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Fermented Rice Germ Extract Ameliorates Abnormal Glucose Metabolism via Antioxidant Activity in Type 2 Diabetes Mellitus Mice

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Abstract: Rice germ is an abundant source of ferulic acid, which is known for its anti-oxidant activity. This study aimed to evaluate the regulatory effects of fermented rice germ extracts on hepatic glucose metabolism in C57BL/KsJ-db/db mice. Rice germ was fermented with *Lactobacillus plantarum* and extracted with 30% ethanol (RG_30E) or 50% ethanol (RG_50E). Mice were fed modified AIN-93 diets containing fermented rice germ extracts and ferulic acid for 8 weeks. RG_50E significantly reduced food intake as well as liver weight and RG_30E and RG_50E improved glucose homeostasis, as indicated by fasting blood glucose levels and glucose tolerance. Hepatic triglyceride and total cholesterol levels were significantly decreased in db/db mice fed RG_30E and RG_50E. The antioxidant capacity of RG_30E and RG_50E was confirmed by a decrease in malondialdehyde levels and an increase in hepatic superoxide dismutase activity. The expression of genes related to glycolysis and gluconeogenesis was significantly regulated by RG_30E and RG_50E. These results suggest that fermented rice germ extracts have the potential to regulate hypoglycemia and hepatic glucose metabolism in type 2 diabetes db/db mice.

Keywords: rice germ; ferulic acid; type 2 diabetes mellitus; hypoglycemia effect; hepatic glucose metabolism

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1. Introduction

Diabetes mellitus is a metabolic disorder classified into insulin-dependent type 1 diabetes mellitus and non-insulin-dependent type 2 diabetes mellitus (T2DM) [1]. T2DM is a chronic condition characterized by continued hyperglycemia due to compromised glucose uptake into cells, a consequence of decreased insulin sensitivity. Previous studies have shown that persistent hyperglycemia can cause diseases in organs such as the liver, kidney, and heart. In particular, diabetes-induced liver damage accumulates over time, culminating in liver diseases such as fatty liver and non-alcoholic fatty hepatitis [2,3]. According to several studies, T2DM severely affects glucose metabolism and oxidative stress in the liver. First, under fasting conditions, the liver breaks down glycogen and produces glucose to maintain normal blood glucose levels. After a meal, liver cells synthesize glycogen to regulate blood glucose. When levels of blood glucose are high, it is taken up by liver cells via GLUT2, followed by its conversion into glucose-6-phosphate by glucokinase (GK), which is synthesized as glycogen. Conversely, under low blood glucose level, glucose-6-phosphatase (G6PC) and phosphoenolpyruvate carboxykinase (PEPCK) drive gluconeogenesis for glucose synthesis [4–6]. Second, recent studies have shown that the overproduction of reactive oxygen species (ROS), which results in oxidative stress, is involved in insulin resistance. Thus, compounds with antioxidant activity have been shown to improve blood glucose levels in T2DM patients [7,8]. Currently available

pharmacological agents for the treatment of T2DM have limited tolerability, decreased efficacy, and cause side effects such as vomiting, weight gain, diarrhea, and hypoglycemia. Therefore, the identification of natural compounds suppressing the underlying molecular causes of T2DM is of great necessity [9,10].

Rice germ is known to help lower blood glucose. Apart from γ-aminobutyric acid (GABA) and γ-oryzanol, rice germ also contains a large amount of phenolic acids, including ferulic acid. Fermented rice harbors larger amounts of phenolic compounds when compared to raw rice. In particular, ferulic acid levels are significantly increased during fermentation [11,12]. This phenolic compound is known to have antidiabetic, antioxidant, anti-inflammatory, as well as anticancer effects. According to previous studies, ferulic acid inhibits oxidative damage to cell membranes and DNA by scavenging ROS such as nitrogen peroxide, superoxide, and hydroxyl oxygen [13–15]. Ohnishi et al. found that ferulic acid suppressed hyperglycemia by inhibiting lipid peroxidation by scavenging ROS in streptozotocin - induced Sprague-Dawley (SD) rat [16]. Moreover, ferulic acid was shown to decrease levels of thiobarbituric acid reactive substances (TBARS) and upregulate the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione (GSH) [17,18]. This study investigated the effects of fermented rice gum extracted with 30% ethanol or 50% ethanol on T2DM based on hepatic glucose metabolism and oxidative stress in C57BL/KsJ-db/db mice.

2. Materials and Methods

2.1. Preparation of Sample

Lactobacillus plantarum Korea Agricultural Culture Collection (KACC) 15357 was used as a fermentation strain, purchased from the Korean Collection for Type Cultures (KCTC, Korea). *L. plantarum* was cultured on MRS medium (*Lactobacilli* MRS broth, BD Difco, Germany) at 37 °C for 24 h. The culture medium was homogenized at 5000 rpm for 10 min and then sterilized at 95 °C for 15 min for fermentation. Thereafter, 3% (*w/v*) of the *L. plantarum* culture medium was inoculated into rice fermentation medium composed of 15% (*w/v*) rice germ and 15% (*w/v*) glucose. Fermented rice germ was produced via anaerobic fermentation for 24 h at 37 °C. The fermented rice germ was then extracted at 60 °C for 6 h using prethanol A (Duksan, Seoul, Korea). Prethanol A and fermented rice germ were mixed at a ratio of 1:1 and 1:2, followed by vacuum extraction and concentration using Cosmos 660 (Gyeongseo E&P, Incheon, Korea). The sample used in this study differed in the ratio of extracted prethanol A and fermented rice germ. The sample with a ratio of prethanol A and fermented rice germ of 1: 2 was denoted as RG_30E, and the sample with a ratio of 1:1 was denoted as RG_50E. For the dried powder, sample:maltodextrin was dried in a ratio of 1:4 and used in the experiment.

2.2. Animals and Diets

Five-week-old male C57BL/6KsJ-db/+ mice (db/+ mice) and C57BL/6KsJ-db/db mice (db/db mice) were purchased from JOONGAH BIO (Suwon, Korea). The experiment was approved according to the guidelines published by Kyungpook National University (KNU-2020-0045). The mice were housed in an environment of 23 ± 2 °C under a 12-h light/dark cycle. All mice were allowed free access to food and tap water during adaptation for 1 week and were then randomly divided into five groups (n = 8): db/+ control (db/+ mice and normal AIN-93 diet), db/db control (db/db mice and normal AIN-93 diet), RG_30E (db/db mice and modified diet with RG_30E), RG_50E (db/db mice and modified diet with RG_50E), and FA (db/db mice and modified diet with ferulic acid). Table 1 shows the composition of diets used in this experiment. The modified diets were based on AIN-93, prepared by mixing with ferulic acid (Sigma-Aldrich, St. Louis, MO, USA). The ferulic acid content was determined based on the amount of ferulic acid in fermented rice germ extracts (Supplementary Information). Body weight and food intake were measured every week for 8 weeks. After 8 weeks, the liver, epididymal adipose tissue, and kidney were excised and weighed. Blood samples were collected in EDTA tubes and centrifuged for

10 min at 1500× *g* and 4 °C to obtain plasma. All tissues and blood samples were stored at –80 °C until analysis.

Table 1. Composition of experimental diets.

	Dietary Group (g/kg Diet)			
Ingredients (g/kg)	**Normal Diet**	**FA Diet**	**RG_30E Diet**	**RG_50E Diet**
Casein	200	200	200	200
L-Cystine	3	3	3	3
Corn starch	384.81	384.81	384.81	384.81
Maltodextrin	157.36	157.36	118.02	118.02
Sucrose	87.32	87.32	87.32	87.32
Soybean Oil	70	70	70	70
Cellulose	50	50	50	50
AIN-93G-MX	35	35	35	35
AIN-93-VX	10	10	10	10
Choline Bitartrate	2.5	2.5	2.5	2.5
TBHQ, antioxidant	0.014	0.014	0.014	0.014
RG_30E			39.34	
RG_50E				39.34
Ferulic acid		0.008		
Total (g/kg)	1000.004	1000.012	1000.004	1000.004

2.3. Fasting Blood Glucose Level

Mice were fasted for 12 h prior to sample collection, and blood samples were obtained from the tail vein at 0, 3, 5, and 8 weeks. Blood glucose levels were measured using a New Accu-Chek Active GB glucometer (Roche Diagnostics, Mannheim, Germany).

2.4. Oral Glucose Tolerance Test (OGTT)

For the OGTT, glucose (2 g/kg body weight) was orally administered to mice that had been fasted for 12 h. Blood was collected from the tail vein at 0, 15, 30, 45, 60, 90, and 120 min. The blood glucose level was measured using a New Accu-Chek Active GB glucometer (Roche Diagnostics, Mannheim, Germany). The area under the blood glucose concentration–time curve (AUC) was calculated using the trapezoidal method [19] for 120 min.

2.5. Hepatic Lipid Level

Hepatic triglyceride (TG) and total cholesterol (TC) levels were measured using a commercial ELISA kit (Asan Pharmaceuticals, Seoul, Korea). Liver tissue lipids were extracted using a previously described method, with slight modifications [20]. Liver tissue was homogenized with Folch solution and centrifuged at 3000 rpm and 4 °C for 10 min. The supernatant was evaporated and dissolved in chloroform (Duksan, Seoul, Korea).

2.6. Lipid Peroxidation

Levels of erythrocyte and liver TBARS (Sigma-Aldrich, St. Louis, MO, USA) were determined according to a previously described method [21]. The livers (100 mg) of mice were homogenized in 1.15% KCl (Junsei Chemical Co., Ltd., Tokyo, Japan). A mixture of 0.9% thiobarbituric acid (Sigma-Aldrich, St. Louis, MO, USA), 8.1% sodium dodecyl sulfate (Sigma-Aldrich, St. Louis, MO, USA), and 20% acetic acid (Sigma-Aldrich, St. Louis, MO, USA), and distilled water was then added to the erythrocyte and tissue homogenate. The mixture was incubated at 95 °C for 1 h and cooled at room temperature. Distilled water and pyridine:n-butanol (1:15, *v/v*) were added and centrifuged at 4000× *g* and 4 °C for 10 min. The supernatant was removed, and absorbance was measured at 532 nm.

2.7. Hepatic Catalase

Hepatic catalase activity was determined using a commercial ELISA kit (Thermo Fisher Scientific, Waltham, MA, USA). The liver was washed with ice-cold Dulbecco's Phosphate-Buffered Saline (DPBS) and homogenized with the assay buffer from the catalase assay kit. The homogenate was centrifuged at 10,000× *g* for 15 min, and the supernatant was assessed according to the manufacturer's protocol.

2.8. SOD Activity

Liver SOD activity was measured using a commercial ELISA kit (Biovision, Milpitas, CA, USA). The liver was homogenized with 0.1 M Tris/HCl (pH 7.4) containing 0.5% Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA), 5 mM β-mercaptoethanol (Sigma-Aldrich, St. Louis, MO, USA), and 0.1 mg/mL phenylmethylsulfonyl fluoride (Sigma-Aldrich, St. Louis, MO, USA). Tissue homogenates were centrifuged at 14,000× *g* and 4 °C for 5 min, and the supernatant was assayed according to the kit manufacturer's protocol.

2.9. Quantitative Reverse Transcriptase-Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from liver tissue using TRIzol reagent (Life Technologies, Rockville, MD, USA) according to the manufacturer's protocol. cDNA was synthesized by reverse transcription using a Transcriptor First-Strand cDNA Synthesis Kit (Life Technologies, Rockville, MD, USA). Gene expression levels were determined via the Universal Probe Library on a Light Cycler 96 system (Hoffmann La Roche, Basel, Switzerland). The qRT-PCR thermocycling conditions were as follows: initial denaturation at 95 °C for 30 s, 40 cycles at 92 °C for 5 s, and 60 °C for 20 s. Relative mRNA expression levels were calculated using the Livak et al. method and normalized to *Gapdh*. Gene primers, purchased from Bioneer (Daejeon, Korea), were as follows: *Gk*, 5′-CACCAGTTCCTCACAGCTCA-3′ (forward) and 5′-GAGCCATGTGGTTCCTCAGT-3′ (reverse); Glucose-6-phosphatase 5′-AACCCATGTAAAGACAGTTGACAG-3′ (forward) and 5′-GGAGTCTTTCTGCTAACTGGAATG-3′ (reverse); Phosphoenolpyruvate carboxykinase, 5′-ATCCCAACTCGAGATTCTGC-3′ (forward) and 5′-CCCAGGCAGGGTCAATAAT-3′ (reverse); *Gapdh*, 5′-AAGAGGGATGCTGCCCTTAC-3′ (forward) and 5′-CCATTTTGTCTACGGGACGA-3′ (reverse).

2.10. Statistical Analysis

Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC, USA). Data are presented as the mean ± standard error (SE). Data were analyzed via one-way analysis of variance (ANOVA), followed by Duncan's multiple range test. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Body Weight, Food Intake, and Organ Weight

Table 2 shows the effects of ferulic acid and fermented rice germ extracts on body weight, food intake, and organ weight. The initial body weight was not significantly different between diabetic groups, except for the db/+ control. After 8 weeks, the final body weight decreased by 4.81% in the FA group compared to the db/db control. Neither RG_30E nor RG_50E affected body weight. However, food intake was significantly lower in the FA and RG_50E groups, with decreases of 14.22% and 12.24%, respectively, when compared to the db/db control group ($p < 0.05$). In addition, liver weight decreased by 10.73%, 6.92%, and 16.96% in the FA, RG_30E, and RG_50E groups, respectively, when compared to the db/db control ($p < 0.05$). The weight of epididymal adipose tissue tended to decrease in the FA, RG_30E, and RG_50E groups, but the decreases were not significant. Further, no significant decrease in kidney weight was observed.

Table 2. Effect of fermented rice germ extracts on body weight, food intake, and organ weights.

	Group [(1)]				
	db/+	**db/db**	**FA**	**RG_30E**	**RG_50E**
Body weight (g)					
Initial weight (g)	22.49 ± 0.76 [b]	37.30 ± 0.33 [a]	37.20 ± 0.70 [a]	38.28 ± 1.15 [a]	36.34 ± 1.77 [a]
Final weight (g)	27.66 ± 0.49 [c]	50.78 ± 1.04 [ab]	48.34 ± 3.82 [b]	52.60 ± 1.53 [a]	51.98 ± 1.15 [a]
Food intake (g/day)	2.87 ± 0.10 [d]	18.14 ± 0.58 [a]	15.56 ± 0.09 [c]	16.83 ± 0.12 [b]	15.92 ± 0.02 [c]
Organ weight (g)					
Liver (g/100 g BW)	0.93 ± 0.03 [c]	2.89 ± 0.13 [a]	2.58 ± 0.11 [ab]	2.69 ± 0.16 [ab]	2.40 ± 0.12 [b]
Epididymal adipose tissue (g/100 g BW)	0.54 ± 0.05 [b]	2.81 ± 0.09 [a]	2.70 ± 0.11 [a]	2.44 ± 0.07 [a]	2.61 ± 0.23 [a]
Kidney (g/100 g BW)	0.30 ± 0.00 [a]	0.35 ± 0.02 [a]	0.35 ± 0.02 [a]	0.33 ± 0.03 [a]	0.30 ± 0.00 [a]

[(1)] db/+, C57BL/6KsJ-db/+ mice and normal diet; db/db, C57BL/6KsJ-db/db mice and normal diet; RG_30E, C57BL/6KsJ-db/db mice and modified diet with RG_30E; RG_50E, C57BL/6KsJ-db/db mice and modified diet with RG_50E; FA, C57BL/6KsJ-db/db mice and modified diet with ferulic acid. Data are presented as mean ± standard error (SE) (n = 8). Data with different letters determined by Duncan's multiple range test were significantly different at $p < 0.05$.

3.2. Fasting Blood Glucose Levels

At week 0, there was no difference in blood glucose levels between all groups (Figure 1). However, 8 weeks later, db/db mice had higher blood glucose levels than db/+ mice; additionally, there were differences between each group of db/db mice. At week 3, blood glucose levels in the RG_30E and RG_50E groups were significantly lower (29.68% and 25.71%, respectively) compared to the db/db control ($p < 0.05$). However, there was no significant difference between groups except the db/+ control group at 5 weeks. At 8 weeks, only RG_50E mice had a significantly lower blood glucose level compared to the db/db control, with a decrease of 12.37% ($p < 0.05$).

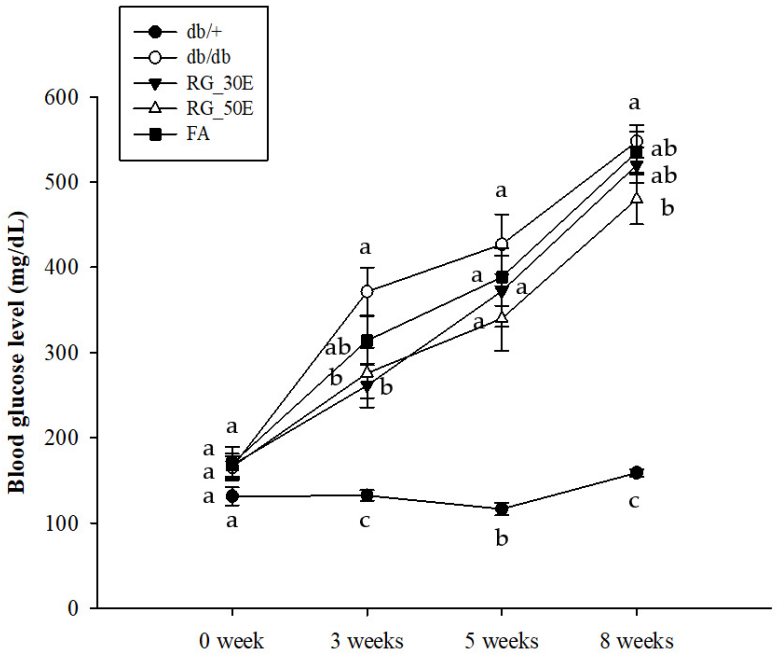

Figure 1. The effects of fermented rice germ extracts on blood glucose levels in db/+ mice and db/db mice over 8 weeks. db/+, C57BL/6KsJ-db/+ mice and normal diet; db/db, C57BL/6KsJ-db/+ mice and normal diet; RG_30E, C57BL/6KsJ-db/+ mice and modified diet with RG_30E; RG_50E, C57BL/6KsJ-db/+ mice and modified diet with RG_50E; FA, C57BL/6KsJ-db/+ mice and modified diet with ferulic acid. Data with letters are significantly different ($p < 0.05$) according to Duncan's multiple range test.

3.3. OGTT

Figure 2 shows changes in blood glucose levels over time after a single administration of glucose (2 g/kg body weight) to mice at 8 weeks. A significant decrease in blood glucose levels was observed in FA (594.63 ± 19.21 mg/dL) and RG_50E (592.75 ± 13.27 mg/dL) mice at 60 min when compared to db/db controls (Figure 2a, $p < 0.05$). The RG_50E diet significantly decreased glucose levels by 20.68% compared to db/db control mice for 120 min ($p < 0.05$). In addition, the AUC value was significantly lower (5.14% decrease) in the RG_50E group compared to the db/db control (Figure 2b, $p < 0.05$).

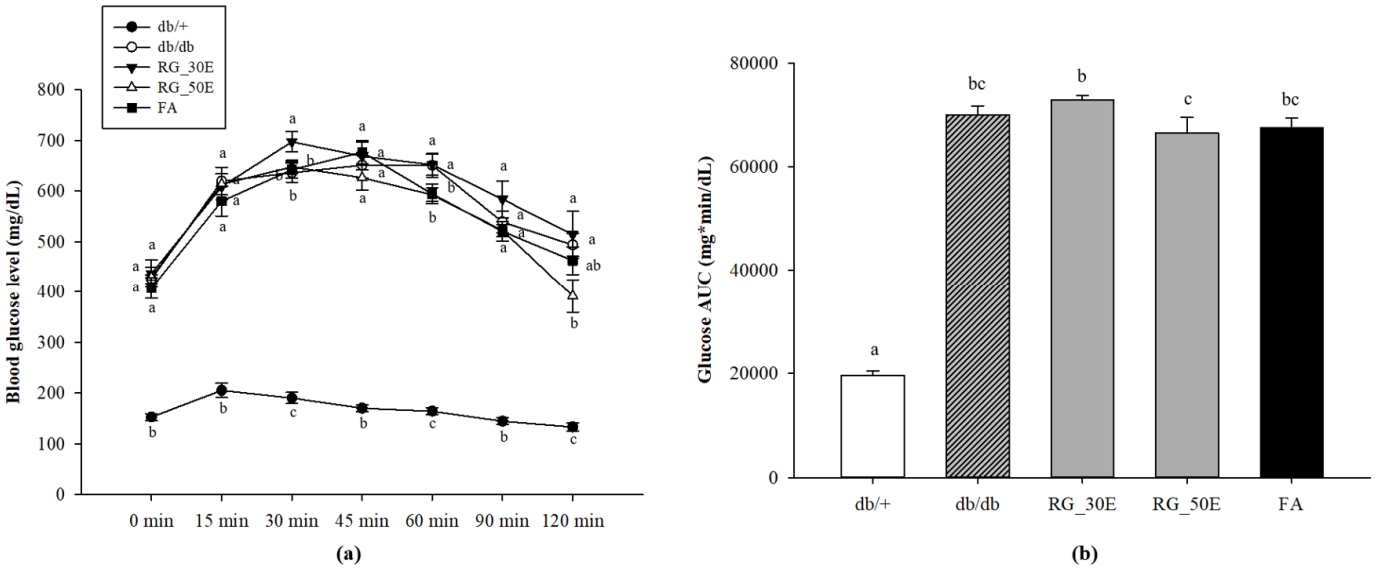

Figure 2. The effects of fermented rice germ extracts on the glucose tolerance in db/+ mice and db/db mice. (**a**) Fasting blood glucose levels were measured for 120 min after oral administration; (**b**) The area under the curve (AUC) of blood glucose levels. db/+, C57BL/6KsJ-db/+ mice and normal diet; db/db, C57BL/6KsJ-db/+ mice and normal diet; RG_30E, C57BL/6KsJ-db/+ mice and modified diet with RG_30E; RG_50E, C57BL/6KsJ-db/+ mice and modified diet with RG_50E; FA, C57BL/6KsJ-db/+ mice and modified diet with ferulic acid. Data with letters are significantly different ($p < 0.05$) according to Duncan's multiple range test.

3.4. Hepatic TG and TC Concentration

The effect of fermented rice germ extracts on hepatic lipid accumulation was assessed by measuring TG and TC levels (Figure 3). Hepatic TG levels were significantly lower (decreased by 25.49%, 52.23%, and 53.33%, respectively) in FA, RG_30E, and RG_50E mice compared to the db/db controls ($p < 0.05$). Hepatic TC exhibited a tendency to decrease in the FA, RG_30E, and RG_50E groups compared to the db/db controls. A significant decrease was observed only in RG_50E mice, wherein TC levels were 37.05% lower ($p < 0.05$).

3.5. Oxidative Stress-Related Biomarkers

Antioxidant effects were evaluated by measuring malondialdehyde (MDA) levels as well as catalase and SOD activity. Compared to the db/db control, erythrocyte MDA levels were significantly lower (49.63%, 23.03%, and 37.36% lower) in the FA, RG_30E, and RG_50E group, respectively (Figure 4a, $p < 0.05$). Hepatic MDA levels were 83.26% and 38.43% lower in FA and RG_50E mice compared to the db/db controls (Figure 4b, $p < 0.05$). FA, RG_30E, and RG_50E mice exhibited a significant increase of 97.86%, 92.04%, and 92.00%, respectively, in hepatic SOD activity (Figure 4d, $p < 0.05$). In contrast, hepatic catalase did not increase significantly in FA, RG_30E, or RG_50E mice (Figure 4c, $p < 0.05$).

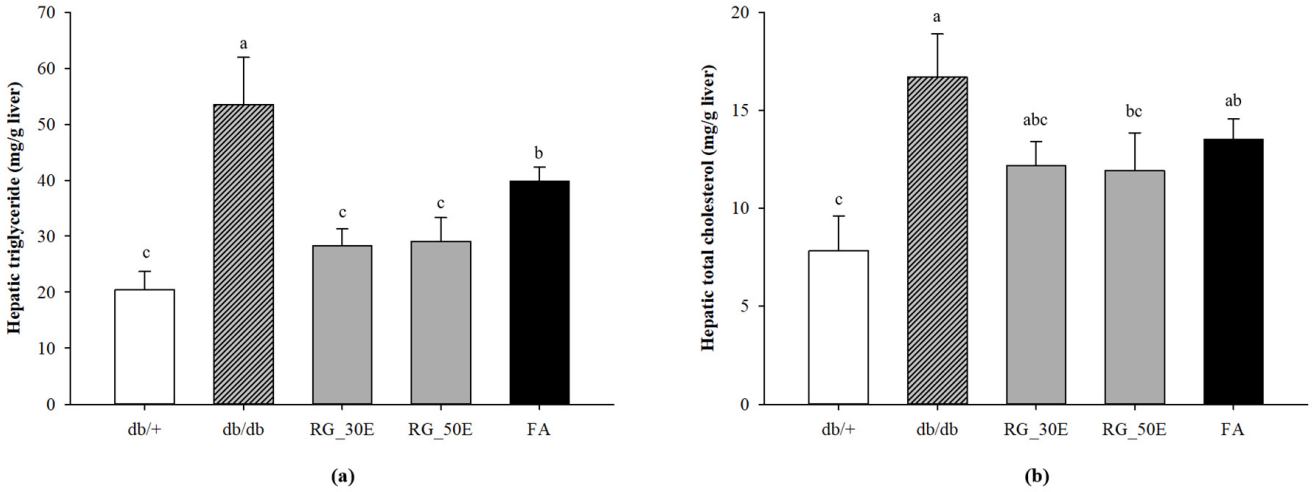

Figure 3. The effects of fermented rice germ extracts on hepatic lipid profiles in db/+ mice and db/db mice. (**a**) Hepatic triglyceride levels and (**b**) Hepatic total cholesterol levels. db/+, C57BL/6KsJ-db/+ mice and normal diet; db/db, C57BL/6KsJ-db/+ mice and normal diet; RG_30E, C57BL/6KsJ-db/+ mice and modified diet with RG_30E; RG_50E, C57BL/6KsJ-db/+ mice and modified diet with RG_50E; FA, C57BL/6KsJ-db/+ mice and modified diet with ferulic acid. Data with letters are significantly different ($p < 0.05$) according to Duncan's multiple range test.

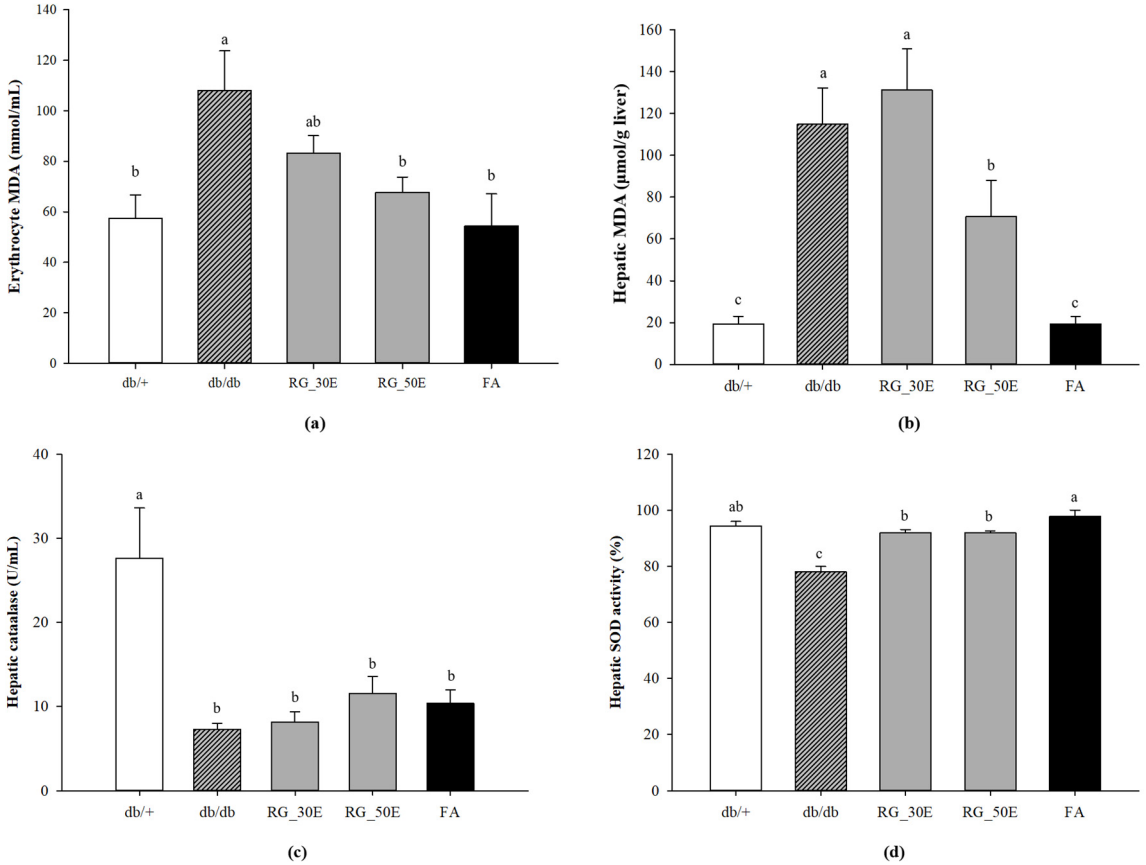

Figure 4. The effects of fermented rice germ extracts on malondialdehyde (MDA) levels and hepatic antioxidant enzymes in db/+ mice and db/db mice. (**a**) Erythrocyte MDA concentration; (**b**) Hepatic MDA concentration; (**c**) Hepatic catalase; (**d**) Hepatic superoxide dismutase (SOD) activity. db/+, C57BL/6KsJ-db/+ mice and normal diet; db/db, C57BL/6KsJ-db/+ mice and normal diet; RG_30E, C57BL/6KsJ-db/+ mice and modified diet with RG_30E; RG_50E, C57BL/6KsJ-db/+ mice and modified diet with RG_50E; FA, C57BL/6KsJ-db/+ mice and modified diet with ferulic acid. Data with letters are significantly different ($p < 0.05$) according to Duncan's multiple range test.

3.6. Expression of Genes Related Glycolysis and Gluconeogenesis

The expression levels of glucokinase (*Gk)*, which is involved in glycolysis, were increased 1.47-, 2.02-, and 4.12-fold in FA, RG_30E, and RG_50E mice, respectively, compared to the db/db controls ($p < 0.05$, Figure 5a). To confirm the effect of fermented rice germ extracts on gluconeogenesis, Glucose-6-phaspahtase (*G6pc*) and phosphoenolpyruvate carboxykinase (*Pepck*) expression was assessed (Figure 5b,c). *G6pc* expression was significantly reduced 7.11-, 1.56-, and 5.08-fold in FA, RG_30E, and RG_50E mice, respectively, compared to the db/db controls ($p < 0.05$). *Pepck* expression decreased 17.15-, 14.04-, and 24.78-fold in FA, RG_30E, and RG_50E mice compared to the db/db control ($p < 0.05$).

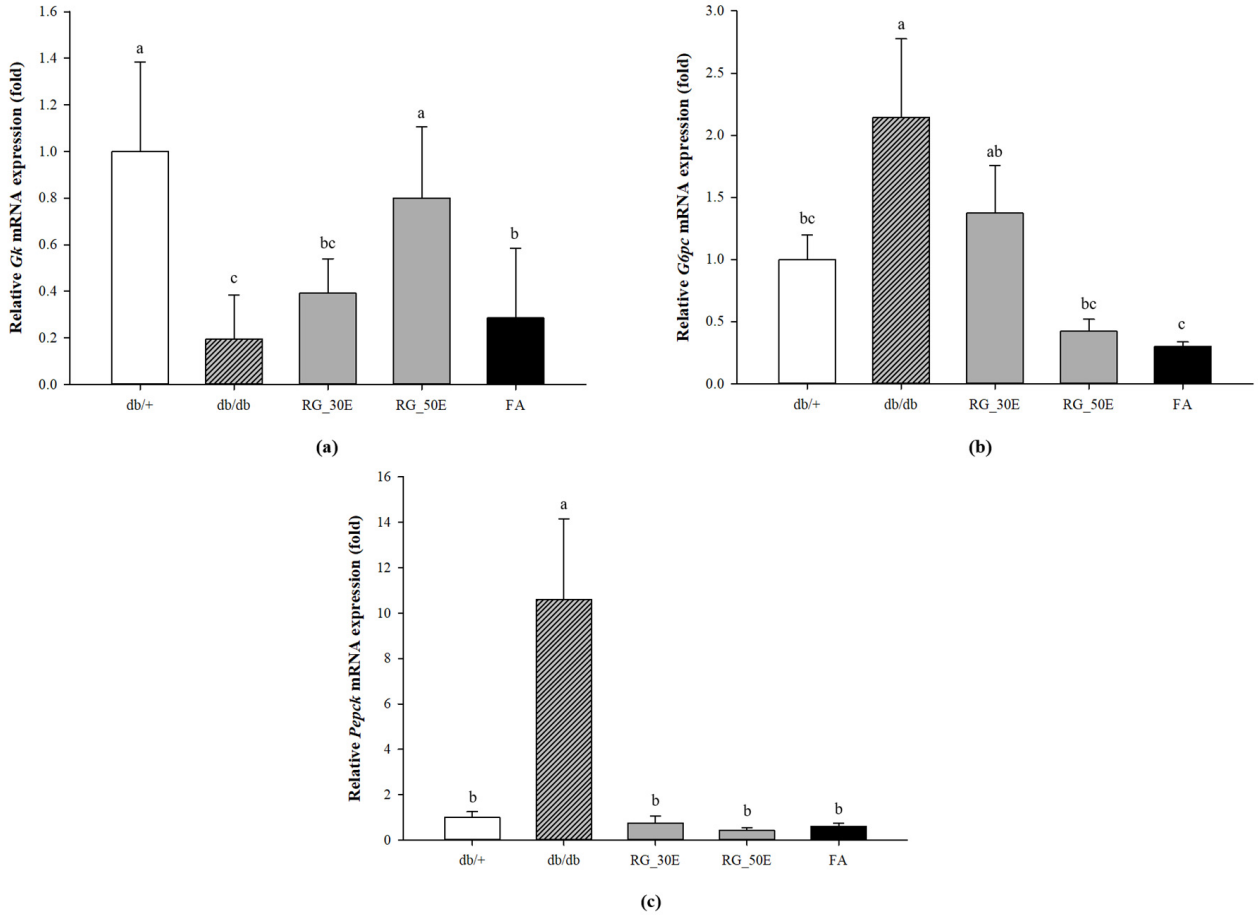

Figure 5. The effects of fermented rice germ extracts on hepatic glycolysis and gluconeogenesis in db/+ mice and db/db mice. (**a**) Relative mRNA expression of glucokinase (*Gk*); (**b**) Relative mRNA expression of glucose-6-phosphatase; and (**c**) Relative mRNA expression of phosphoenolpyruvate carboxykinase (*Pepck*). db/+, C57BL/6KsJ-db/+ mice and normal diet; db/db, C57BL/6KsJ-db/+ mice and normal diet; RG_30E, C57BL/6KsJ-db/+ mice and modified diet with RG_30E; RG_50E, C57BL/6KsJ-db/+ mice and modified diet with RG_50E; FA, C57BL/6KsJ-db/+ mice and modified diet with ferulic acid. Data with letters are significantly different ($p < 0.05$) according to Duncan's multiple range test.

4. Discussion

This study was conducted to investigate the effects of fermented rice germ extracts on hyperglycemia and hepatic glucose metabolism in T2DM mice. The db/db mice were used as a T2DM model, as they develop obesity and dyslipidemia due to a mutated leptin receptor gene. Further, the db/db mouse is the most popular animal model used for the study of blood glucose, diabetes, and obesity. Currently, db/db mice are widely employed in T2DM research [4,8,9,22,23].

Previous studies have reported that ferulic acid and fermented rice germ extracts affect hyperglycemia and hepatic glucose metabolism in patients with diabetes. In particular,

ferulic acid, a compound abundant in rice germ, was shown to regulate glucose metabolism in the liver [24,25]. Rice contains a variety of phenols, which are often metabolized and not bioavailable. Fermentation using *L. plantarum* increases the amount of physiologically active compounds such as ferulic acid, α-tocopherol, and γ-oryzanol in rice, improving its antioxidant activity [11,12,26]. In addition, ethanol can effectively extract phytochemicals or phenolic compounds from plants when used with water. This is related to polarity, as the use of an ethanol–water mixture lowers polarity, creating an environment where low-polarity phenol chemicals can be dissolved [27,28]. In this study, fermented rice germ was extracted with two different ethanol:water ratios to determine the more effective one with regard to glucose metabolism.

Diabetic mice fed diets with ferulic acid or other modified diets did not lose weight compared to the db/db controls. However, the significant decrease in food intake and liver weight observed in FA, RG_30E, and RG_50E mice indicated that ferulic acid and fermented rice germ extracts could regulate the diabetes-associated increase in food intake and liver weight. Epididymal adipose tissue weight was not significantly different between groups but showed a tendency to decrease in FA, RG_30E, and RG_50E mice (Table 2). The intake of fermented rice germ, which is a rich source of minerals, vitamins, and phytochemicals, has been reported to reduce body weight, liver weight, and fat as a result of lower food intake [29,30].

Dyslipidemia, a condition characterized by elevated TG, very low-density lipoprotein (VLDL) cholesterol, and TC and decreased high-density lipoprotein (HDL) levels is a frequent symptom of T2DM patients [23]. RG_30E and RG_50E mice had lower hepatic TG and TC levels than those in the FA group. Further, RG_50E mice exhibited lower hepatic TC levels than their RG_30E counterparts (Figure 3). Fermented rice was previously shown to reduce TC levels [31]. Lowering TG and TC levels can reduce the risk of developing cardiovascular disease in T2DM patients [32,33].

To confirm the effect of fermented rice germ extracts on hyperglycemia, blood glucose levels in the fasting state (Figure 1) and OGTT (Figure 2) were assessed. Lower blood glucose levels were observed in RG_30E and RG_50E mice than in those of the FA group. In particular, blood glucose levels were significantly lowered at 3, 5, and 8 weeks after RG_50E intake. Further, RG_50E significantly reduced the AUC value following oral glucose administration. Ferulic acid in rice germ was previously shown to suppress the activities of G6PC and PEPCK, both of which are involved in liver gluconeogenesis, highlighting the potential of this phenolic compound in T2DM [34,35].

To explore the involvement of ferulic acid in glycolysis and gluconeogenesis, we measured the expression of genes related to glucose metabolism in the liver. GK plays an important role in the regulation of glycolysis. Increased *Gk* expression decreases blood glucose levels by storing glucose in the form of glycogen or utilizing glucose. In contrast, G6pc and PEPCK are major enzyme regulators of gluconeogenesis. A decrease in *G6pc* and *Pepck* expression indicates reduced glucose synthesis in the liver, subsequently resulting in lower blood glucose levels [34,36,37]. We observed that *Gk* expression was significantly upregulated in FA, RG_30E, and RG_50E mice. In particular, *Gk* expression in the RG_50E group increased to a similar degree as in db/+ mice, which were non-diabetic controls. According to Son et al., mice fed ferulic acid were reported to regulate the hepatic glucose production by engaging in the activities of GK, G6PC, and PEPCK [34]. The result of this study also confirmed that ferulic acid significantly reduced the expression of the hepatic *Gk* gene. *G6pc* expression was significantly decreased in FA and RG_50E mice, while *Pepck* expression was lower in the FA, RG_30E, and RG_50E groups. These results indicate that the fermented rice germ extract regulates the expression of *G6pc* and *Pepck*, thereby inhibiting glucose synthesis.

Ferulic acid has both antihyperglycemic and antioxidant properties. It has been shown to alleviate hyperglycemia and oxidative stress by maintaining the redox balance and inhibiting mitochondrial apoptosis pathways [18,38,39]. Ferulic acid removes the free radicals present in aromatic rings, thus acting as an antioxidant and reducing MDA

levels. The decrease in antioxidant enzyme activity in tissues promotes ROS-mediated lipid peroxidation and protein oxidation. As one such antioxidant enzyme, SOD inhibits ROS generation by converting superoxide anions into hydrogen peroxide [40,41]. We observed that ferulic acid and fermented rice germ extracts reduced MDA levels and improved catalase and SOD activity, confirming previous observations. While no effect on hepatic catalase activity was observed (Figure 4c), MDA levels in erythrocytes and the liver were significantly decreased in the FA and RG_50E groups, indicative of decreased lipid peroxidation (Figure 4a,b). Further, hepatic SOD activity was significantly increased in the FA, RG_30E, and RG_50E groups (Figure 4d). Thus, RG_50E significantly reduced MDA levels and enhanced SOD activity, exhibiting a greater antioxidant effect than RG_30E.

The db/db mice, which are animal models of type 2 diabetes, have mutation in the leptin receptor involved in dietary intake regulation and homeostasis [23]. In this study, compared to the db/db group, FA, RG_30E, and RG_50E did not confirm the tendency of a decrease in body weight. This is a limitation of this study. GABA is one of the compounds in rice germ [42]. Pu et al. reported that GABA was expressed in a subpopulation of neuropeptide Y (NPY) neurons involved in feed stimulation [43]. In particular, it was suggested that GABA and NPY interact to regulate of feeding. The relationship between GABA in rice germ and NPY must be a further study confirming the effect of weight loss by controlling food intake.

Yu et al. reported that rice germ contains large amounts of vitamin E and γ-oryzanol [44]. γ-oryzanol is a mixture of ferulic acid esters of phytosterols and triterpenoids [45]. Therefore, this study focused on ferulic acid which was known compound as hydrolyzed compound of γ-oryzanol, and it was conducted based on the study that antioxidants such as ferulic acid have antidiabetic effects [46,47]. This study has a limitation that fermented rice germ extracts still contained a small amount of ferulic acid. However, fermented rice germ extracts contain ferulic acid as well as various phenolic compounds and antioxidants, and it is expected that the effect of higher hepatic glucose metabolism was confirmed due to the interaction with other compounds. Since we have not verified the glucose metabolism between other compounds in fermented rice germ extracts, further research must be confirmed.

In conclusion, we demonstrated the efficacy of fermented rice germ extracts in improving hyperglycemia and hepatic glucose metabolism in T2DM mice. Fermented rice germ extracts lowered blood glucose levels as well as OGTT values and modulated hepatic glucose metabolism via regulating *Gk*, *G6pc*, and *Pepck* expression. In addition, fermented rice germ extracts lowered hepatic TG and TC as well as MDA levels, while enhancing SOD activity in liver tissue, highlighting their antioxidant effect. The current findings indicate the potential of fermented rice germ extracts for the prevention and alleviation of T2DM by regulating hepatic glucose metabolism.

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Abbreviations

RG_30E	fermented rice germ extract with 30% ethanol
RG_50E	fermented rice germ extract with 50% ethanol
FA	ferulic acid
T2DM	type 2 diabetes mellitus
GK	glucokinase
G6PC	glucose-6-phosphatase
PEPCK	phosphoenolpyruvate carboxykinase
ROS	reactive oxygen species
GSH	glutathione
AUC	area under the curve
TG	triglyceride
TC	total cholesterol
OGTT	oral glucose tolerance test
NPY	neuropeptide Y

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