authors have no conflicts of interest to declare. The authors provided personal resources for funding this project.

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