

Article

Dietary Intervention with *Hibiscus sabdariffa* L. Beverage Residue Attenuates Dyslipidemia and Hepatic Steatosis in Late-Stage Type 2 Diabetic Rats

Evelyn Regalado-Rentería ¹, Jesús E. Serna-Tenorio ², David G. García-Gutiérrez ¹, Rosalía Reynoso-Camacho ³,
Miriam A. Anaya-Loyola ² and Iza F. Pérez-Ramírez ^{1,*}

¹ Department of Clinical Chemistry and Molecular Diagnosis, School of Chemistry, Universidad Autónoma de Querétaro, Querétaro 76010, Mexico; evelyn.regalado.renteria@gmail.com (E.R.-R.); davidggtz@gmail.com (D.G.G.-G.)

² Department of Research in Human Nutrition, School of Natural Sciences, Universidad Autónoma de Querétaro, Querétaro 76230, Mexico; eduardoserna1808@gmail.com (J.E.S.-T.); aracely.anaya@uaq.mx (M.A.A.-L.)

³ Department of Research and Graduate Studies in Food Science, School of Chemistry, Universidad Autónoma de Querétaro, Querétaro 76010, Mexico; rrcamachomx@yahoo.com.mx

* Correspondence: iza.perez@uaq.mx

Abstract

Roselle beverage residue (RBR), a by-product of *Hibiscus sabdariffa* L. processing, retains bioactive compounds, including soluble and insoluble dietary fiber and polyphenols. Its antihyperglycemic effect in type 2 diabetes mellitus (T2DM) has been previously demonstrated; however, its role in lipid metabolism remains unknown. This study assessed the preventive and therapeutic potential of RBR on dyslipidemia and hepatic steatosis in a rodent model of late-stage T2DM characterized by hyperglycemia and hypoinsulinemia. Male Wistar rats with T2DM induced by a high-fat and high-fructose diet combined with streptozotocin received 6% RBR supplementation as either a preventive intervention (starting at week 1 in healthy rats or week 9 in insulin-resistant rats) or a therapeutic intervention (starting at week 14 in diabetic rats). After 17 weeks, RBR supplementation significantly reduced serum triglycerides and total cholesterol, attenuating hepatic lipid accumulation regardless of the timing of intervention. Hepatic *Acadm* expression, involved in fatty acid β -oxidation, was significantly upregulated in rats treated with RBR from week 1 and 9, whereas no significant modulation was observed for genes related to fatty acid synthesis or uptake. These findings suggest that RBR supplementation may contribute to improving lipid metabolism and hepatic steatosis in a rat model of late-stage T2DM.

Keywords: type 2 diabetes mellitus; dyslipidemia; hepatic steatosis; dietary intervention; *Hibiscus sabdariffa*; roselle; residue; polyphenols; dietary fiber; Wistar rats



Academic Editor: Dongmin Liu

Received: 6 February 2026

Revised: 28 March 2026

Accepted: 1 April 2026

Published: 8 April 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and

conditions of the [Creative Commons](https://creativecommons.org/licenses/by/4.0/)

[Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

Type 2 diabetes mellitus (T2DM), a highly prevalent chronic metabolic disease affecting 463 million individuals worldwide, represents a major global health burden, with rapidly increasing prevalence across both developed and developing countries [1]. T2DM is characterized by insulin resistance and progressive pancreatic β -cell dysfunction, leading to chronic hyperglycemia and multiple metabolic complications. Among these, metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease (NAFLD), affects approximately one quarter of the global adult population [2,3].

The pathogenesis of T2DM-associated MASLD involves alterations in lipid metabolism, including dysregulation of genes and proteins involved in hepatic fatty acid synthesis, uptake, and β -oxidation [4].

Current clinical management of T2DM and its metabolic complications relies largely on pharmacological interventions. These therapies are effective for glycemic control and may improve hepatic steatosis; however, long-term treatment has been associated with adverse effects, including gastrointestinal symptoms, weight gain, hypoglycemia, or hepatic dysfunction [5]. Consequently, there is growing interest in dietary supplements with the potential to complement pharmacological therapies by modulating metabolic pathways involved in T2DM and its associated complications [6].

Roselle (*Hibiscus sabdariffa* L.), a member of the Malvaceae family, is widely cultivated in tropical and subtropical regions and is traditionally used for the preparation of foods and beverages. It has been widely investigated for its potential metabolic benefits, particularly due to its high content of dietary fiber and polyphenols. Several studies have reported hypoglycemic, hypolipidemic, and hepatoprotective effects of roselle extracts in experimental models and clinical trials [7,8].

In parallel, the valorization of agro-industrial residues as sources of functional dietary components has emerged as a promising and sustainable approach to support metabolic health [9]. Regarding roselle processing, the residue generated during decoction preparation (roselle beverage residue, RBR) retains high amounts of both soluble and insoluble dietary fiber as well as non-extractable polyphenols (NEPP), despite thermal processing [10].

We previously demonstrated the anti-hyperglycemic and hypoglycemic effect of RBR supplementation in T2DM rats [11]. Moreover, RBR showed a similar efficacy as compared to roselle calyxes in preventing body weight gain, insulin resistance, and hepatic steatosis in a diet-induced obesity rodent model [12]. However, the potential of RBR in modulating lipid metabolism in the context of late-stage T2DM remains unknown. Furthermore, the effects of RBR supplementation initiated at different stages of T2DM development have not been explored. Therefore, this study aimed to evaluate the preventive and therapeutic potential of RBR supplementation on MASLD in a rodent model of late-stage T2DM.

2. Materials and Methods

2.1. Preparation of Roselle Beverage Residue

Roselle (*Hibiscus sabdariffa* L.) calyxes were obtained from local farmers in Guerrero, México. RBR used in this study was generated during the preparation of roselle decoction and subsequently dried for its incorporation into the experimental diet. The preparation procedure and chemical characterization have been previously described in detail by Serna-Tenorio et al. [10].

2.2. Animal Model and Experimental Design

All experimental procedures involving animals were conducted according to the Guide for the Care and Use of Laboratory Animals. The protocol was reviewed and approved by the Bioethics Committee of the School of Natural Sciences of the Autonomous University of Querétaro (approval number: 53FCN2022). The detailed experimental conditions were previously described by Regalado-Rentería et al. [11].

Briefly, male Wistar rats ($n = 50$, 180–200 g) were randomly distributed into five experimental groups ($n = 10$ per group). The healthy control group was fed *ad libitum* with a standard diet (SD), while the remaining groups were fed *ad libitum* with a high-fat and high-fructose diet (HFFD; Table S1) and received a single intraperitoneal injection of streptozotocin (30 mg/kg) at week 13. One week later, diabetes was confirmed by fasting glucose levels > 150 mg/dL. A late-stage T2DM-like phenotype was observed at week 17,

characterized by persistent hyperglycemia accompanied by hypoinsulinemia resulting from pancreatic islet atrophy and glomerular hyperfiltration [11].

Three experimental groups received daily RBR supplementation (6% mixed with HFFD) beginning at different time points: from week 1 (preventive intervention in healthy rats), week 9 (preventive intervention in insulin-resistant rats) or week 14 (therapeutic intervention in T2DM rats), until the end of week 17 [11]. Daily food consumption was recorded to estimate the daily intake of dietary fiber and polyphenols based on the reported bioactive composition of RBR [10].

2.3. Biochemical Measurements

At the end of the experiment, fasted animals were euthanized after an overnight fast (12 h), and blood samples and liver tissues were collected [11]. Serum triglycerides, total cholesterol, and high-density lipoprotein (HDL) levels were determined using commercial kits (Spinreact, Girona, Spain) in an automated chemistry analyzer (Selectra Pro M, ELITech Group, Paris, France). The atherogenic index was calculated as $\log(\text{triglycerides}/\text{HDL})$ and the Castelli risk index as the ratio of total cholesterol to HDL.

2.4. Histological Analysis

Liver tissue was rinsed in 0.85% saline solution and divided into two portions. One portion was snap-frozen in liquid nitrogen and stored at -70°C for gene expression analysis. The second portion was fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned for histological analysis. Sections were stained with hematoxylin and eosin (H&E) and observed at $40\times$ magnification. Six sections per animal were analyzed.

2.5. RNA Extraction and qRT-PCR Analysis

For gene expression analysis, total RNA was extracted from liver samples, reverse transcribed to cDNA and analyzed by quantitative real-time PCR under the conditions described by Regalado-Rentería et al. [11]. RNA purity and integrity were assessed prior to cDNA synthesis by spectrophotometry and agarose gel electrophoresis, respectively. Genes involved in lipid metabolism were evaluated using the following primers: *Acadm* (Fw-TACTGCGTGACAGAACCC; Rv-TTTTCCGATGTGTATTCCC), *Cd36* (Fw-AGGAAAGCCTGTGTACATTCT; Rv-GTCCTATGCTCATCTTCGTTAGG), *Cpt1a* (Fw-CCGAGCCAGGAGATATAGATAGA; Rv-GAATCTGACTGGGTGGGATTAG), *Fatp5* (Fw-TGCCAAGCTTCGTGCTAATA; Rv-TGATAGGATGGCTGGCTTTG), *Acaca* (Fw-AGGAAGATGGTGTCCGCTCTG; Rv-GGGGAGATGTGCTGGGTCAT) and *Fasn* (Fw-CCATTTCATTGCCCTTAGCC; Rv-GTAACACATGCTGCTCAAACGA). Primer specificity was confirmed by melting curve analysis. Relative mRNA expression was calculated by normalization against β -actin as housekeeping gene according to the $2^{-\Delta\Delta\text{Ct}}$ method [13].

2.6. Data Analysis

Statistical analyses were performed using JMP software version 16 (JMP Statistical Discovery LLC, Cary, NC, USA). Outliers were identified through box-and-whisker plots, and extreme values (>3 IQR) were excluded. Two animals from the T2DM control group were identified as extreme outliers and excluded from the statistical analyses. Normality and homogeneity of variance were assessed using the Kolmogorov–Smirnov test and Levene’s test, respectively. Parametric data were analyzed by one-way ANOVA followed by Tukey’s test for multiple comparisons and Dunnett’s test when comparing to the control groups. Non-parametric variables were analyzed using the Kruskal–Wallis test followed by the all-pairs Wilcoxon (Steel–Dwass) test for multiple comparisons and Dunn’s test when comparing to the control group.

3. Results

3.1. RBR-Derived Dietary Fiber and Polyphenol Daily Supplementation

The estimated daily intake of dietary fiber and both extractable and non-extractable polyphenols from RBR supplementation is shown in Table 1. Insoluble dietary fiber was the major fraction, while caffeoylquinic acid derivatives represented the most abundant polyphenols, followed by dihydroxybenzoic and coumaroylquinic acids. No significant differences in food consumption were observed among the RBR-supplemented groups, nor when compared to the unsupplemented diabetic control group.

Table 1. Dietary fiber and polyphenols daily supplementation from roselle beverage residue.

Component	Daily Supplementation ¹
Dietary fiber (g/day)	
Total dietary fiber	1.18–2.37
Soluble dietary fiber	0.27–0.53
Insoluble dietary fiber	0.93–1.86
Extractable polyphenols (mg/day)	
Delphinidin hexoside	0.27–0.53
Delphinidin sambubioside	0.53–1.06
Kaempferol	0.11–0.22
Kaempferol hexoside-rhamnoside	0.09–0.18
Kaempferol pentoside-hexoside	0.34–0.68
Myricetin	0.22–0.43
Myricetin hexoside	0.12–0.25
Myricetin rhamnoside	0.06–0.11
Myricetin rutinoside	0.03–0.06
Quercetin	1.04–2.07
Quercetin hexoside	0.30–0.59
Quercetin hexoside-rhamnoside	0.49–0.98
Quercetin pentoside-rutinoside	0.02–0.03
Coumaric acid	0.08–0.17
Coumaroylquinic acid isomer I	2.28–4.57
Coumaroylquinic acid isomer II	3.08–6.17
Dihydroxybenzoic acid isomer I	0.47–0.95
Dihydroxybenzoic acid isomer II	0.16–0.33
Dihydroxybenzoic acid hexoside	7.25–14.50
Gallic acid	0.50–1.01
Gallic acid ethyl ester	0.18–0.36
Hydroxybenzoic acid	2.66–5.33
Methylgallic acid	0.14–0.28
Trigallic acid hexoside	0.11–0.23
Vanillic acid	0.15–0.30
Caffeic acid	0.43–0.86
Caffeic acid hexoside	0.15–0.30
Caffeoylquinic acid isomer I (chlorogenic acid)	20.61–41.22
Caffeoylquinic acid isomer II	13.04–26.07
Ferulic acid	0.03–0.06
Feruloylquinic acid isomer I	1.50–3.00
Feruloylquinic acid isomer II	1.09–2.19
Feruloylquinic acid isomer III	1.29–0.59
Sinapic acid hexoside	0.24–0.49
Acid hydrolysable polyphenols (mg/day)	
Dihydroxybenzoic acid isomer I	0.01–0.02
Hydroxybenzoic acid	0.04–0.08

Table 1. *Cont.*

Component	Daily Supplementation ¹
Ferulic acid	0.00–0.01
Sinapic acid	0.01–0.02
Alkaline hydrolysable polyphenols (mg/day)	
Hydroxybenzoic acid hexoside	0.07–0.14
Coumaric acid	0.02–0.04
Caffeic acid hexoside	0.00–0.01

¹ RBR was supplemented at 6% in the high-fat and high-fructose diet. The average food intake was 30 g/day, which was monitored daily throughout the experiment.

3.2. RBR Supplementation Improves Dyslipidemia and Atherogenic Risk in Late-Stage T2DM Rats

At the time of the primary preventive intervention (week 1), animals had not yet developed metabolic alterations and were considered metabolically healthy. Insulin resistance progressively developed during the high-fat and high-fructose diet intervention, whereas diabetes was induced at week 13 and confirmed at week 14. All outcomes presented in the following sections correspond to the metabolic status at week 17, when HFFD-fed rats displayed a late-stage TDM phenotype.

The preventive and therapeutic effect of RBR intervention on serum lipid levels is shown in Table 2. T2DM rats showed a significant increase in triglyceride and cholesterol levels as compared to the healthy control group (9.4- and 2.1-fold, $p < 0.05$). RBR supplementation initiated at all time points significantly reduced serum triglyceride and cholesterol levels as compared to the T2DM control group (3.2 to 4.5- and 1.9 to 2.0-fold, respectively), with similar values than the healthy control group.

Table 2. Effect of roselle beverage residue on serum lipid profile in late-stage T2DM rats.

Parameter	Control Groups		RBR Intervention Groups		
	Healthy	T2DM	From Week 1	From Week 9	From Week 14
Triglycerides ¹	69.30 ± 1.86 ^{b,†}	649.80 ± 76.22 ^a	145.29 ± 10.05 ^{b,†}	185.89 ± 13.18 ^{b,†}	204.43 ± 20.66 ^{b,†}
Cholesterol ¹	54.6 ± 0.86 ^{b,†}	114.00 ± 6.88 ^a	61.43 ± 1.87 ^{b,†}	56.63 ± 1.79 ^{b,†}	61.29 ± 2.83 ^{b,†}
HDL ¹	47.60 ± 0.73 ^{b,†}	74.83 ± 4.00 ^a	61.00 ± 2.28 ^{a,b}	51.38 ± 1.57 ^{a,b}	60.11 ± 2.25 ^{a,b}
Atherogenic index	0.15 ± 0.01 ^{c,†}	0.77 ± 0.10 ^a	0.30 ± 0.02 ^{b,c,†}	0.53 ± 0.03 ^{a,b}	0.46 ± 0.03 ^{a,b}
Castelli risk index	1.12 ± 0.01 ^a	1.21 ± 0.06 ^a	1.02 ± 0.01 ^a	1.10 ± 0.01 ^a	1.19 ± 0.01 ^a

¹ Data are expressed as mg/dL. Data are shown as mean ± standard deviation ($n = 10$ per group except T2DM control, $n = 8$ after outlier exclusion). Different letters indicate significant ($p < 0.05$) differences between samples by Tukey's test or Kruskal–Wallis test. [†] Indicates significant ($p < 0.05$) difference as compared to the HFFD group by Dunnet's test or Wilcoxon test. RBR: roselle beverage residue; T2DM: type 2 diabetes mellitus.

No significant differences were detected in HDL levels between the RBR-supplemented groups and the T2DM control group. Regarding the atherogenic index, only rats supplemented with RBR from week 1 showed a significant reduction compared with the T2DM control group, indicating that the most pronounced effect was observed with the primary preventive intervention. In contrast, no significant differences were found in the Castelli risk index among any of the experimental groups. Although total cholesterol levels increased in the T2DM group and decreased with RBR supplementation, HDL levels followed a similar pattern, resulting in relatively similar cholesterol-to-HDL ratios across groups.

3.3. RBR Supplementation Attenuates Hepatic Steatosis in Late-Stage T2DM Rats

The liver histological analysis of all experimental groups is shown in Figure 1. The healthy control group (Figure 1A) exhibited normal hepatic architecture, with well-defined hepatocytes and sinusoids. In contrast, the T2DM control group (Figure 1B) showed marked hepatic steatosis (lipid accumulation > 30% of hepatocytes), accompanied by sinusoidal

congestion and apparent Kupffer cell hyperplasia. The combination of hepatic steatosis, dyslipidemia, and sustained hyperglycemia (as reported previously [11]) is consistent with the histopathological and metabolic features of MASLD, a feature of late-stage T2DM. In all RBR-supplemented groups (Figure 1C: starting at week 1, Figure 1D: starting at week 9, Figure 1E: starting at week 14), decreased lipid vacuoles and Kupffer cell activation are observed as compared to the T2DM control group, regardless of the timing of intervention. These observations are based on morphological analysis of liver sections, since hepatic triglyceride content was not quantitatively determined in this study.

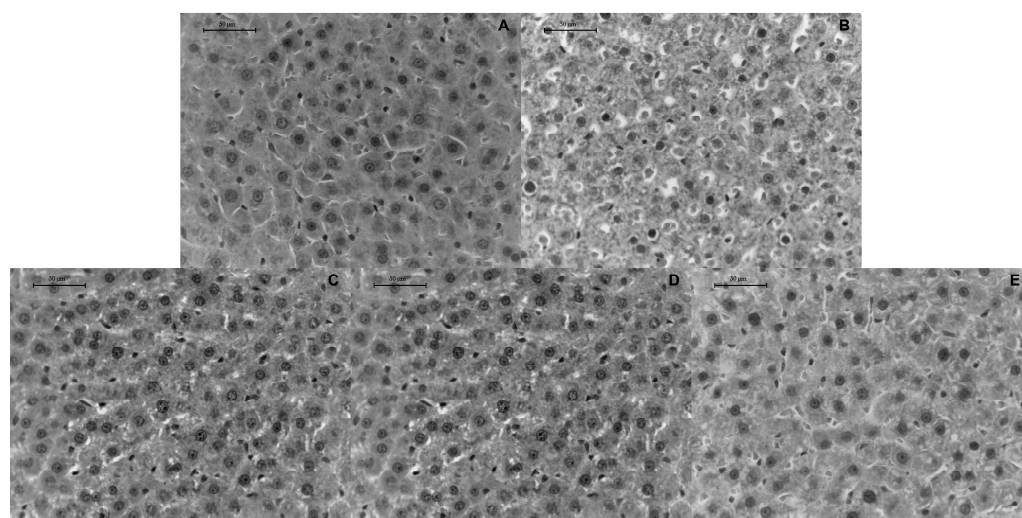


Figure 1. Histological analysis of the effect of roselle beverage residue on metabolic associated fatty liver disease in late-stage T2DM rats. (A) Healthy control group, (B) T2DM control group, (C) RBR from week 1, (D) RBR from week 9, and (E) RBR from week 14. RBR: roselle beverage residue; T2DM: type 2 diabetes mellitus. Images shown are representative of ten biological replicates per group, with six sections analyzed per animal. Micrographs were acquired at 40× magnification. Scale bar = 50 µm.

3.4. Modulation of Hepatic Lipid Metabolism Genes by RBR Supplementation in Late-Stage T2DM Rats

The relative expression of genes involved in hepatic lipid metabolism is shown in Figures 2–4. Regarding genes associated with de novo fatty acid synthesis (Figure 2), *Fasn* expression was decreased in all the T2DM groups as compared to the healthy control group; however, no significant differences were observed. In contrast, *Acaca* expression was significantly lower in the group supplemented with RBR from week 14 (therapeutic strategy) as compared to the healthy control group, but no significant differences were found between the T2DM control and the RBR-supplemented groups.

The T2DM control group showed slight downregulation of both genes involved in fatty acid uptake, *Fatp5* and *Cd36*, as compared to the healthy group, but no significant differences were found (Figure 3). No significant effect was observed with RBR supplementation. Regarding genes involved in fatty acid β-oxidation (Figure 4), *Cpt1a* expression was statistically similar among all the experimental groups. Finally, *Acadm* expression was significantly increased with RBR starting at week 1 or week 9 (primary and secondary preventive interventions, respectively) as compared to the T2DM control group (2.8- and 3.3-fold, respectively), showing expression values higher than the healthy group.

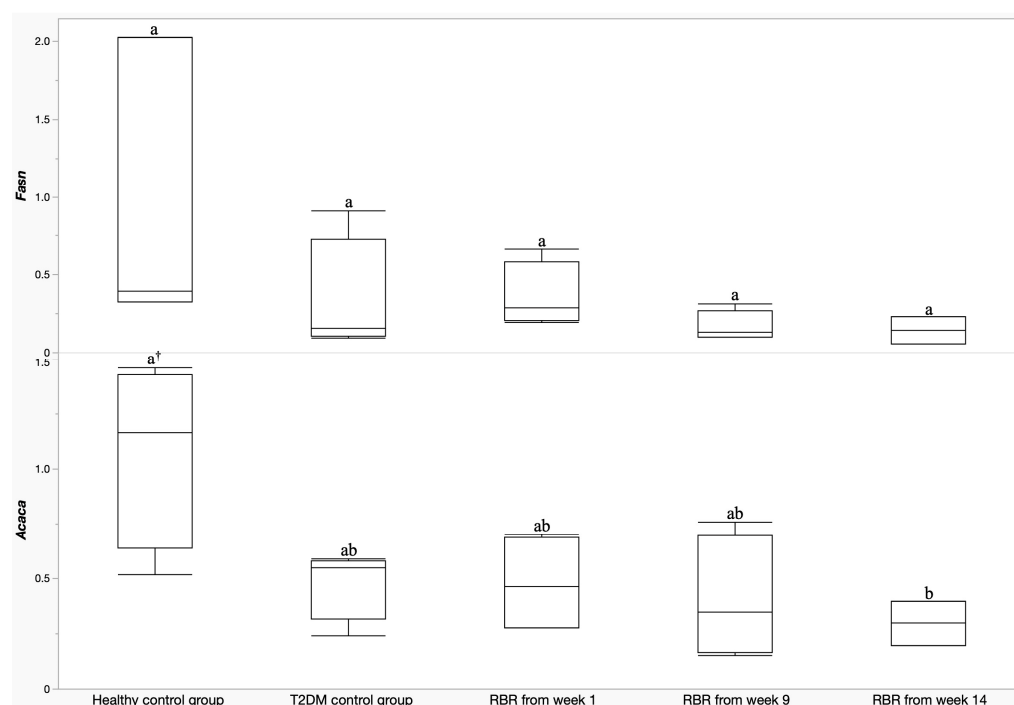


Figure 2. Relative expression of genes involved in hepatic fatty acid biosynthesis. Data are shown as mean values, and error bars represent the interquartile range ($n = 10$ per group except T2DM control, $n = 8$ after outlier exclusion). Different letters indicate significant ($p < 0.05$) differences between samples by Tukey's test or Kruskal–Wallis test. † Indicates significant ($p < 0.05$) difference as compared to the HFFD group by Dunnet's test or Wilcoxon test. RBR: roselle beverage residue; T2DM: type 2 diabetes mellitus; *Fasn*: fatty acid synthase; *Acaca*: Acetyl-CoA carboxylase alpha.

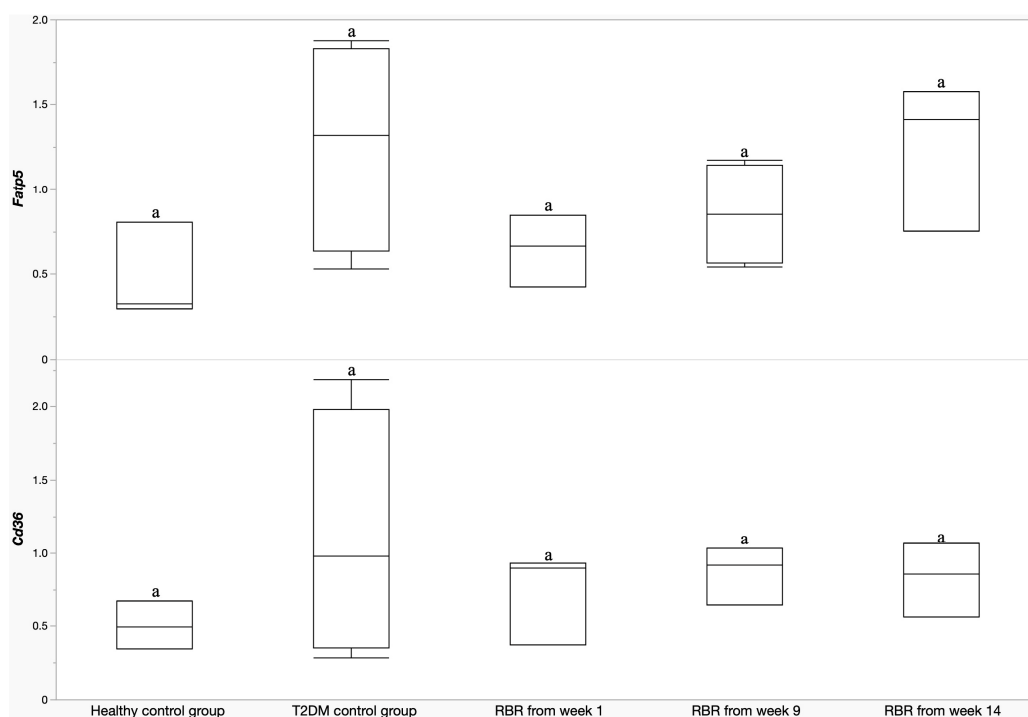


Figure 3. Relative expression of genes involved in hepatic fatty acid transport. Data are shown as mean values, and error bars represent the interquartile range ($n = 10$ per group except T2DM control, $n = 8$ after outlier exclusion). Different letters indicate significant ($p < 0.05$) differences between samples by Tukey's test or Kruskal–Wallis test. RBR: roselle beverage residue; T2DM: type 2 diabetes mellitus; *Fatp5*: fatty acid transport protein-5; *Cd36*: cluster of differentiation 36.

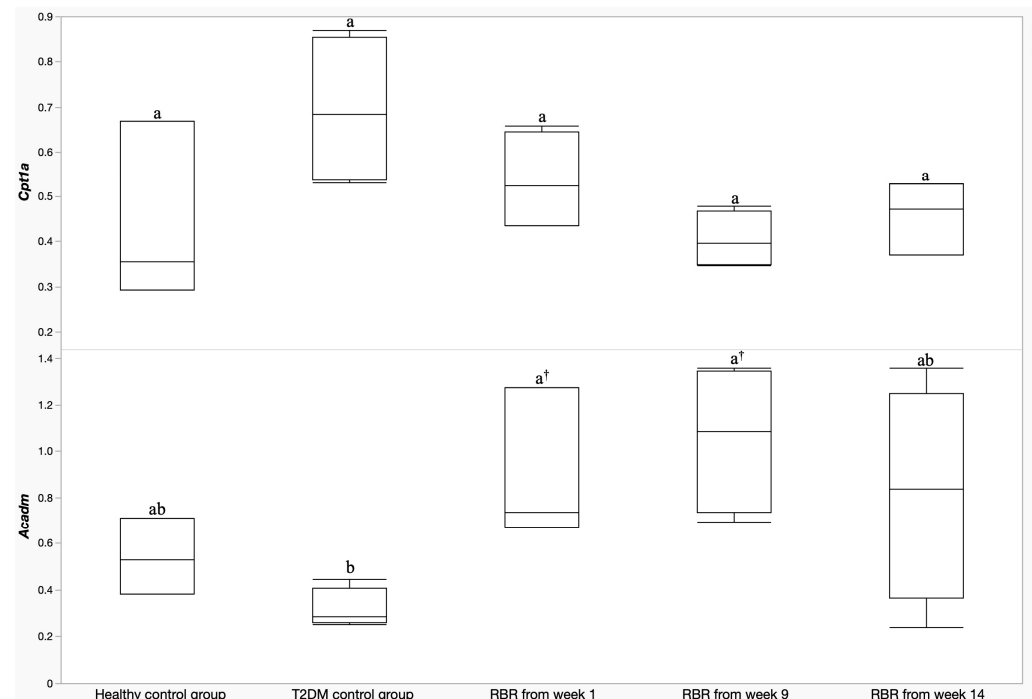


Figure 4. Relative expression of genes involved in hepatic fatty acid β -oxidation. Data are shown as mean values, and error bars represent the interquartile range ($n = 10$ per group except T2DM control, $n = 8$ after outlier exclusion). Different letters indicate significant ($p < 0.05$) differences between samples by Tukey's test or Kruskal–Wallis test. † Indicates significant ($p < 0.05$) difference as compared to the HFFD group by Dunnet's test or Wilcoxon test. RBR: roselle beverage residue; T2DM: type 2 diabetes mellitus; *Cpt1a*: carnitine palmitoyltransferase 1a; *Acadm*: medium-chain acyl-CoA dehydrogenase.

4. Discussion

This study describes the metabolic effects of RBR, a polyphenol- and dietary fiber-rich by-product of *Hibiscus sabdariffa* L. processing, when used as a dietary supplement in a rodent model of late-stage TD2M. Previous studies have primarily focused on the aqueous extracts or infusions of roselle calyxes, highlighting their content of anthocyanins, organic acids, and flavonoids. In contrast, we previously demonstrated that RBR retains substantial amounts of soluble and insoluble dietary fiber as well as both extractable and non-extractable polyphenols, particularly caffeoylquinic acid derivatives [10], suggesting that this by-product may represent an alternative dietary supplement.

Poor glycemic control exacerbates dyslipidemia and contributes to lipid-related metabolic dysfunction, including hepatic steatosis. Hyperglycemia and insulin resistance increases lipolysis in adipose tissue, increasing the flux of free fatty acids to the liver and promoting triglyceride accumulation. Previous studies have demonstrated that interventions improving glycemic control also reduce serum lipid levels and hepatic fat accumulation. These effects are largely mediated by improved insulin sensitivity and attenuation of inflammation and oxidative stress associated with the progression of hepatic steatosis [14].

In a previous study, we reported that RBR improved glucose metabolism in diabetic rats with comparable efficacy when administered as either a preventive or therapeutic intervention. These effects were partly associated with the modulation of genes involved in glucose signaling pathways [9]. Based on these findings, we hypothesized that RBR supplementation could also exert beneficial effects on lipid metabolism and hepatic fat accumulation in diabetic rats, mediated not only through improved glucose homeostasis but also through the direct metabolic effects of dietary fiber and polyphenols [15,16].

Consistent with this hypothesis, RBR supplementation significantly reduced serum triglycerides and total cholesterol levels, restoring values close to those observed in the healthy control group. Although HDL levels remained unchanged, the atherogenic index was significantly improved only in rats supplemented with RBR from week 1. Attenuation of dyslipidemia was accompanied by apparent reduced hepatic lipid accumulation and attenuation of structural alterations such as Kupffer cell hyperplasia and sinusoidal congestion. Notably, rats supplemented with RBR from week 1 showed pronounced improvements, suggesting that early dietary intervention may limit the progression of hepatic lipid accumulation under T2DM conditions. These findings are consistent with previous studies reporting hypolipidemic and hepatoprotective effects of nutraceutical strategies based on polyphenol- and dietary fiber-rich compounds in experimental models and clinical studies of MASLD [17].

At the molecular level, hepatic gene expression analysis showed that *Acadm*, which encodes the mitochondrial enzyme medium-chain acyl-CoA dehydrogenase (MCAD), was significantly upregulated in rats supplemented with RBR as a preventive intervention. MCAD catalyzes the first step of mitochondrial β -oxidation of medium-chain fatty acids, generating acetyl-CoA, which is further oxidized to produce ATP. Therefore, increased *Acadm* expression suggests reduced availability of substrates for triglyceride synthesis and contributes to decreased hepatic and circulating triglyceride levels [18,19].

In contrast, genes associated with lipogenesis (*Fasn* and *Acaca*) and fatty acid transport (*Cd36* and *Fatp5*) were not significantly modulated by RBR supplementation. Notably, *Cd36* and *Fatp5* showed a tendency toward higher expression in the T2DM control group compared with healthy rats, whereas a slight downregulation of *Fasn* and *Acaca* was observed, but no significant differences were observed. These findings may reflect the late-stage T2DM phenotype of this model, characterized by hypoinsulinemia secondary to pancreatic β -cell dysfunction induced by streptozotocin, which may limit the activation of hepatic insulin-dependent lipogenic pathways [20].

The mechanisms underlying these effects are likely multifactorial. Soluble dietary fiber present in RBR may modulate lipid metabolism by binding bile acids and promoting their excretion, thereby promoting hepatic conversion of cholesterol into bile acids. In addition, its viscosity may reduce the digestion and absorption of carbohydrates and lipids. Insoluble dietary fiber, which represents the major fraction in RBR, is mainly associated with increased fecal bulk and enhanced colonic transit, which may contribute to reduced lipid absorption [15]. Furthermore, the colonic fermentation of dietary fiber can produce short-chain fatty acids such as butyrate, which may activate peroxisome proliferator-activated receptor alpha (PPAR α), a nuclear receptor that upregulates genes involved in fatty acid β -oxidation, including *Acadm* [21].

Polyphenols present in RBR may also contribute to these metabolic effects. Chlorogenic acid, the major polyphenol found in RBR, has been reported to improve dyslipidemia and reduce hepatic triglyceride accumulation [22]. Moreover, chlorogenic acid has been shown to activate AMP-activated protein kinase (AMPK) signaling [23] and increase hepatic PPAR α levels [24]. On the other hand, some dihydroxybenzoic acids, also found in RBR, have been reported to reduce lipid accumulation in adipocytes by promoting lipolysis and fatty acid β -oxidation [24] and to decrease lipid accumulation in cardiac tissue through AMPK activation [25]. Nevertheless, their effects on hepatic lipid metabolism have not been reported; thus, these mechanisms may not directly reflect hepatic metabolic responses.

Although the present study demonstrated improvements in dyslipidemia and hepatic steatosis in late-stage T2DM rats following RBR supplementation, several limitations should be acknowledged. First, the causal relationships between individual RBR components and the molecular effects observed remain to be elucidated. Although gene expression

analysis suggested enhanced fatty acid β -oxidation, as reflected by increased *Acadm* expression, MCAD enzymatic activity and hepatic levels of intermediate metabolites were not directly measured. In addition, hepatic lipid content was not quantitatively assessed, and therefore histological findings should be interpreted as descriptive. Differences in the bioavailability and metabolism of dietary polyphenols between rodents and humans may limit the direct extrapolation of these findings to clinical contexts. Furthermore, the experimental model used in this study reproduces key features of late-stage T2DM; nevertheless, it may not fully reproduce the complex and progressive pathophysiology of MASLD in humans. Furthermore, the experimental design did not include an HFFD control group without streptozotocin administration, which could have helped to distinguish diet-induced metabolic alterations from those associated with streptozotocin-mediated diabetes. Finally, because RBR supplementation was initiated at different stages of disease development but continued until the end of the experiment, the intervention groups also differed in the duration of exposure. Therefore, the observed effects cannot be attributed exclusively to the timing of the intervention.

5. Conclusions

RBR, a dietary fiber- and polyphenol-rich by-product of *Hibiscus sabdariffa* L., ameliorated dyslipidemia and hepatic steatosis in a rodent model of late-stage T2DM. These effects were partly associated with the upregulation of *Acadm*, a key gene involved in mitochondrial fatty acid β -oxidation, particularly in rats receiving preventive RBR supplementation. However, differences in intervention duration among groups should be considered when interpreting these findings. These findings highlight the potential of RBR as a sustainable functional ingredient to support the management of metabolic alterations associated with T2DM. Nevertheless, these findings should be interpreted considering the limitations discussed above. Further studies are necessary to elucidate the underlying mechanisms and evaluate its translational relevance in human populations.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/nutraceuticals6020023/s1>, Table S1: Composition of the high-fat and high-fructose diet (HFFD) used to induce insulin resistance in the rat model.

Author Contributions: Conceptualization, M.A.A.-L. and I.F.P.-R.; formal analysis, E.R.-R., J.E.S.-T. and I.F.P.-R.; investigation, E.R.-R. and J.E.S.-T.; resources, D.G.G.-G., R.R.-C., M.A.A.-L. and I.F.P.-R.; writing—original draft preparation, E.R.-R. and I.F.P.-R.; writing—review and editing, J.E.S.-T., D.G.G.-G., R.R.-C. and M.A.A.-L.; supervision, M.A.A.-L. and I.F.P.-R.; project administration, M.A.A.-L. and I.F.P.-R.; funding acquisition, I.F.P.-R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the School of Chemistry of the Universidad Autónoma de Querétaro, grant number FQU202404.

Institutional Review Board Statement: The animal study protocol was approved by the Bioethics Committee of the School of Natural Sciences of the Universidad Autónoma de Querétaro (53FCN2022 on 5 August 2022).

Informed Consent Statement: Not applicable.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Acknowledgments: E.R.-R. and J.E.S.-T. are grateful to SECIHTI for the postdoctoral and master's granted scholarships awarded, respectively. We thank Ericka Alejandra de los Ríos Arellano for the histology analysis carried out in the Microscopy Unit of the Institute of Neurobiology of the National Autonomous University of Mexico (INB-UNAM).

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

T2DM	Type 2 diabetes mellitus
HFFD	High-fat high-fructose diet
MASLD	Metabolic dysfunction-associated steatotic liver disease
NAFLD	Non-alcoholic fatty liver disease
RBR	Roselle beverage residue
NEPP	Non-extractable polyphenols
HDL	High-density cholesterol
<i>Fasn</i>	Fatty acid synthase
<i>Acaca</i>	Acetyl CoA carboxylase alpha
<i>Fatp5</i>	Fatty acid transport protein-5
<i>Cd36</i>	Cluster of differentiation 36
<i>Cpt1a</i>	Carnitine palmitoyltransferase 1a
<i>Acadm</i>	Medium-chain acyl-CoA dehydrogenase

References

1. Saeedi, P.; Petersohn, I.; Salpea, P.; Malanda, B.; Karuranga, S.; Unwin, N.; Colagiuri, S.; Guariguata, L.; Motala, A.A.; Ogurtsova, K.; et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res. Clin. Pract.* **2019**, *157*, 107843. [CrossRef]
2. Diabetes Around the World in 2021. Available online: <https://diabetesatlas.org> (accessed on 23 January 2025).
3. Eslam, M.; Newsome, P.N.; Sarin, S.K.; Anstee, Q.M.; Targher, G.; Romero-Gomez, M.; Zelber-Sagi, S.; Wong, V.W.-S.; Dufour, J.-F.; Schattenberg, J.M.; et al. A new definition for metabolic dysfunction-associated fatty liver disease: An international expert consensus statement. *J. Hepatol.* **2020**, *73*, 202–209. [CrossRef] [PubMed]
4. Xie, Z.; Li, H.; Wang, K.; Lin, J.; Wang, Q.; Zhao, G.; Jia, W.; Zhang, Q. Analysis of transcriptome and metabonome profiles alterations in fatty liver induced by high fat diet in rat. *Metabl. Clin. Exp.* **2010**, *59*, 554–560. [CrossRef] [PubMed]
5. Grunberger, G. Should side effects influence the selection of antidiabetic therapies in type 2 diabetes? *Curr. Diabetes Rep.* **2017**, *17*, 21. [CrossRef]
6. Fernandes, I.; Oliveira, J.; Pinho, A.; Carvalho, E. The role of nutraceutical containing polyphenols in diabetes prevention. *Metabolites* **2022**, *12*, 184. [CrossRef]
7. Almajid, A.; Bazroon, A.; AlAhmed, A.; Bakhurji, O. Exploring the health benefits and therapeutic potential of roselle (*Hibiscus sabdariffa*) in human studies: A comprehensive review. *Cureus* **2023**, *15*, e49309. [CrossRef]
8. Somaratne, G.M.; Lakshani, S.G.A.; Chamodini, A.G.K.; Kariyawasam, K.P. Unveiling the health benefits of roselle calyx extracts: Functional compounds and their role in non-communicable diseases. *Pharmacol. Res.* **2025**, *9*, 100407. [CrossRef]
9. Varzakas, T.; Zakynthinos, G.; Verpoort, F. Plant food residues as a source of nutraceuticals and functional foods. *Foods* **2016**, *5*, 88. [CrossRef] [PubMed]
10. Serna Tenorio, J.E.; Sotelo-González, A.M.; Reynoso-Camacho, R.; Anaya-Loyola, M.A.; Pérez Ramírez, I.F. Comprehensive characterization of the overlooked residue generated during roselle calyxes brewing with potential use as functional ingredient. *Biotechnia* **2023**, *25*, 208–220. [CrossRef]
11. Regalado-Rentería, E.; Serna-Tenorio, J.E.; García-Gutiérrez, D.G.; Reynoso-Camacho, R.; García, O.P.; Anaya-Loyola, M.A.; Pérez-Ramírez, I.F. Preventive and therapeutic efficacy of roselle beverage residue in late-stage type 2 diabetic rats. *Beverages* **2024**, *10*, 40. [CrossRef]
12. Amaya-Cruz, D.; Pérez-Ramírez, I.F.; Pérez-Jiménez, J.; Nava, G.M.; Reynoso-Camacho, R. Comparison of the bioactive potential of Roselle (*Hibiscus sabdariffa* L.) calyx and its by-product: Phenolic characterization by UPLC-QTOF MSE and their anti-obesity effect in vivo. *Food Res. Int.* **2019**, *126*, 108589. [CrossRef]
13. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **2001**, *25*, 402–408. [CrossRef] [PubMed]
14. García-Compeán, D.; Kumar, R.; del Cueto-Aguilera, Á.N.; Maldonado-Garza, H.J.; Villarreal-Pérez, J.Z. Body weight loss and glycemic control on the outcomes of patients with NAFLD. The role of new antidiabetic agents. *Ann. Hepatol.* **2023**, *28*, 100751. [CrossRef] [PubMed]

15. Nie, Y.; Luo, F. Dietary fiber: An opportunity for a global control of hyperlipidemia. *Oxid. Med. Cell. Longev.* **2021**, *2021*, 5542342. [[CrossRef](#)]
16. Bayram, H.M.; Majoo, F.M.; Ozturkcan, A. Polyphenols in the prevention and treatment of non-alcoholic fatty liver disease: An update of preclinical and clinical studies. *Clin. Nutr. ESPEN* **2021**, *44*, 1–14. [[CrossRef](#)] [[PubMed](#)]
17. Vrentzos, E.; Pavlidis, G.; Korakas, E.; Kountouri, A.; Pliouta, L.; Dimitriadis, G.D.; Lambadiari, V. Nutraceutical strategies for metabolic dysfunction-associated steatotic liver disease (MASLD): A path to liver health. *Nutrients* **2025**, *17*, 1657. [[CrossRef](#)]
18. Tahri-Joutey, M.; Andreoletti, P.; Surapureddi, S.; Nasser, B.; Cherkaoui-Malki, M.; Latruffe, N. Mechanisms mediating the regulation of peroxisomal fatty acid beta-oxidation by PPAR α . *Int. J. Mol. Sci.* **2021**, *22*, 8969. [[CrossRef](#)]
19. Badmus, O.O.; Hillhouse, S.A.; Anderson, C.D.; Hinds, T.D., Jr.; Stec, D.E. Molecular mechanisms of metabolic associated fatty liver disease (MAFLD): Functional analysis of lipid metabolism pathways. *Clin. Sci.* **2022**, *136*, 1347–1366. [[CrossRef](#)]
20. Jiang, S.; Young, J.L.; Wang, K.; Qian, Y.; Cai, L. Diabetic-induced alterations in hepatic glucose and lipid metabolism: The role of type 1 and type 2 diabetes mellitus. *Mol. Med. Rep.* **2020**, *22*, 603–611. [[CrossRef](#)]
21. Sukkar, A.H.; Lett, A.M.; Frost, G.; Chambers, E.S. Regulation of energy expenditure and substrate oxidation by short-chain fatty acids. *J. Endocrinol.* **2019**, *242*, R1–R8. [[CrossRef](#)]
22. Nguyen, V.; Taine, E.G.; Meng, D.; Cui, T.; Tan, W. Chlorogenic acid: A systematic review on the biological functions, mechanistic actions, and therapeutic potentials. *Nutrients* **2024**, *16*, 924. [[CrossRef](#)]
23. Ong, K.W.; Hsu, A.; Tan, B.K.H. Anti-diabetic and anti-lipidemic effects of chlorogenic acid are mediated by AMPK activation. *Biochem. Pharmacol.* **2013**, *85*, 1341–1351. [[CrossRef](#)] [[PubMed](#)]
24. Wagner, W.; Sobierajska, K.; Pulaski, L.; Stasiak, A.; Ciszewski, W.M. Whole grain metabolite 3,5-dihydroxybenzoic acids is a beneficial nutritional molecule with the feature of a double-edged sword in human health: A critical review and dietary considerations. *Crit. Rev. Food Sci. Nutr.* **2024**, *64*, 8786–8804. [[CrossRef](#)] [[PubMed](#)]
25. García-Díez, E.; López-Oliva, M.E.; Pérez-Jiménez, J.; Martín, M.A.; Ramos, S. Metabolic regulation of (–)-epicatechin and the colonic metabolite 2, 3-dihydroxybenzoic acid on the glucose uptake, lipid accumulation and insulin signalling in cardiac H9c2 cells. *Food Funct.* **2022**, *13*, 5602–56153. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.