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Antidiabetic Effect of Hydroalcoholic Extract of *Myrtus communis* L. Fruit in a Type 2 Diabetes Mouse Model

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Abstract

This study aimed to determine the effects of *Myrtus communis* L. fruit extract on the diabetes-related glycemic and lipid profile, some serum biochemical parameters, and mRNA expression of some endogenous substances in type 2 diabetes. A total of 54 healthy male Swiss albino mice were divided into 5 groups. Except for the mice in the healthy control group, the mice in the other group were fed high-fat diets, and streptozotocin (50 mg/kg) was administered. No medication was administered to the animals in the diabetes control group. Metformin (200 mg/kg), *Myrtus communis* L. fruit extract (2 g/kg), and metformin + *Myrtus communis* L. fruit extract were administered orally to D+ metformin, D + *Myrtus communis* L. fruit extract, and D + *Myrtus communis* L. fruit extract+ metformin groups for 14 days, respectively. In serum samples, glucose, triglyceride, cholesterol, aspartate aminotransferase, alanine aminotransferase , and blood urea nitrogen were measured with an autoanalyzer and hemoglobin A1C (Hb1Ac) was measured with an Hb1Ac analyzer. Adiponectin, insulin, leptin, and adenosine monophosphate-activated protein kinase levels were analyzed in an ELISA reader in serum samples. The mRNA expressions of glucose transporter-1 (GLUT-1), glucose transporter-4 (GLUT-4), peroxisome proliferator-activated receptor-α (PPAR-α), and peroxisome proliferator-activated receptor-γ (PPAR-γ) in the liver and striated muscles were determined with real-time polymerase chain reaction. Compared to the diabetic control group, *Myrtus communis* L. fruit extract significantly improved serum glucose, cholesterol, and triglyceride levels ($p < .05$) and significantly increased serum adiponectin, AMPK, and leptin levels ($p < .05$). The *Myrtus communis* L. fruit extract Myrtus communis significantly increased GLUT-1, PPAR-α, and PPAR-γ mRNA expressions in striated muscle tissue ($p < .05$) but did not cause any changes in the liver. In conclusion, it may be stated that the *Myrtus communis* L. fruit extract had a significant positive impact on both glucose and lipid metabolism. Further studies are needed to elucidate the mechanisms of action of *Myrtus communis* L. fruit extract and its application in human diabetes treatment.

Keywords: Glucose transporter, mouse, *Myrtus communis*, peroxisome proliferator-activated receptor, type 2 diabetes mellitus

Introduction

Diabetes mellitus is a chronic metabolic disease characterized by hyperglycemia. It results from either the autoimmune destruction of pancreatic β-cells leading to insufficient insulin production (type 1 diabetes) or a combination of insulin resistance and inadequate compensatory insulin secretion (type 2 diabetes). For 2021, the IDF reported that 537 million adults were living with diabetes, and this number is projected to rise to 783 million by 2045 (IDF, 2021). It negatively affects tissues and organs, causing complications such as nephropathy, neuropathy, and retinopathy (Okur et al., 2017).

Leptin, an adipokine secreted from adipose tissue, plays a role in regulating appetite and enhancing insulin sensitivity, thus influencing glycemia (Aktaş et al., 2013; Welters & Lammert, 2014). Adiponectin, another key adipokine, promotes glucose uptake and fatty acid oxidation in tissues by activating AMP-activated protein kinase (AMPK), contributing to overall metabolic regulation (Zhou et al., 2009).

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors that regulate genes involved in energy metabolism (Kota et al., 2005). There are three isoforms: PPAR-α, PPAR-β, and PPAR-γ. PPAR-α primarily regulates lipid metabolism, while PPAR-γ enhances insulin sensitivity and lipid storage (Kota et al., 2005; Sharma et al., 2009; Song et al., 2012). Activation of PPARs influences glucose transport by increasing the expression and translocation of glucose transporter (GLUT)-1 and GLUT-4 to cell membranes (Chung et al., 2010; Kota et al., 2005). GLUT-4, predominant in muscle and adipose tissues, and GLUT-1, present in all tissues, are critical for glucose uptake and metabolism (Abel et al., 2001; Ciaraldi et al., 2005; Herman et al., 2022; Kampmann et al., 2011; Pragallapati & Manyam, 2019)

The mechanism of action of metformin, which is a cornerstone in treating type 2 diabetes (T2DM), is still not fully understood. It is believed that the effects of metformin on blood sugar and other metabolic processes are linked to its impact on AMPK, gluconeogenesis, GLUT-4, hormone secretion, and digestive microbiota (Collier et al., 2006; Ma et al., 2022; Petakh et al., 2023).

Plants have been used to treat diseases since ancient times and provide 25% of current drugs. The World Health Organization (WHO) supports herbal treatment studies because some synthetic antidiabetic drugs do not produce sufficient effectiveness and have some side effects (Talebianpoor et al., 2019). *Myrtus communis* L. (MC), the common myrtle, is a species of flowering plant in the family *Mytaceae*. The plant has white and black fruits. It is native to Southern Europe,

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the Mediterranean coast, North Africa, Western Asia, and India, and is also cultivated. In folk medicine, both the leaves and fruits of MC are utilized. MC fruits have antinociceptive, antimicrobial, antimutagenic, antioxidant, antihyperlipidemic, and hypoglycemic effects, and it is stated that these effects may be related to various flavanols and flavanol glycosides in their composition (Asgarpanah & Ariamanesh, 2015; Issa & Blue, 2015; Khan et al., 2014; Sepici et al., 2004; Tas et al., 2018; Talebianpoor et al., 2019). The phytochemicals in MC fruit affect some signaling pathways in the liver, skeletal muscle, and fat tissue, increase insulin release and sensitivity, improve glucose uptake into tissues, and reduce glucose absorption from the intestine (Tas et al., 2018).

Previous studies have shown the antidiabetic effects of MC leaf extracts (Sepici et al., 2004; Tas et al., 2018). It has been hypothesized that the hydroalcoholic extract of MC fruit has effects on the glycemic and lipid profiles of mice with T2DM at the molecular level. The study aimed to compare the possible effects of MC fruit extract on diabetes and lipid metabolism with metformin and to determine whether it interacts with metformin. It also aimed to determine the potential of MC fruit to be used as a food supplement and drug in the prevention and treatment of T2DM.

Materials and Methods

The research was conducted in agreement with the ethical guidelines and policies approved by the Selçuk University Experimental Medicine Research and Application Center Ethics Committee (Approval no: 2020/42, Date: October 30, 2020), in accordance with the Guide for the Care and Use of Laboratory Animals.

Animals and Study Design

In this study, 54 male Swiss albino mice aged 6 weeks (31±1.92 g) were divided into 5 groups. The healthy control (SC) group consisted of 6 mice to serve as a baseline for comparisons, while each experimental group consisted of 12 mice to allow for more robust statistical analysis. During the experimental period, mice were kept in polysulfone cages in a central facility under controlled conditions (12 h light/dark cycle, room temperature of 24±1°C, and 60% atmospheric humidity) at the Selçuk University Experimental Medicine Research and Application Center and permitted water and food (Bilyem, Ankara, Türkiye) ad libitum.

Experimental Type 2 Diabetes Induction in Mice

Type 2 Diabetes Mellitus (T2DM) was induced in the mice following a two-step method (Yu et al., 2017). First, the mice were fed a high-fat diet (58% kcal fat, 17% kcal protein, 25% kcal carbohydrate) for 10 weeks as per Bas et al. (2012). Second, multiple low doses of streptozotocin (STZ) were administered intraperitoneally (50 mg/kg on day 1, followed by 25 mg/kg on days 2-7). Glucose levels were measured in blood samples taken from the tail tip post-STZ application, and mice with fasting blood glucose levels above 250 mg/dL were classified as having T2DM. To avoid the lethal consequences of STZ-induced hypoglycemia, mice were provided with 10% sucrose drinking water for 6 days after STZ injection.

Preparation of Hydroalcoholic Extract of *Myrtus communis* Fruits

MC fruit was supplied from the Gazipaşa district of Antalya province. *Myrtus communis* fruit extract (MFE) was prepared by drying and powdering the fruits, then soaking the powder in 70% ethanol (ISOLAB, Germany). The mixture was condensed using a rotary evaporatör (Heidolph, Laborota 4000-efficient, Germany), and the final extract was dried and stored at +4°C until use, following the method recommended by Talebianpoor et al. (2019).

Designing Experiments and Substance Applications

Group HC (Healthy Control)
Mice were given normal saline (0.2 mL/mouse) by oral gavage once daily for 2 weeks while having access to water and standard commercial food ad libitum.

Group DC (Diabetes Control)
T2DM was confirmed in these mice, and they were given normal saline (0.2 mL/mouse) by oral gavage once daily for 2 weeks.

Group D+Met (Diabetes+Metformin)
T2DM was confirmed, and mice received metformin HCl (200 mg/kg) dissolved in distilled water with 0.01% carboxymethylcellulose and 0.1% Tween™ 80, administered by oral gavage once daily for 2 weeks.

Group D+MFE (Diabetes+*Myrtus communis L.* fruit extract)
T2DM was confirmed, and mice received MFE (2 g/kg) dissolved in distilled water, administered by oral gavage once daily for 2 weeks. The study of Elfellah et al. (1984) was considered when determining the dose of MFE.

Group D+MFE+Met (Diabetes+*Myrtus communis L.* fruit extract+ Metformin)
T2DM was confirmed, and mice were given both MFE (2 g/kg) and metformin HCl (200 mg/kg) by oral gavage once daily for 2 weeks.

Collection of Samples

The blood and tissue samples were taken from 6 mice in each group for measurements. Fasting blood samples were taken into heparin-containing tubes and gel micro tubes from the periorbital region, and the hearts of all animals under xylazine (10 mg/kg, intraperitoneal, Xylazinbio 2%, Bioveta, Czech Republic) + ketamine (90 mg/kg, intraperitoneal, Ketasol 10%, Richter Pharma AG, Austria) anesthesia. The mice were then euthanized via cervical dislocation. Following sacrifice, the liver, gastrocnemius, and soleus muscle tissues of the mice were taken. The tissue samples taken were immediately frozen in liquid nitrogen and stored at -80°C until analysis was performed. The blood samples in gel (serum-separated) microtubes were centrifuged at 4000 rpm, and the obtained serums were divided into eppendorf tubes and frozen at –80°C.

Biochemical Analysis

HbA1c measurements were made within 4 h on blood samples collected in heparin tubes using the HbA1c analyzer (Trinity Biotech, Premier Hb9210, Ireland). Biochemical parameters (ALT, AST, fasting glucose, BUN, creatinine, cholesterol, triglyceride) from serum samples were measured by an autoanalyzer (Abbott c8000, Chicago, USA). Insulin (Mouse Insulin, Standard Curve Range: 10-2000 µIU/mL, Cat No: E0062Mo, Bioassay Technology Laboratory, Shanghai, China), AMPK (Mouse AMPK, Standard Curve Range: 1.57–100 ng/mL, Cat No: ELK9450, ELK Biotechnology, Colorado, USA), adiponectin (Mouse Adiponectin, Standard Curve Range: 0.2-60 mg/L, Cat No: E0246Mo, Bioassay Technology Laboratory, Shanghai, China), and leptin (Mouse Leptin, Standard Curve Range: 5-2000 ng/L, Cat No: E0652Mo, Bioassay Technology Laboratory, Shanghai, China) parameters were analyzed in an ELISA reader (Bio-Tek Instruments Inc., MWGt Lambda Scan 200) by commercially available ELISA kits.

Molecular Analysis

Total RNA was isolated from tissue samples with Purezole (Biorad, USA) according to the prospectus. Total RNA samples (1 µg) 28S and 18S band intensities were observed using agarose gel electrophoresis. Nanodrop (Thermo Scientific, NanoDrop TM 1000 Spectrophotometer) was used to determine the quality and quantity (2±0.1 for A260/A280 and 2.0-2.4 for A260/A230) of total RNA obtained. RNA amounts were determined as ng/µL. DNase-I (Thermo Scientific, Vilnius, Lithuania) was applied to remove genomic DNAs. The obtained mRNAs were converted into cDNA with the iScript cDNA synthesis kit (Bio-Rad, USA) following the manufacturer's protocol. cDNA was stored at -20°C for RT-PCR. The obtained cDNAs were amplified in RT-PCR with specific primers (PPAR-α, PPAR-γ, GLUT-1, and GLUT-4). In our study, all primers for RT-PCR were obtained from Oligomer. Specific primers are as follows: GLUT-1, 5'-CCA GCT GGG AAT CGT CGT T-3' (forward) and 5'-CAA GTC TGC ATT GCC CAT GAT-3' (reverse); GLUT-4, 5'-TCT TAT TGC AGC GCC TGA GTC-3' (forward) and 5'-GCC AAG CAC AGC TGA GAA TAC A-3' (reverse); PPAR- α, 5'-TCG GAC TCG GTC TTC TTG AT-3' (forward) and 5'-TCT TCC CAA AGC TCC TTC AA-3' (reverse); PPAR- γ, 5'-CAC AAT GCC ATC AGG TTT GG-3' (forward) and 5'-GCT GGT CGA TAT CAC TGG AGA TC-3' (reverse); GAPDH, 5'-TGT GTC CGT CGT GGA TCT GA-3' (forward) and 5'-TTG CTG TTG AAG TCG CAG GAG-3' (reverse); β-actin, 5'-CTC CGG CAT GTG CAA-3' (forward) and 5'-CCC ACC ATC ACA CCC T-3' (reverse). β-actin for GLUT-1, PPAR-α, PPAR-γ, and GAPDH for GLUT-4 were used as housekeeping genes. Negative controls were used in each experiment. Measurements for all samples were run in triplicate. RT-PCR reactions were performed using SYBR Green qPCR Master Mix according to the manufacturer's instructions with RT-PCR Detection System (Biorad CFX Connect Real-Time PCR Detection System, USA). The data normalization process was performed according to Livak and Schmittgen (2001) via the $2^{-\Delta\Delta Ct}$ method.

Statistical Analysis

The data obtained were analyzed statistically with the Statistical Package for Social Sciences version 22.0 software (IBM Corp.; Armonk, NY, USA). Parametric data were evaluated using a one-way analysis of variance (ANOVA) and a post-hoc Duncan test. $p < .05$ value was considered statistically significant.

Results

The effects of MFE on adiponectin, AMPK, insulin, and leptin are presented in Table 1. Compared to the HC group, induced diabetes did not cause any significant changes in adiponectin, AMPK, insulin, and leptin levels ($p > .05$). Compared to the HC and DC groups, metformin treatment did not cause a significant change in the mentioned parameters ($p > .05$). Compared to the DC group, MFE and MFE+metformin treatment caused a statistically significant ($p < .05$) increase only in AMPK, and MFE treatment caused a statistically significant ($p < .05$) increase in adiponectin, AMPK, and leptin levels.

The effects of MFE on some serum biochemical parameters are presented in Table 2. Compared to the HC group, diabetes caused statistically significant ($p < .05$) increases in serum glucose, HbA1c, triglyceride, cholesterol, AST, ALT, BUN, and creatinine levels. While metformin significantly ($p < .05$) reduced the increase in serum glucose, HbA1c, triglyceride, and cholesterol levels caused by diabetes, it did not cause any change ($p > .05$) in other measured parameters. *Myrtus communis* fruit extract and MCE+metformin treatments caused significant ($p < .05$) decreases in the increases in serum glucose, triglyceride, and cholesterol levels caused by diabetes; they caused a further increase in ALT levels ($p < .05$).

The effects of MFE on GLUT-1, GLUT-4, PPAR-α, and PPAR-γ mRNA expression in the liver are presented in Figure 1 and in the muscle are presented in Figure 2. Compared with the SC group, diabetes and metformin showed a significant ($p < .05$) increase in GLUT-1 expression in the liver, whereas there was no difference ($p > .05$) between groups in GLUT-4 expression. In the liver, diabetes caused a significant ($p < .05$) decrease in PPAR-α expression, and this decrease was not corrected by MFE and metformin treatments. On the other hand, diabetes caused an insignificant ($p > .05$) decrease in PPAR-γ expression in the liver, and metformin significantly corrected this decrease. Compared with other groups, MFE significantly ($p < .05$) increased GLUT-1 expression in striated muscle, and there was no difference between groups in terms of GLUT-4. Diabetes did not affect PPAR-α expression in striated muscle; however, MFE and MFE+metformin treatment significantly ($p < .05$) increased the expression of this receptor. Compared with SC and DC groups, MFE and MFE+metformin treatments significantly ($p < .05$) increased the PPAR-γ expression in the striated muscle.

In addition, the administration of MFE did not cause any adverse effects in mice.

Table 1.

Effect of metformin HCl (200 mg/kg), Myrtus communis hydroalcoholic extract (2 g/kg), and their combinations on serum adiponectin, AMPK, insulin, and leptin levels in mice with experimental type 2 diabetes (mean ± SD)

	Experimental Groups				
Parameters	**HC**	**DC**	**D+Met**	**D+MFE**	**D+MFE+Met**
Adiponectin (mg/L)	14.14 ± 2.19^{ab}	11.50 ± 1.51^{bc}	11.29 ± 1.25^{bc}	15.70 ± 3.78^{a}	10.00 ± 2.77^{c}
AMPK (ng/mL)	4.75 ± 0.58^{c}	3.63 ± 0.51^{c}	5.45 ± 0.27^{bc}	10.96 ± 4.23^{a}	8.36 ± 3.64^{ab}
Insulin (µIU/mL)	663.29 ± 150.18^{a}	659.31 ± 128.37^{a}	686.44 ± 152.54^{a}	864.70 ± 216.98^{a}	763.02 ± 154.22^{a}
Leptin (ng/L)	306.30 ± 34.15^{b}	295.17 ± 43.26^{b}	320.03 ± 42.36^{b}	384.74 ± 54.80^{a}	315.46 ± 41.34^{b}

Note: ^{a,b,c}Values within a row with different superscripts differ significantly at $p < .05$. AMPK = Adenosine monophosphate-activated protein kinase; DC = Diabetes control; D+Met = Diabetes + 200 mg/kg + metformin HCl; D+MFE = Diabetes+2 g/kg *Myrtus communis* L. fruit hydroalcoholic extract; D+MFE+Met = Diabetes + 2 g/kg *Myrtus communis L*. fruit hydroalcoholic extract + 200 mg/kg metformin HCl; HC = Healthy control.

Table 2.

Effect of metformin HCl (200 mg/kg), Myrtus communis hydroalcoholic extract (2 g/kg), and their combinations on biochemical parameters in mice with experimental type 2 diabetes (mean ± SD)

	Experimental Groups				
Parameters	**HC**	**DC**	**D+Met**	**D+MFE**	**D+MFE+Met**
Glucose (mg/dL)	121.33 ± 12.69^{c}	339.17 ± 22.35^{a}	274.83 ± 20.03^{b}	286.67 ± 19.40^{b}	270.17 ± 14.90^{b}
HbA1c (%)	5.33 ± 0.15^{c}	11.01 ± 0.74^{a}	9.15 ± 1.34^{b}	10.46 ± 0.24^{a}	10.29 ± 0.47^{a}
Triglyceride (mg/dL)	102.50 ± 13.40^{c}	328.17 ± 77.08^{a}	238.33 ± 25.05^{b}	233.50 ± 46.50^{b}	225.33 ± 39.31^{b}
Cholesterol (mg/dL)	93.33 ± 4.27^{c}	145.17 ± 27.47^{a}	122.17 ± 12.45^{b}	126.83 ± 7.93^{b}	118.50 ± 9.44^{b}
AST (U/L)	80.67 ± 7.94^{b}	211.83 ± 30.08^{a}	194.83 ± 44.10^{a}	216.00 ± 68.78^{a}	173.17 ± 37.16^{a}
ALT (U/L)	59.50 ± 7.50^{c}	141.17 ± 24.12^{b}	137.00 ± 20.94^{b}	170.83 ± 28.50^{a}	154.67 ± 13.31^{ab}
BUN (mg/dL)	13.33 ± 1.51^{c}	16.50 ± 1.52^{ab}	14.00 ± 2.37^{bc}	17.00 ± 2.83^{a}	14.17 ± 2.32^{bc}
Creatinine (mg/dL)	0.37 ± 0.14^{b}	0.60 ± 0.19^{a}	0.50 ± 0.13^{ab}	0.43 ± 0.14^{ab}	0.48 ± 0.15^{ab}

Note: ^{a,b,c}Values within a row with different superscripts differ significantly at $p < .05$. HC=Healthy control; DC=Diabetes control; D+Met=Diabetes+200 mg/kg metformin HCl; D+MFE=Diabetes+2 g/kg *Myrtus communis* L. fruit hydroalcoholic extract; D+MFE+Met=Diabetes+2 g/kg *Myrtus communis L.* fruit hydroalcoholic extract+200 mg/kg metformin HCl; AST=Aspartate aminotransferase; ALT=Alanine aminotransferase; BUN=Blood urea nitrogen.

Discussion

MFE significantly improved adiponectin and AMPK levels, unlike metformin. *Myrtus communis* fruit extract and metformin both reduced serum glucose, triglyceride, and cholesterol levels, but their effects on other markers like ALT and BUN differed. *Myrtus communis* fruit extract had a notable impact on GLUT-1 and PPAR-α mRNA expressions in striated muscle, distinct from metformin's effects.

This study's results revealed that MFE significantly improved the decrease in adiponectin and AMPK levels caused by diabetes, increased the leptin level, and did not cause any change in the insulin level (Table 1). Although there is no data related to the effect of the MC plant on AMPK, it has been reported that quercetin found in this plant activates AMPK (Eid et al., 2015). Similar to our results, myricetin found in this plant's leaves caused a 15.77% increase in adiponectin levels in mice that were made obese with a high-fat diet (Su et al., 2016). MC leaf extract and myricetin were found to reduce serum leptin levels in rats and mice fed a high-fat diet (Su et al., 2016; Ozyilmaz Yay et al., 2023). The divergence between our results and the results of these studies may be due to differences in the animal and plant parts used and the methods of the studies. We determined that MFE significantly ($p < .05$) improved the increase in serum glucose, triglyceride, and cholesterol levels caused by diabetes, did not cause significant changes in HbA1c, AST, BUN, and creatinine levels, and increased the ALT level (Table 2). Although there

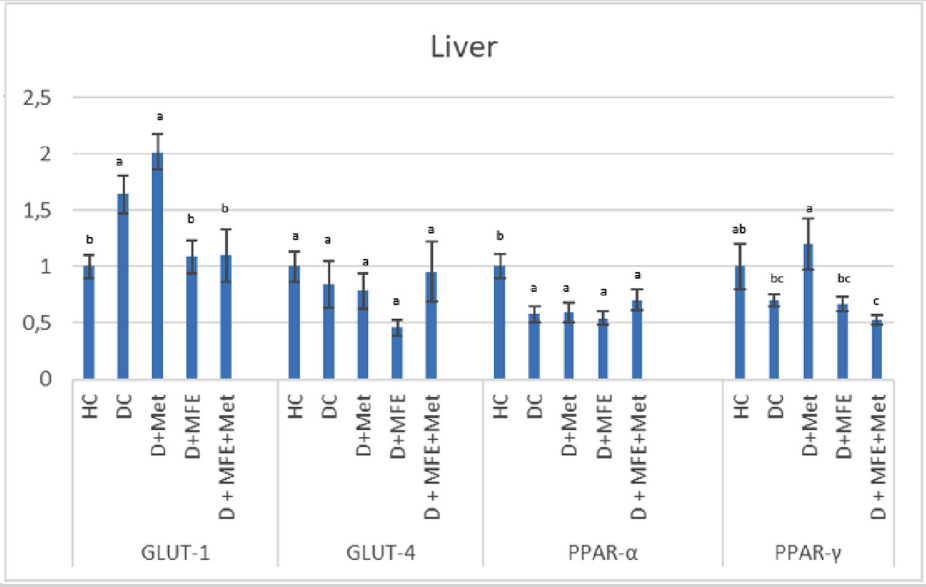

Figure 1.

Fold-change values in liver GLUT-1, GLUT-4, PPAR-α, and PPAR-γ mRNA expressions in type 2 diabetic mice administered Myrtus communis L. fruit hydroalcoholic extract and metformin (2-ΔΔCt ± SE of Mean of log).

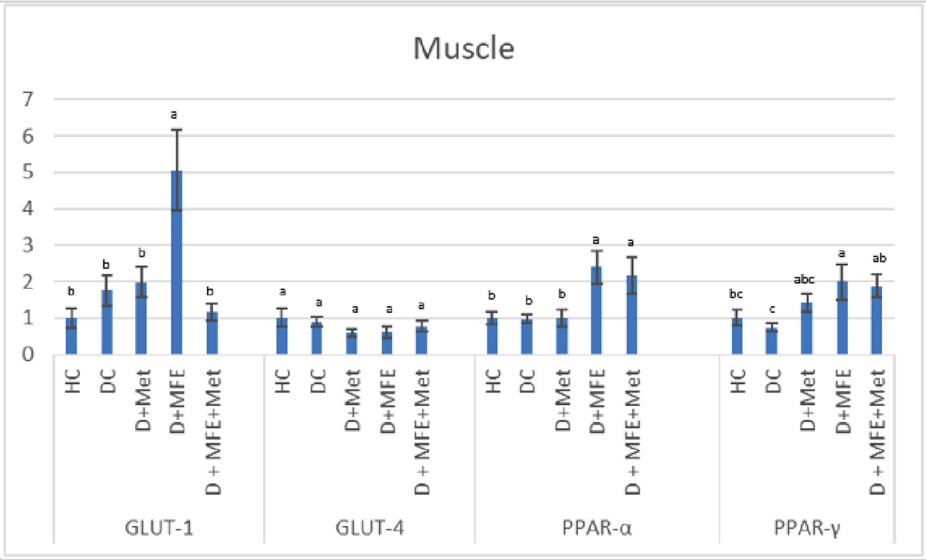

Figure 2.
Fold-change values in muscle GLUT-1, GLUT-4, PPAR-α, and PPAR-γ mRNA expressions in type 2 diabetic mice administered Myrtus communis L. fruit hydroalcoholic extract and metformin (2-ΔΔCt ± SE of Mean of log).

are no studies on the effect of myrtle fruit on blood biochemistry, leaf extracts of the plant have been reported to reduce serum cholesterol and triglyceride levels in experimental animals, similar to our results (Kanpalta et al., 2022; Ozyilmaz Yay et al., 2023). In addition, it was found that myricetin reduced the plasma glucose, cholesterol, and triglyceride levels in mice that were made obese with a high-fat diet (Su et al., 2016). The decrease in serum glucose, triglyceride, and cholesterol levels caused by MFE may be related to its ability to increase adiponectin, leptin, and AMPK levels in the serum and to upregulate GLUT-1, PPAR-α, and PPAR-γ mRNA expressions in the striated muscle. PPAR-γ regulates adiponectin expression (Sun et al., 2021; Ahmadian et al., 2013). It has been stated that adiponectin and AMPK stimulate the uptake of glucose and fatty acids by tissues, and adiponectin reduces hepatic glucose production and increases cholesterol efflux (Wu et al., 2003; Long & Zierath, 2006; Yanai & Yoshida, 2019). Leptin reduces appetite, increases insulin sensitivity, and controls lipid levels and glycemia (Aktaş et al., 2013; Welters & Lammert, 2014). In addition, PPAR-α and PPAR-γ regulate the expression of genes critical for glucose uptake and lipid metabolism, respectively (Yoon, 2009; Ahmadian et al., 2013).

According to the study results, it was determined that metformin did not cause a significant change in the serum levels of adiponectin, AMPK, insulin, and leptin in diabetic mice (Table 1). Administration of metformin to db/db mice for 4 weeks improved hyperinsulinemia but did not cause a change in plasma adiponectin levels (Fujita et al., 2005). It was reported that administration of metformin for 14 days to diabetic obese rats with high plasma leptin and insulin levels caused a significant decrease in insulin levels, but did not cause a change in leptin levels (Aubert et al., 2011). It was found that metformin increased AMPK activity in human skeletal muscle with type 2 diabetes and in an in vitro study on human umbilical cord-mesenchymal stem cells (Bajetto et al., 2023; Musi et al., 2002). We determined that metformin significantly improved serum glucose, HbA1c, triglyceride, and cholesterol levels in diabetic mice, but did not cause an improvement in AST, ALT, BUN, and creatinine levels. Similar to our results, it has been shown that metformin reduces plasma cholesterol levels in humans and rats (Hu et al., 2021; Liu et al., 2022). Through in vivo and in vitro studies, Duan et al. (2023) determined that metformin improved glucose and lipid metabolism disorders in ob/ob mice. Similar to our findings, it is stated that metformin does not have a significant effect on plasma creatinine (Bakris et al., 2016). A meta-analysis study revealed that metformin did not affect plasma ALT and significantly reduced AST levels (Jalali et al., 2020). It is reported that the administration of metformin at a dose of 70 mg/kg to diabetic rats for 13 weeks significantly improved the elevated plasma BUN and creatinine levels (Zhang et al., 2017). The dissimilarity between our results and the mentioned study results may be due to differences in the animal species used and the metformin dosage regimen applied. It has also been reported that administering metformin to healthy mice for 30 days does not cause a change in plasma urea value (Arruda et al., 2020).

We determined that diabetes increased GLUT-1 mRNA expression in the liver but not GLUT-4 expression (Figure 1) Similar to our results, GLUT-1 mRNA expression has been reported to be increased in the liver, kidney, and heart of diabetic rats (Sokolovska et al., 2012). It was found that metformin increased GLUT-1 mRNA expression in the liver in diabetic mice, and MFE did not affect GLUT-1 mRNA expression. It was determined that metformin and MFE did not affect GLUT-4 mRNA expression in diabetic mice. In addition, metformin and MFE did not correct the decrease in PPAR-α mRNA expression caused by diabetes in the liver. We determined that metformin increased the PPAR-γ mRNA expression in the liver (Figure 1). Diabetes did not significantly increase GLUT-1 mRNA expression in the striated muscles. *Myrtus communis* fruit extract caused significant upregulation in GLUT-1 mRNA expression in striated muscle in diabetic mice, whereas metformin caused non-significant upregulation. We did not detect any changes in the GLUT-4 mRNA expression of striated muscle. It was determined that diabetes had no significant effect on mice's striated muscle PPAR-α

and PPAR-γ mRNA expression. *Myrtus communis* fruit extract significantly upregulated PPAR-α and PPAR-γ mRNA expression in diabetic mice's striated muscle. Although there is no research on the effect of myrtle leaves and fruit on GLUT and PPAR, studies have been conducted on the effects of myricetin, quercetin, and kaempferol polyphenols, which are found in myrtle and other plants. Su et al. (2016) found that a high-fat diet increased PPAR-γ mRNA expression in white adipocytes of mice and that this was reversed by myricetin found in myrtle leaves. It was found that kaempferol downregulated the mRNA expression of PPAR-γ in the liver of mice that were fed a fatty diet (Zang et al., 2015). In an in vitro study conducted by Eid et al. (2015), quercetin was found to upregulate the mRNA expression of GLUT-4 in striated muscle cells, but not in the liver. It has been reported that administering metformin at a dose of 250 mg/kg for 4 weeks to rats with type 2 diabetes upregulated GLUT-4 mRNA expression in skeletal muscle and liver (Shen et al., 2020). The difference in our results and the mentioned study may be due to differences in animal species and the metformin dosage regimen used. It has been shown that giving metformin to healthy mice at 300 mg/kg per day upregulates the mRNA expression of PPAR-α in the kidney (Arruda et al., 2020). The discrepancy between our findings and the study above may be due to differences in tissue, metformin dosage regimen, and experimental model. Liu et al. (2022) found that administering metformin at a dose of 30 mg/kg for 6 weeks to rats fed a fatty diet for 28 weeks caused a decrease in the upregulation of PPAR-γ and GLUT-1 mRNA expressions in the heart caused by the fatty diet. Unlike the study mentioned above, the reason for metformin causing upregulation rather than downregulating GLUT-1 and PPAR-γ mRNA expression in our study may be due to differences in species, tissue, and study design. Duan et al. (2023) found that administration of 200 mg/kg of metformin to ob/ob mice increased PPAR-γ mRNA expression in bone marrow adipose tissue and improved glucose and lipid metabolism, similar to our results. Metformin was reported not upregulating PPAR-γ mRNA expression in diabetic human liver fat tissue (Tiikkainen et al., 2004). Previous in vitro studies have also investigated the effect of metformin on GLUT and PPAR expressions. Different concentrations of metformin were found to remarkably reverse LPS-induced increases in PPAR-γ mRNA expression in rat vascular smooth muscle (Qu & Qu, 2019). Metformin has been reported to increase PPAR-γ expression in human umbilical cord-mesenchymal stem cells (Bajetto et al., 2023). Grisouard et al. (2010) determined that metformin increased GLUT-4 expression via AMPK but not GLUT-1 in human adipose tissue.

When the effects of MFE and metformin on blood biochemistry were compared, it was determined that the two substances had similar effects on glucose, triglyceride, cholesterol, AST, and creatine. On the other hand, the effects of the two substances on BUN and ALT differed (Table 2). The effect of MFE on adiponectin, AMPK, and leptin was found to be different from metformin, and its effect on insulin was similar to metformin (Table 1). The effect of MFE on striated muscle GLUT-1 and PPAR-α mRNA expression was found to be different from metformin, and its effect on PPAR-γ was similar (Figure 2). The effect of MFE on liver GLUT-1 and PPAR-γ mRNA expression was found to be different from metformin, and its effect on PPAR-α was similar (Figure 1). When the results of our study were evaluated, it was determined that there was no significant interaction between MFE and metformin on glucose and lipid metabolism.

Conclusion and Recommendations

In conclusion, the administration of *Myrtus communis* fruit extract (MFE) demonstrates significant therapeutic potential in managing diabetes and modulating lipid metabolism, exhibiting effects comparable to the pharmaceutical agent metformin. Specifically, MFE effectively mitigates hyperglycemia and hyperlipidemia by reducing elevated serum glucose, triglyceride, and cholesterol levels typically induced by diabetes. Additionally, MFE counteracts diabetes-associated decreases in serum adiponectin, AMPK, and leptin levels, suggesting a restoration of key metabolic regulators. At the molecular level, MFE enhances the mRNA expression of GLUT-1 and PPAR-γ and PPAR-α within striated muscle tissue, indicative of improved glucose and lipid metabolism. However, it is notable that these regulatory effects on GLUT and PPAR expression are not observed in hepatic tissue, suggesting tissue-specific mechanisms of action for MFE. These findings underscore the potential of MFE as a complementary treatment strategy in diabetes management, meriting further investigation into its mechanistic pathways and clinical applications.

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