

## Myricetin May Improve Cardiac Dysfunction Possibly Through Regulating Blood Pressure and Cellular Stress Molecules in High-Fructose-Fed Rats

### ABSTRACT

**Background:** The aim of this study was to examine the effect of myricetin on cardiac dysfunction caused by high fructose intake.

**Methods:** Fructose was given to the rats as a 20% solution in drinking water for 15 weeks. Myricetin was administered by oral gavage for the last 6 weeks. Systolic blood pressure was measured by tail-cuff method. The effects of isoprenaline, phenylephrine, and acetylcholine on cardiac contractility and rhythmicity were recorded in the isolated right atrium and left ventricular papillary muscles. In addition to biochemical measurements, the cardiac expressions of cellular stress-related proteins were determined by western blotting.

**Results:** Myricetin improved systolic blood pressure but did not affect body weight, plasma glucose, and triglyceride levels in fructose-fed rats. The impairment of isoprenaline- and phenylephrine-mediated increases in atrial contraction and sinus rate in fructose-fed rats was restored by myricetin treatment. Isoprenaline, phenylephrine, and acetylcholine-mediated papillary muscle contractions were not changed by fructose or myricetin administration. The expression of the mitochondrial fission marker dynamin-related protein 1 and the mitophagic marker PTEN-induced kinase 1 (PINK1) was enhanced in the fructose-fed rat, and myricetin treatment markedly attenuated PINK1 expression. High-fructose intake augmented phosphorylation of the proinflammatory molecule Nuclear factor kappa B (NF- $\kappa$ B) and the stress-regulated kinase JNK1, but myricetin only reduced NF- $\kappa$ B expression. Moreover, myricetin diminished the elevation in the expression of the pro-apoptotic Bax.

**Conclusion:** Our results imply that myricetin has a protective role in cardiac irregularities induced by a high-fructose diet through reducing systolic blood pressure, improving cardiac adrenergic responses, suppressing PINK1, NF- $\kappa$ B, and Bax expression, and thus reflecting a potential therapeutic value.

**Keywords:** Fructose, myricetin, cardiac dysfunction, blood pressure, cellular stress molecules

### INTRODUCTION

Dietary fructose consumption, in the form of high-fructose corn syrup or sucrose, has gradually increased in daily diets, especially in beverages, over the last few decades.<sup>1,2</sup> Accumulating evidence has revealed that high fructose intake is associated with the development of insulin resistance, dyslipidemia, obesity, diabetes, and hypertension.<sup>3,4</sup> Chronic fructose consumption raises blood pressure and leads to cardiovascular complications, which are reported to be a major cause of morbidity and mortality worldwide.<sup>5-7</sup>

Previous studies have reported that different dietary interventions, including a high intake of fructose, lead to cardiac metabolic alterations affecting the performance of the heart.<sup>6,7</sup> Additionally, the changes in adrenergic receptor density and responsiveness, as well as contractile proteins, are implicated in impaired cardiac function induced by high fructose consumption.<sup>8-11</sup> Moreover, excess fructose intake profoundly affects cardiac parameters through numerous mechanisms,

### ORIGINAL INVESTIGATION

Nur Banu Bal<sup>1</sup> 

Ceren Güney<sup>2</sup> 

Onur Gökhan Yıldırım<sup>3</sup> 

Fatma Akar<sup>1</sup> 

Emine Demirel-Yılmaz<sup>4</sup> 

<sup>1</sup>Department of Pharmacology, Faculty of Pharmacy, Gazi University, Ankara, Türkiye

<sup>2</sup>Department of Pharmacology, Faculty of Pharmacy, Düzce University, Düzce, Türkiye

<sup>3</sup>Department of Pharmacy Services, Vocational School of Health Services, Artvin Çoruh University, Artvin, Türkiye

<sup>4</sup>Department of Medical Pharmacology, Faculty of Medicine, Ankara University, Ankara, Türkiye

#### Corresponding author:

Nur Banu Bal  
✉ nurbanubal@gazi.edu.tr

Received: September 19, 2023

Accepted: October 02, 2023

Available Online Date: January 01, 2024

**Cite this article as:** Bal NB, Güney C, Yıldırım OG, Akar F, Demirel-Yılmaz E. Myricetin may improve cardiac dysfunction possibly through regulating blood pressure and cellular stress molecules in high-fructose-fed rats. *Anatol J Cardiol.* 2024;28(1):55-64.



Copyright©Author(s) - Available online at anatoljcardiol.com.  
Content of this journal is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.

DOI:10.14744/AnatolJCardiol.2023.3866

such as increased oxidative stress, mitochondrial dysfunction, inflammation, and apoptosis.<sup>12,13</sup> It is well known that mitochondrial health is crucial for the maintenance of cardiac function and energy homeostasis. Recent studies demonstrated that mitochondrial dynamics (fusion and fission) are changed due to the overproduction of reactive oxygen species in the hearts of rats with diet-induced metabolic syndrome.<sup>14,15</sup> Additionally, mitophagy, which is a specific mitochondrial quality control mechanism, selectively eliminates dysfunctional mitochondria through the PTEN-induced kinase 1 (PINK1)/parkin pathway. Excessive or insufficient mitophagy can disrupt mitochondrial homeostasis and redox balance, leading to dysfunction in organs.<sup>16</sup> Although a high intake of fructose has been shown to impair the mitophagic process in organs such as the liver and kidney,<sup>17,18</sup> its effect on the heart has not been examined yet. Moreover, chronic fructose consumption causes low-grade inflammatory status in several tissues, thus leading to systemic inflammation. Nuclear factor kappa B (NF- $\kappa$ B) is a redox-sensitive pro-inflammatory factor and thus contributes to the development of organ damage associated with cardiometabolic diseases.<sup>19,20</sup> Apoptotic and mitogen-activated signaling pathways (MAPK) have also been exhibited to play an influential role in the progression of fructose-induced cardiac dysfunction.<sup>21,22</sup> Given this, it is thought that therapeutic approaches to prevent oxidative stress, mitochondrial dysfunction, inflammation, and apoptosis may be beneficial in preventing cardiac functional alterations induced by high fructose consumption.

It is known that high consumption of flavonoid-rich foods with antioxidant properties may be favorable for various pathologies, including cardiovascular and metabolic diseases.<sup>23</sup> Myricetin (3,3',4',4',5',5',7-hexahydroxyflavone) is a flavonoid compound with a high antioxidant capacity found in several vegetables, fruits, and herbs.<sup>23,24</sup> In addition to antioxidant action, it has been demonstrated to have a variety of pharmacological activities, such as anti-inflammatory, antiviral, and anticancer.<sup>24,25</sup> Myricetin also ameliorated insulin resistance and increased blood pressure caused by high intake of dietary fructose.<sup>24,26,27</sup> Moreover, myricetin exhibited a cytoprotective effect on oxidative stress-induced apoptosis through the regulation of MAPK signaling.<sup>28</sup> However, the effects of myricetin on high-fructose-diet-induced cardiac dysfunction and its possible mechanistic action have not been examined yet. In the current

study, we evaluated the potential effects of myricetin on systolic blood pressure, cardiac functions (adrenergic and cholinergic responses), and protein expression of some key molecules associated with cellular stress response.

## METHODS

### Animals and Diets

The animal studies were organized according to the Guide for the Care and Use of Laboratory Animals (NIH). The animal care and experimental procedures were approved by the local ethics committee for animal experiments (G.Ü.ET-23.050). Male Wistar rats (3-week-old) were housed in temperature- (22°C  $\pm$  1°C) and humidity-controlled rooms with a light–darkness cycle (12 h:12 h). The rats were fed with a standard rodent chow consisting of a mixture of 62% starch, 23% protein, 7% cellulose, 4% fat, standard vitamins, and salt. After a 1-week acclimatization period, rats were randomly segregated into 4 groups containing 6 rats each: control, CMC (carboxymethylcellulose), fructose, and fructose + myricetin. Fructose (Danisco Sweeteners OY, Kotka, Finland) was administered to rats as a 20% solution (w/v) in drinking water ad libitum for 15 weeks, and the fructose solution was freshly prepared daily.

### Myricetin Treatment and Blood Pressure Measurements

Myricetin (Santa Cruz Biotechnology) was suspended in a 0.5% CMC solution and administered by oral gavage during the last 6 weeks of the 15-week experimental period. The dose and duration of myricetin administration (50 mg/kg/day, for 6 weeks) were chosen based on previous studies.<sup>29,30</sup> The same volume of water and 0.5% CMC solution was orally given to the control and CMC groups, respectively. The body weight of rats was recorded weekly during the experiment. Systolic blood pressure measurements with the tail-cuff apparatus started 1 week before myricetin treatment and continued every week until the end of the study.

Upon completion of 6 weeks of treatment, the rats were anesthetized with a mixture of ketamine and xylazine (80 and 10 mg/kg, respectively, i.p.). The blood samples were collected from the abdominal aorta and quickly centrifuged. Plasma samples were stored at –80°C until plasma glucose and triglyceride levels were measured by standard enzymatic techniques. The left papillary muscles and right atrium were immediately dissected and placed in a cold Krebs solution (containing 112 mM NaCl, 5 mM KCl, 25 mM NaHCO<sub>3</sub>, 0.5 mM MgCl<sub>2</sub>, 1.2 mM NaH<sub>2</sub>PO<sub>4</sub>, 2.5 mM CaCl<sub>2</sub>, and 11.5 mM dextrose, pH: 7.4). The left ventricular tissue was rapidly frozen in liquid nitrogen and stored at –80°C for the western blot experiments.

### Isolated Organ Bath Experiments

The effects of myricetin on cardiac rhythmic activity and contractile responses to adrenergic and cholinergic stimulation were evaluated according to the previously published procedure.<sup>31</sup> The left ventricular papillary muscles and right atrium were prepared and mounted between holders connected to the Grass FT03 force transducer in an organ bath containing Krebs solution warmed to 37°C and gassed with a mixture of 95% oxygen and 5% carbon dioxide. The papillary muscles

## HIGHLIGHTS

- A high intake of fructose leads to increased systolic blood pressure, impaired adrenergic cardiac contractile and rhythmic activity, and activated cellular stress responses.
- Myricetin treatment decreased systolic blood pressure and improved  $\alpha$ - and  $\beta$ -adrenergic responses of isolated cardiac tissues in fructose-fed rats.
- The positive effects of myricetin on cardiac functions may be linked to suppression of PINK1, NF- $\kappa$ B, and Bax.

were stimulated with the Harvard Apparatus Advanced Stimulator after being placed between the gold electrodes. Initial tension (2 g) was applied, and then tissues were allowed to equilibrate for 40 minutes, with washing every 10 minutes. Responses of tissues were determined using force-displacement transducers (Grass FT03) connected with the Grass polygraph system (model 79D). Cumulative concentration responses to acetylcholine ( $10^{-8}$ - $10^{-5}$  M), phenylephrine ( $10^{-8}$ - $10^{-5}$  M), and isoproterenol ( $10^{-11}$ - $10^{-7}$  M) were recorded. These results were presented as a percentage of the baseline contraction magnitude and sinus rate. The atrial contractile responses were calculated as developed tension and resting tension. The developed tension is the contraction amplitude of the atrial muscle at each beat, whereas the resting tension represents the basal tension of the atrium as modified by the spontaneous beat.

### Western Blot Experiments

Left ventricle tissues were homogenized in 4-fold volumes of homogenization buffer, including 150 mM NaCl, 50 mM Tris, 1% NP40 (v/v), 0.5% sodium deoxycholate, 0.1% SDS, and a protease and phosphatase inhibitor cocktail. The homogenates were centrifuged at 1500 g for 20 minutes at 4°C, and then the supernatants were collected. The protein concentration of samples was determined by the Lowry method.<sup>32</sup> Equal proteins (40 µg) for each group were separated with 10%-12% polyacrylamide gel by electrophoresis and transferred to polyvinylidene fluoride membranes using semi-dry electroblotting. After being blocked with 3% bovine serum albumin or 5% nonfat dried milk for 60 minutes, the membranes were incubated with the primary antibodies: DRP1 (sc-271583, 1:1000), Mfn2 (sc-515657, 1:1000), PINK1 (sc-517353, 1:1000), NF-κB (BioLegend 622602, 1:250), p-NF-κB p65 (sc-136548, 1:100), JNK1 (ab199380, 1:1000), p-JNK1 (ab47337, 1:1000), Bax (E-AB-13814, 1:1000), Bcl-2 (sc-7382, 1:500), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (sc-32233, 1:2000) for overnight at 4°C. After the washing step, membranes were treated with horseradish peroxidase-conjugated secondary antibodies for 60 minutes at room temperature. After that, the blots were exposed to western blotting chemiluminescence substrate solution

for 3-4 minutes. Images of the membranes were obtained with the Azure chemiluminescence imaging system (Azure™ Imaging Systems, c300). The intensity of the bands was estimated using the ImageLab6.1 program. For normalization, GAPDH proteins were used as an internal control.

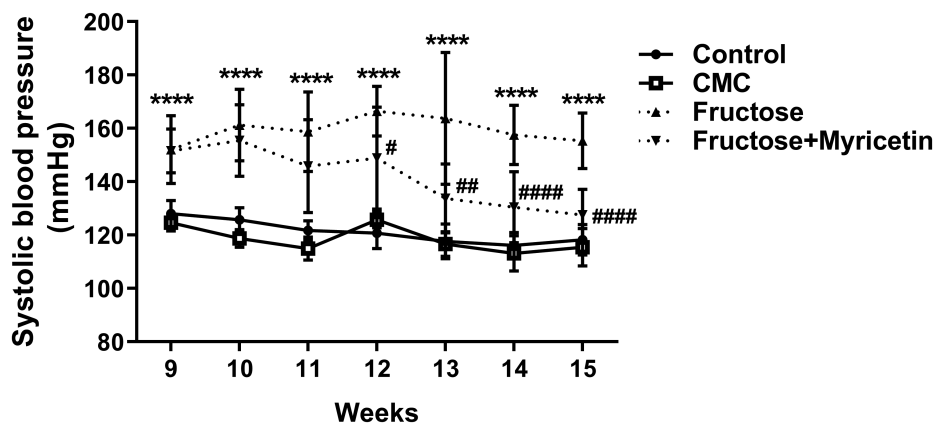
### Statistical Analysis

The findings were expressed as the mean ± standard deviation (SD). The compliance of all groups to the normal distribution was evaluated using the Shapiro-Wilk test. In biochemical and metabolic findings, One-way analysis of variance (ANOVA) post hoc Dunnett test was used for the parametric data, and the Kruskal-Wallis followed by Dunn's post-test was used for the nonparametric data. In isolated organ bath results, a repeated measures two-way ANOVA followed by the Bonferroni post hoc test was used. At a level of  $P < .05$ , values were accepted as statistically significant. All analyses were performed using the statistical program GraphPad Prism software, version 8.0 (La Jolla, CA, USA).

### RESULTS

Fructose administration in drinking water to rats significantly increased systolic blood pressure, and the measurement was started in the ninth week of the experiment. Myricetin treatment significantly lowered the systolic blood pressure of fructose-fed rats from the third week onward (Figure 1). Fructose-fed animals had higher final body weight, plasma glucose, and triglyceride levels than the control group, but myricetin supplementation did not affect these parameters (Table 1).

The effect of high fructose consumption on cardiac functions (contractility and rhythmicity) was studied in isolated right atrium and left ventricular papillary muscle tissues. In the right atrium of fructose-fed animals, the isoprenaline-induced increase in developed tension was lower, but the sinus rate was greater than in the control animals. Myricetin treatment restored these responses in the right atrial tissue of fructose-fed rats. Contraction of the papillary muscle and resting tension of the right atrium with isoprenaline stimulation did not differ between groups (Figure 2).



**Figure 1.** Effects of myricetin on the systolic blood pressure of rats. Myricetin reversed the increase in systolic blood pressure caused by high intake of fructose. \*\*\*\* $P < .0001$  (control vs. fructose group); # $P < .05$ , ## $P < .01$  (fructose vs. fructose + myricetin group). Values are presented as mean ± SD,  $n = 5-6$ .

| Table 1. Effects of Dietary Fructose and Myricetin on Body Weight, Systolic Blood Pressure, Plasma Glucose, and Triglyceride Levels of Rats |                      |                  |                       |                                   |
|---------------------------------------------------------------------------------------------------------------------------------------------|----------------------|------------------|-----------------------|-----------------------------------|
| Parameters                                                                                                                                  | Control (n = 6 Rats) | CMC (n = 5 Rats) | Fructose (n = 6 Rats) | Fructose + Myricetin (n = 6 Rats) |
| Initial body weight (g)                                                                                                                     | 92.14 ± 15.92        | 95.00 ± 3.69     | 90.00 ± 16.74         | 91.89 ± 8.05                      |
| Final body weight (g)                                                                                                                       | 361.43 ± 48.19       | 360.00 ± 21.90   | 432.00 ± 46.91**      | 427.86 ± 36.61                    |
| Final systolic blood pressure (mm Hg)                                                                                                       | 118.22 ± 5.74        | 115.49 ± 7.00    | 155.38 ± 10.46****    | 127.57 ± 9.57####                 |
| Plasma glucose (mg/dL)                                                                                                                      | 101.53 ± 13.08       | 105.43 ± 12.20   | 147.45 ± 18.34**      | 152.50 ± 18.06                    |
| Plasma triglyceride (mg/dL)                                                                                                                 | 97.72 ± 18.08        | 93.08 ± 30.05    | 233.07 ± 49.00**      | 174.42 ± 56.71                    |

Results are presented as mean ± SD.  
CMC, carboxymethylcellulose.  
\*\**P* < .01. \*\*\*\**P* < .0001 (control vs. fructose group). ####*P* < .0001 (fructose vs. fructose + myricetin group).

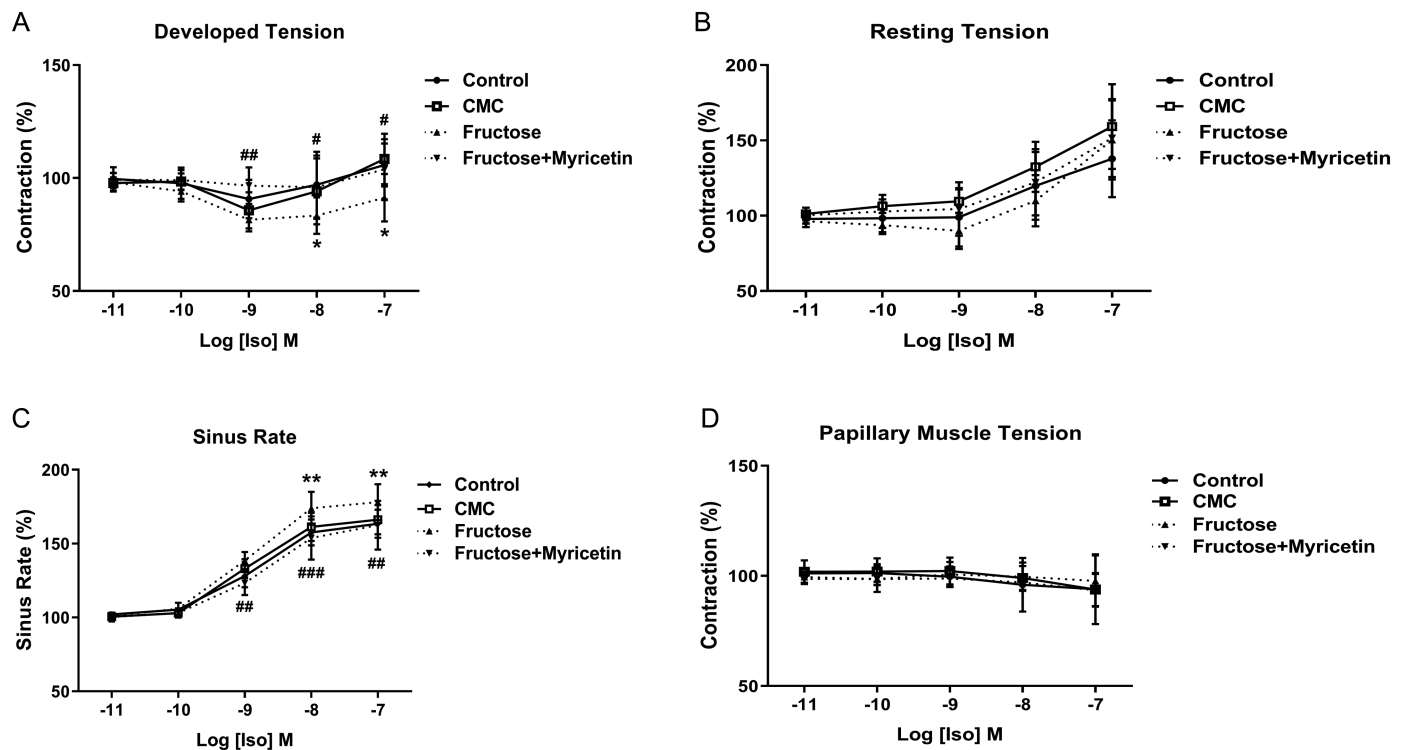
In the right atrium of the fructose-fed group, the phenylephrine-stimulated increase in developed tension was less, but the sinus rate was higher than in the control group. Myricetin application improved the phenylephrine-induced contractile responses and sinus rate of the right atrium. The phenylephrine-induced contractions of the left papillary muscle did not differ between the groups (Figure 3).

The acetylcholine-mediated suppression of resting tension in the right atrium was lower in the fructose-fed group compared to the control group. Myricetin did not affect the acetylcholine-mediated responses of the right atrium and left papillary muscle (Figure 4).

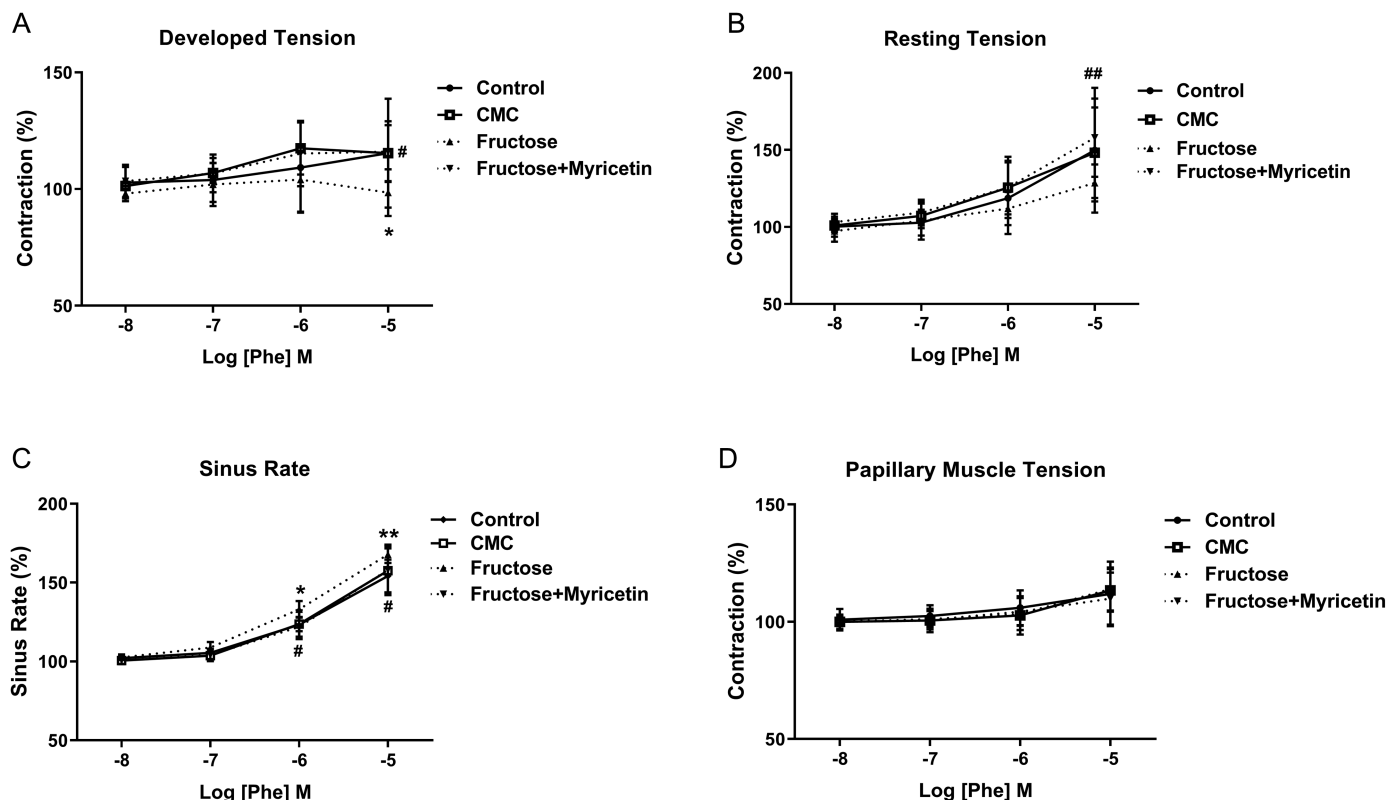
Cardiac mitochondrial homeostasis was evaluated by determining the expression of fission, fusions, and

mitophagy-related proteins. Expression of dynamin-related protein 1 (DRP1), a mitochondrial fission marker, was significantly upregulated in the hearts of fructose-fed rats. There was a tendency to decrease in DRP1 in the myricetin-treated group, but it was not statistically significant. The expression of Mitofusin2 (Mfn2), an indicator of fusion, was similar in all groups. The increase in cardiac PINK1 expression of fructose-fed rats was attenuated by myricetin treatment (Figure 5).

Nuclear factor kappa B and p-NF-κB protein expressions were examined to assess cardiac inflammatory status. Although there was a trend for an increase in cardiac NF-κB expression in fructose-fed rats, it did not reach a statistically significant level. On the other hand, p-NF-κB expression was considerably augmented in the cardiac tissue of the fructose



**Figure 2.** Effects of myricetin on the isoprenaline-induced responses in the right atrium and left ventricular papillary muscle isolated from the rat heart. (A) developed tension, (B) resting tension, (C) sinus rate, and (D) papillary muscle contraction. In fructose-fed rats, the reduction in isoprenaline-mediated contraction (developed tension) and the elevation in isoprenaline-induced sinus rate were reversed by myricetin. \**P* < .05, \*\**P* < .01 (Control vs. Fructose group); #*P* < .05, ##*P* < .01, ###*P* < .0001 (fructose vs. fructose + myricetin group). Values are presented as mean ± SD, n = 5-6. Iso, isoprenaline.



**Figure 3.** Effects of myricetin on the phenylephrine-mediated responses in the right atrium and left ventricular papillary muscle isolated from the rat heart. (A) developed tension, (B) resting tension, (C) sinus rate, and (D) papillary muscle contraction. Myricetin treatment restored the reduction of phenylephrine-induced contractile responses and the elevation of phenylephrine-stimulated sinus rate in the right atrium of the fructose-fed rats. \* $P < .05$ , \*\* $P < .01$  (control vs. fructose group); # $P < .05$ , ## $P < .01$  (fructose vs. fructose + myricetin group). Values are presented as mean  $\pm$  SD,  $n = 5-6$ . Phe, phenylephrine.

group. Myricetin shows a beneficial effect by decreasing cardiac NF- $\kappa$ B protein expression (Figure 6).

Activation of JNK1, which is known as a stress-regulated molecule from the MAPK family, was assessed by measuring JNK1 and p-JNK1 protein expressions. The JNK1 protein level did not differ between the groups. While high intakes of dietary fructose enhanced cardiac p-JNK1 expression, myricetin did not affect JNK1 and p-JNK1 expression (Figure. 7).

Cardiac apoptosis was estimated by quantifying the protein expressions of Bax and Bcl-2 involved in the apoptotic pathway. The pro-apoptotic marker Bax expression was significantly increased in the hearts of fructose-fed rats. Myricetin application diminished the cardiac expression of this protein. The Bcl-2 protein expression was similar in all groups (Figure 8).

In all the findings obtained from this study, no significant difference was observed between the control and CMC group; therefore, the CMC group was not used for statistical comparisons.

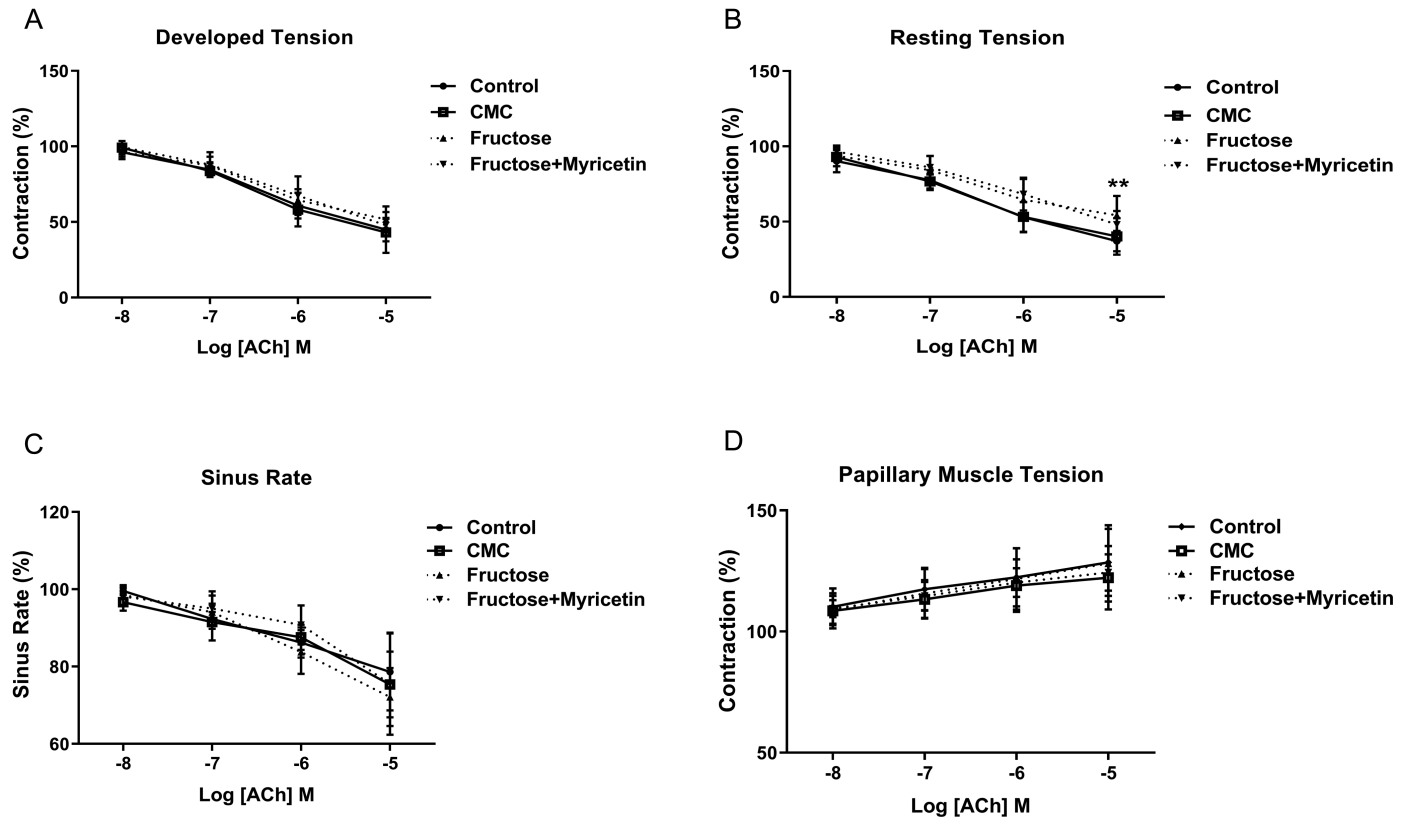
## DISCUSSION

Increased consumption of fructose could be linked to the globally rising prevalence of metabolic and cardiovascular diseases. Fructose feeding leads to both functional and structural cardiac abnormalities accompanied by oxidative,

inflammatory, and apoptotic insults.<sup>8-14</sup> Myricetin, showing antioxidant, antiaging, anti-inflammatory, antidiabetic, and anticancer activities,<sup>23-28</sup> could be proposed as a potential flavonoid for the treatment of various cardiac pathologies. In this study, the possible effects of myricetin on blood pressure and cardiac function, as well as its mechanistic action in high-fructose-fed rats, were evaluated for the first time. Our results demonstrated that myricetin treatment reduced the systolic blood pressure, restored the impaired  $\alpha$ - and  $\beta$ -adrenergic responses of isolated cardiac tissues, and improved cellular stress-related protein expressions such as PINK1, NF- $\kappa$ B, and Bax in high-fructose-fed rats.

High fructose consumption has been reported to raise blood pressure through several mechanisms, including dysfunction of the endothelium, stimulation of the sympathetic nervous system, and elevation of salt absorption.<sup>33-36</sup> Consistently, this study demonstrated that administration of a 20% fructose solution for 15 weeks markedly elevated the systolic blood pressure of rats. Myricetin with potential biological activity has been shown to lower blood pressure in Deoxycorticosterone acetate-salt and fructose-fed rats.<sup>26,37</sup> In the current study, we showed that myricetin (50 mg/kg/day) reduced systolic blood pressure without changing metabolic parameters in high fructose intake.

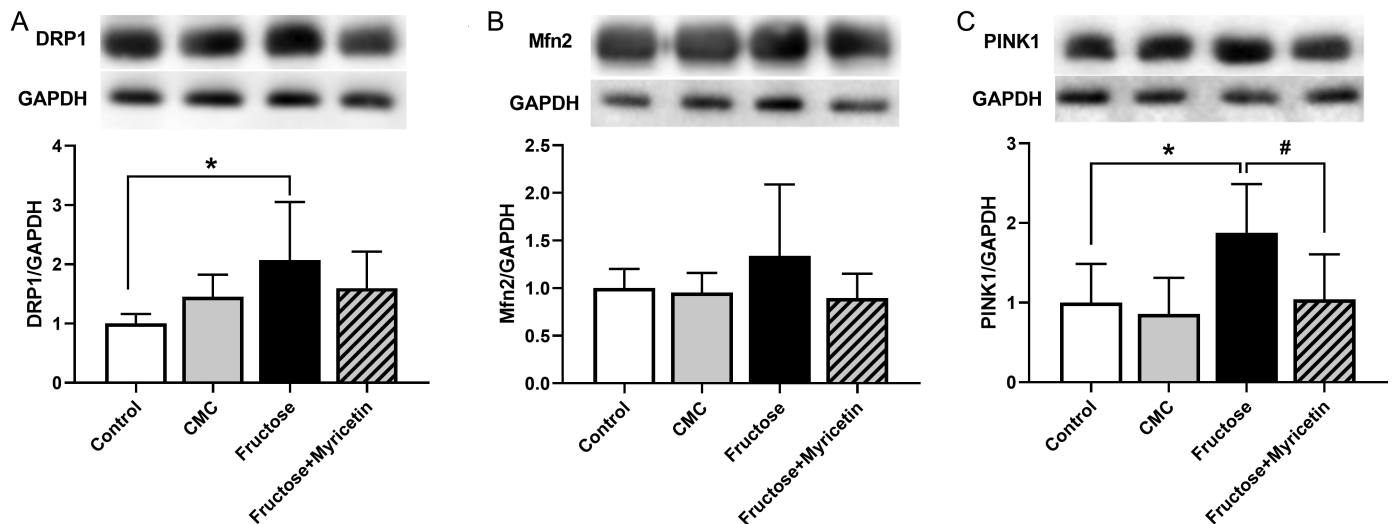
Several studies have reported that high fructose intake leads to a detrimental influence on cardiac structure and



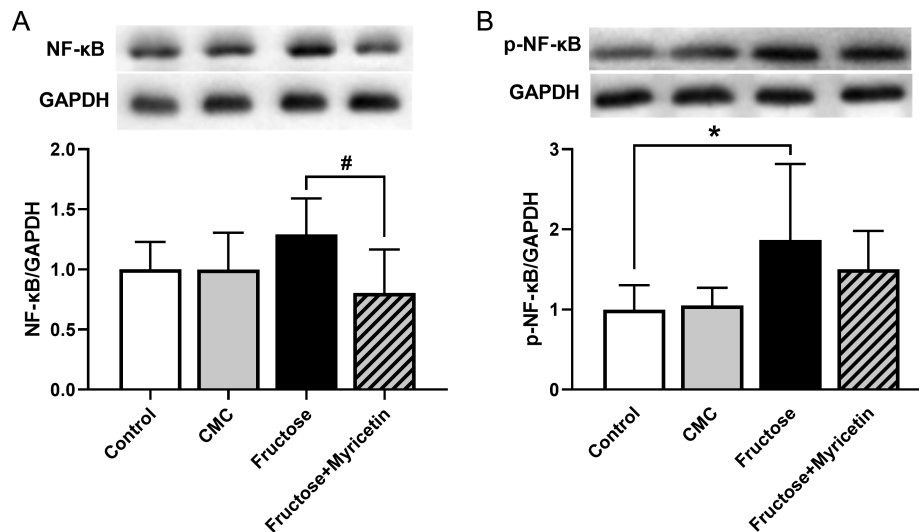
**Figure 4.** Effects of myricetin on the acetylcholine-stimulated responses in the right atrium and left ventricular papillary muscle isolated from the rat heart. (A) developed tension, (B) resting tension, (C) sinus rate, and (D) papillary muscle contraction. Acetylcholine-induced suppression of resting tension was markedly lower in fructose-fed rats than in the control rats.  $**P < .01$  (control vs. fructose group). Values are presented as mean  $\pm$  SD,  $n = 5-6$ . ACh, acetylcholine.

function.<sup>14,38,39</sup> The changes in contractility and rhythmic activity in response to adrenergic stimulation have been reported to play an important role in impaired cardiac function caused by high fructose feeding, but the findings have

some inconsistency. Kamide et al<sup>9</sup> reported that high-fructose diet increased  $\alpha 1$ -receptor density but not  $\beta$ -receptor density in the left ventricle of rats. Moreover, fructose feeding did not alter the chronotropic response to noradrenaline



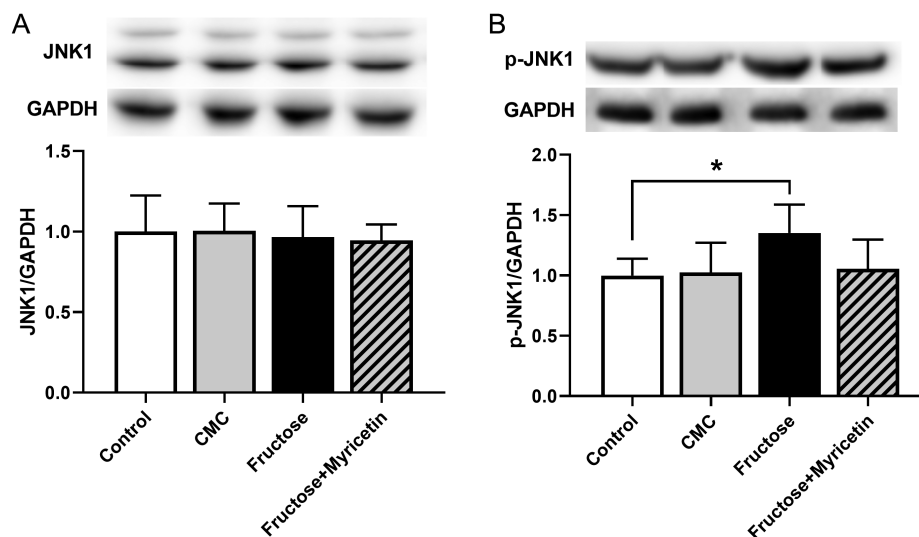
**Figure 5.** Effects of myricetin on the protein expression of DRP1 (A), Mitofusin2 (B), and PINK1 (C) in the heart. In the heart tissue of fructose-fed rats, DRP1 and PINK1 expression were increased while Mitofusin2 expression was unchanged. Myricetin treatment only decreased cardiac PINK1 expression.  $*P < 0.05$  (control vs. fructose group);  $\#P < 0.05$  (fructose vs. fructose + myricetin group). Values are presented as mean  $\pm$  SD,  $n = 5-6$ .



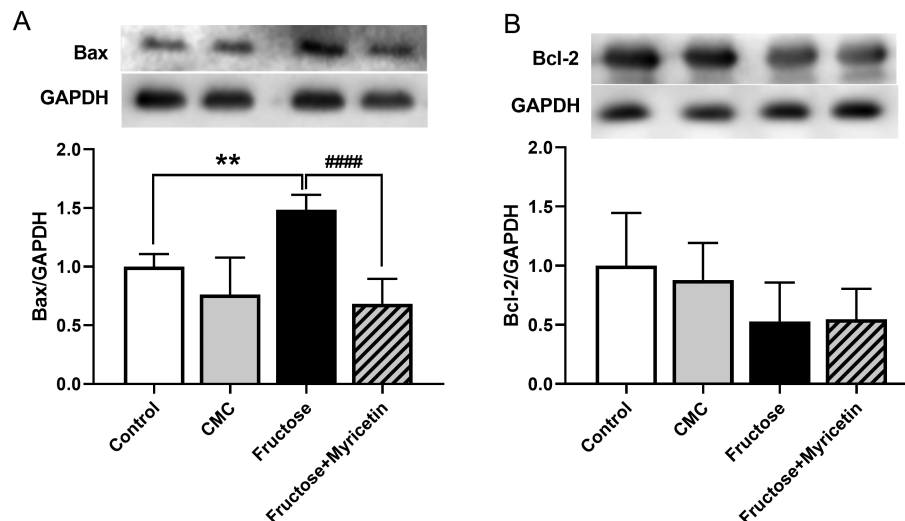
**Figure 6.** Effects of myricetin on the protein expression of NF-κB (A) and p-NF-κB (B) in the heart. p-NF-κB expression was enhanced in the heart of fructose-fed rats. Myricetin administration prevented the increase in NF-κB expression. \* $P < .05$  (control vs. fructose group); # $P < .05$  (fructose vs. fructose + myricetin group). Values are presented as mean  $\pm$  SD,  $n = 5-6$ .

stimulation in the isolated atrium.<sup>10</sup> However, other studies demonstrated that high-fructose consumption produces a significant positive chronotropic effect in the isolated atria of fructose-fed rats.<sup>11</sup> In the present study, we show that high-fructose intake decreased the contractile responses (developed tension) to isoprenaline and phenylephrine in the right atrium. Consistent with our decreased atrial contractility response, chronotropic responses to isoprenaline and phenylephrine were markedly augmented in fructose-fed rats. However, the  $\alpha$  and  $\beta$ -adrenergic responses of the left ventricular papillary muscles were not affected by the high intake of fructose. Importantly, myricetin treatment reversed the changes in atrial contractility and sinus rate induced by isoprenaline and phenylephrine stimulation.

Although several studies have examined the adrenergic receptor density and responsiveness in cardiac tissues in high-fructose-fed animals, the muscarinic function has been less addressed. Lee et al<sup>40</sup> indicated that both mRNA and protein expressions of muscarinic cholinergic receptors in cardiac tissue were unchanged in fructose-fed rats. In the current study, fructose feeding further suppressed the acetylcholine-induced decrease in resting tension of the right atrium. However, myricetin did not affect acetylcholine-mediated responses in the right atrium and left papillary muscle tissues. These results propose that myricetin treatment in high fructose-fed rats can improve impaired cardiac function by restoring  $\alpha$  and  $\beta$  receptor-mediated contractions and rhythmic activity in the atrium of the heart.



**Figure 7.** Effects of myricetin on the protein expression of JNK1 (A) and p-JNK1 (B) in the heart. The elevation in cardiac p-JNK1 expression induced by high intake of fructose was not affected by myricetin. \* $P < .05$  (control vs. fructose group). Values are presented as mean  $\pm$  SD,  $n = 5-6$ .



**Figure 8.** Effects of myricetin on the protein expression of Bax (A) and Bcl-2 (B) in the heart. The increase in cardiac Bax expression caused by high intake of fructose was ameliorated by myricetin administration. The Bcl-2 expression were similar in all groups.  $**P < .01$  (control vs. fructose group);  $####P < .0001$  (fructose vs. fructose + myricetin group). Values are presented as mean  $\pm$  SD,  $n = 5-6$ .

Mitochondria, which account for 30% of cardiomyocyte volume, are the main organelles for cellular metabolism through the production of adenosine triphosphate and control of redox status.<sup>16,41</sup> Mitochondrial homeostasis, which is produced by mitochondrial biogenesis, dynamics, and mitophagy, is essential in the maintenance of the physiological functions of the heart.<sup>12,42</sup> Mitochondrial dynamics, regulated by the balance of fusion (association of mitochondria) and fission (division of mitochondria), are considered the critical linkage between mitochondrial biogenesis and mitophagy. High fructose consumption has been reported to disrupt the balance of mitochondrial dynamics in the heart.<sup>14,43</sup> Consistent with the above studies, we observed that the fission protein DRP1 was significantly enhanced while the fusion protein Mfn2 was unchanged in the hearts of the high fructose-fed rats. Mitochondrial dynamics are closely linked to the mitophagic process because proper fission of mitochondria is a prerequisite for mitophagy. According to the elevation in DRP1 levels, protein expression of PINK1, which mediates selective removal of damaged mitochondria, was also augmented in the hearts of fructose-fed rats. These results indicate that increased fission induced by a high intake of fructose leads to excessive mitochondrial fragmentation. These findings also revealed that mitophagic activity is enhanced to eliminate the fragmented mitochondria in the hearts of fructose-fed animals. There was a trend to decrease in cardiac DRP1 expression in the myricetin-treated group, but it did not achieve statistical significance. However, myricetin application markedly reduced mitophagic activity, as evidenced by suppressed PINK1 expression in cardiac tissue. Our findings demonstrated that one of the cardioprotective mechanisms of myricetin could be related to the regulation of disrupted fission and mitophagy.

Recent studies have shown that excessive fructose consumption triggers the inflammatory response in heart tissue,

leading to cardiac impairments.<sup>13,19,44</sup> Redox-sensitive NF- $\kappa$ B signaling is the main pathway in the progression of inflammation.<sup>19</sup> In support of these findings, the current study shows that p-NF- $\kappa$ B expression was upregulated in the heart of fructose-fed rats. Previously, myricetin was shown to have anti-inflammatory properties, decreasing NF- $\kappa$ B activation in different cells and tissues.<sup>28,29</sup> Similarly, myricetin treatment significantly attenuated cardiac NF- $\kappa$ B protein expressions, showing anti-inflammatory activity.

Extracellular signal-regulated kinase signaling pathways including c-JUN N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), and p38-MAPK, have been known to play a pivotal role in organ injury and apoptosis caused by harmful challenges, including high-fructose conditions.<sup>22,45</sup> Previous studies also show that inhibition of the stress-regulated protein kinase JNK leads to attenuation of cardiac dysfunction caused by metabolic disorders.<sup>46,47</sup> In the present study, long-term fructose consumption enhanced cardiac p-JNK expression. However, myricetin treatment did not change the expression of this protein. On the other hand, excessive fructose consumption triggers the apoptotic process along with other cellular stress pathways that contribute to cardiac dysfunction.<sup>12,21</sup> Likely in this study, the protein level of the pro-apoptotic marker Bax was elevated in the hearts of fructose-fed rats. Myricetin evidently restored the increase in Bax protein expression in the heart tissue. Consistent with other cardiac abnormalities tested with myricetin,<sup>48,49</sup> suppression of pro-apoptotic protein may mediate the favorable effects of the flavonoid in the heart tissue of fructose-fed rats.

#### Study Limitations

The cardioprotective effect of myricetin may be partly due to its antihypertensive activity; however, there are no data for a mechanistic explanation of the blood pressure-lowering effect of myricetin in this study. Therefore, the potential

mechanisms underlying this effect of myricetin will remain the objective of future studies.

## CONCLUSION

In the fructose-fed rats, myricetin treatment significantly lowered blood pressure and restored cardiac dysfunction by improving  $\alpha$  and  $\beta$  receptor-mediated responses without ameliorating metabolic parameters. Mechanistically, these positive impacts of myricetin on cardiac functions might also be related to the suppression of PINK1, NF- $\kappa$ B, and Bax. Although the relevance of these results in humans remains to be determined, our findings suggest that myricetin may have promising therapeutic potential in high-fructose-diet-induced cardiac dysregulations. Further research is needed to clarify the clinical consequences of myricetin.

**Ethics Committee Approval:** This study was approved by Ethics Committee of Gazi University (Approval No: G.Ü.ET-23.050, Date: 01.06.2023).

**Peer-review:** Internally peer-reviewed.

**Author Contributions:** Concept – N.B.; Design – N.B., C.G., O.Y., F.A., E.Y.; Supervision – N.B., F.A., E.Y.; Resource – N.B., O.Y., F.A., E.Y.; Materials – N.B. and O.Y.; Data Collection and/or Processing – N.B., C.G.; Analysis and/or Interpretation – N.B., C.G.; Literature Review – N.B., C.G.; Writing – N.B., F.A.; Critical Review – N.B., F.A., and E.Y.

**Declaration of Interests:** The authors declare that they have no conflict of interest.

**Funding:** This study was partially supported by grants from the Gazi and Artvin Çoruh University Research Fund (project no: TDK-2022-7661 and 2021.S37.02.01).

## REFERENCES

- Johnson RJ, Segal MS, Sautin Y, et al. Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease, and cardiovascular disease. *Am J Clin Nutr*. 2007;86(4):899-906. [CrossRef]
- Petridi E, Karatzi K, Magriplis E, Charidemou E, Philippou E, Zampelas A. The impact of ultra-processed foods on obesity and cardiometabolic comorbidities in children and adolescents: a systematic review. *Nutr Rev*. 2023;nuad095. [CrossRef]
- Malik VS, Hu FB. The role of sugar-sweetened beverages in the global epidemics of obesity and chronic diseases. *Nat Rev Endocrinol*. 2022;18(4):205-218. [CrossRef]
- Guney C, Bal NB, Akar F. The impact of dietary fructose on gut permeability, microbiota, abdominal adiposity, insulin signaling and reproductive function. *Heliyon*. 2023;9(8):e18896. [CrossRef]
- Pektaş MB, Sadi G, Akar F. Long-term dietary fructose causes gender-different metabolic and vascular dysfunction in rats: modulatory effects of resveratrol. *Cell Physiol Biochem*. 2015;37(4):1407-1420. [CrossRef]
- Jung S, Bae H, Song WS, Jang C. Dietary fructose and fructose-induced pathologies. *Annu Rev Nutr*. 2022;42:45-66. [CrossRef]
- Avagimyan A, Sukiasyan L, Kakturskiy L, et al. Diabefit as a modifier of fructose-induced impairment of cardio-vascular system. *Curr Probl Cardiol*. 2022;47(10):100943. [CrossRef]
- Szűcs G, Sőja A, Péter M, et al. Prediabetes Induced by fructose-Enriched Diet Influences Cardiac Lipidome and Proteome and leads to Deterioration of Cardiac Function prior to the Development of Excessive Oxidative Stress and Cell Damage. *Oxid Med Cell Longev*. 2019;2019:3218275. [CrossRef]
- Kamide K, Rakugi H, Higaki J, et al. The renin-angiotensin and adrenergic nervous system in cardiac hypertrophy in fructose-fed rats. *Am J Hypertens*. 2002;15(1 Pt 1):66-71. [CrossRef]
- Di Verniero CA, Silberman EA, Mayer MA, Opezzo JA, Taira CA, Höcht C. In vitro and in vivo pharmacodynamic properties of metoprolol in fructose-fed hypertensive rats. *J Cardiovasc Pharmacol*. 2008;51(6):532-541. [CrossRef]
- Rodríguez G, Mago N. Inotropic and chronotropic effects of propranolol in isolated atrium of rats with fructose-induced insulin-resistance. *Investig Clin*. 2017;58(1):22-33.
- Park JH, Ku HJ, Kim JK, Park JW, Lee JH. Amelioration of high fructose-induced cardiac hypertrophy by naringin. *Sci Rep*. 2018;8(1):9464. [CrossRef]
- Wang X, Xu Z, Chang R, Zeng C, Zhao Y. High-fructose diet induces cardiac dysfunction via macrophage recruitment in adult mice. *J Cardiovasc Pharmacol Ther*. 2023;28:10742484231162249. [CrossRef]
- Bugga P, Mohammed SA, Alam MJ, et al. Empagliflozin prohibits high-fructose diet-induced cardiac dysfunction in rats via attenuation of mitochondria-driven oxidative stress. *Life Sci*. 2022;307:120862. [CrossRef]
- Nizami HL, Katore PB, Prabhakar P, et al. Paricalcitol attenuates metabolic syndrome-associated heart failure through enhanced mitochondrial fusion. *Oxid Med Cell Longev*. 2022;2022:5554290. [CrossRef]
- Ajoolabady A, Chiong M, Lavandero S, Klionsky DJ, Ren J. Mitophagy in cardiovascular diseases: molecular mechanisms, pathogenesis, and treatment. *Trends Mol Med*. 2022;28(10):836-849. [CrossRef]
- Zhang C, Song Y, Chen L, et al. Urolithin A attenuates hyperuricemic nephropathy in fructose-fed mice by impairing STING-NLRP3 axis-mediated inflammatory response via restoration of parkin-dependent mitophagy. *Front Pharmacol*. 2022;13:907209. [CrossRef]
- Dethlefsen MM, Kristensen CM, Tøndering AS, Lassen SB, Ringholm S, Pilegaard H. Impact of liver PGC-1 $\alpha$  on exercise and exercise training-induced regulation of hepatic autophagy and mitophagy in mice on HFF. *Physiol Rep*. 2018;6(13):e13731. [CrossRef]
- Kosuru R, Kandula V, Rai U, Prakash S, Xia Z, Singh S. Pterostilbene decreases cardiac oxidative stress and inflammation via activation of AMPK/Nrf2/HO-1 pathway in fructose-fed diabetic rats. *Cardiovasc Drugs Ther*. 2018;32(2):147-163. [CrossRef]
- Eleazu CO, Obeten UN, Ozor G, et al. Tert-butylhydroquinone abrogates fructose-induced insulin resistance in rats via mitigation of oxidant stress, NF $\kappa$ B-mediated inflammation in the liver but not the skeletal muscle of high fructose drinking rats. *J Food Biochem*. 2022;46(12):e14473. [CrossRef]
- Cheng SM, Cheng YJ, Wu LY, et al. Activated apoptotic and anti-survival effects on rat hearts with fructose induced metabolic syndrome. *Cell Biochem Funct*. 2014;32(2):133-141. [CrossRef]
- Zhang Y, Zhang L, Zhang Y, Xu JJ, Sun LL, Li SZ. The protective role of liquiritin in high fructose-induced myocardial fibrosis via inhibiting NF- $\kappa$ B and MAPK signaling pathway. *Biomed Pharmacother*. 2016;84:1337-1349. [CrossRef]
- Pekkarinen SS, Stöckmann H, Schwarz K, Heinonen IM, Hopia AI. Antioxidant activity and partitioning of phenolic acids in bulk and emulsified methyl linoleate. *J Agric Food Chem*. 1999;47(8):3036-3043. [CrossRef]
- Niisato N, Marunaka Y. Therapeutic potential of multifunctional myricetin for treatment of type 2 diabetes mellitus. *Front Nutr*. 2023;10:1175660. [CrossRef]

25. Rahmani AH, Almatroudi A, Allemailem KS, et al. Myricetin: A significant emphasis on its anticancer potential via the modulation of inflammation and signal transduction pathways. *Int J Mol Sci.* 2023;24(11):9665. [\[CrossRef\]](#)
26. Godse S, Mohan M, Kasture V, Kasture S. Effect of myricetin on blood pressure and metabolic alterations in fructose hypertensive rats. *Pharm Biol.* 2010;48(5):494-498. [\[CrossRef\]](#)
27. Liu IM, Tzeng TF, Liou SS, Lan TW. Myricetin, a naturally occurring flavonol, ameliorates insulin resistance induced by a high-fructose diet in rats. *Life Sci.* 2007;81(21-22):1479-1488. [\[CrossRef\]](#)
28. Kang KA, Wang ZH, Zhang R, et al. Myricetin protects cells against oxidative stress-induced apoptosis via regulation of PI3K/Akt and MAPK signaling pathways. *Int J Mol Sci.* 2010;11(11):4348-4360. [\[CrossRef\]](#)
29. Coêlho CFF, Souza ILS, Chagas VT, et al. Myricetin improves metabolic outcomes but not cognitive deficit associated to metabolic syndrome in male mice. *Food Funct.* 2021;12(8):3586-3596. [\[CrossRef\]](#)
30. Ahmad SB, Rashid SM, Wali AF, et al. Myricetin (3,3',4',5,5',7-hexahydroxyflavone) prevents ethanol-induced biochemical and inflammatory damage in the liver of Wistar rats. *Hum Exp Toxicol.* 2022;41:9603271211066843. [\[CrossRef\]](#)
31. Bal NB, Bostanci A, Sadi G, Dönmez MO, Uludag MO, Demirel-Yilmaz E. Resveratrol and regular exercise may attenuate hypertension-induced cardiac dysfunction through modulation of cellular stress responses. *Life Sci.* 2022;296:120424. [\[CrossRef\]](#)
32. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem.* 1951;193(1):265-275. [\[CrossRef\]](#)
33. Kubacka M, Kotańska M, Szafarz M, et al. Beneficial effects of non-quinazoline  $\alpha_1$ -adrenolytics on hypertension and altered metabolism in fructose-fed rats. A comparison with prazosin. *Nutr Metab Cardiovasc Dis.* 2019;29(7):751-760. [\[CrossRef\]](#)
34. Aguilera-Mendez A, Hernández-Equihua MG, Rueda-Rocha AC, et al. Protective effect of supplementation with biotin against high-fructose-induced metabolic syndrome in rats. *Nutr Res.* 2018;57:86-96. [\[CrossRef\]](#)
35. Klein AV, Kiat H. The mechanisms underlying fructose-induced hypertension: a review. *J Hypertens.* 2015;33(5):912-920. [\[CrossRef\]](#)
36. Akar F, Uludağ O, Aydın A, et al. High-fructose corn syrup causes vascular dysfunction associated with metabolic disturbance in rats: protective effect of resveratrol. *Food Chem Toxicol.* 2012;50(6):2135-2141. [\[CrossRef\]](#)
37. Borde P, Mohan M, Kasture S. Effect of myricetin on deoxycorticosterone acetate (DOCA)-salt-hypertensive rats. *Nat Prod Res.* 2011;25(16):1549-1559. [\[CrossRef\]](#)
38. Paramesha B, Anwar MS, Meghwani H, Maulik SK, Arava SK, Banerjee SK. Sirt1 and Sirt3 activation improved cardiac function of diabetic rats via modulation of mitochondrial function. *Antioxidants (Basel).* 2021;10(3):338. [\[CrossRef\]](#)
39. Yoo S, Ahn H, Park YK. High dietary fructose intake on cardiovascular disease related parameters in growing rats. *Nutrients.* 2016;9(1):11. [\[CrossRef\]](#)
40. Lee LM, Chang CK, Cheng KC, Kou DH, Liu IM, Cheng JT. Increase of cardiac M2-muscarinic receptor gene expression in type-1 but not in type-2 diabetic rats. *Neurosci Lett.* 2008;441(2):201-204. [\[CrossRef\]](#)
41. Forte M, Schirone L, Ameri P, et al. The role of mitochondrial dynamics in cardiovascular diseases. *Br J Pharmacol.* 2021;178(10):2060-2076. [\[CrossRef\]](#)
42. Uchikado Y, Ikeda Y, Ohishi M. Current understanding of the pivotal role of mitochondrial dynamics in cardiovascular diseases and senescence. *Front Cardiovasc Med.* 2022;9:905072. [\[CrossRef\]](#)
43. Federico M, Zavala M, Vico T, et al. CaMKII activation in early diabetic hearts induces altered sarcoplasmic reticulum-mitochondria signaling. *Sci Rep.* 2021;11(1):20025. [\[CrossRef\]](#)
44. Zhao C, Zhang Y, Liu H, Li P, Zhang H, Cheng G. Fortunellin protects against high fructose-induced diabetic heart injury in mice by suppressing inflammation and oxidative stress via AMPK/Nrf-2 pathway regulation. *Biochem Biophys Res Commun.* 2017;490(2):552-559. [\[CrossRef\]](#)
45. Cheng PW, Lin YT, Ho WY, et al. Fructose induced neurogenic hypertension mediated by overactivation of p38 MAPK to impair insulin signaling transduction caused central insulin resistance. *Free Radic Biol Med.* 2017;112:298-307. [\[CrossRef\]](#)
46. Ding W, Feng H, Li WJ, et al. Apocynin attenuates diabetic cardiomyopathy by suppressing ASK1-p38/JNK signaling. *Eur J Pharmacol.* 2021;909:174402. [\[CrossRef\]](#)
47. Zuo G, Ren X, Qian X, et al. Inhibition of JNK and p38 MAPK-mediated inflammation and apoptosis by ivabradine improves cardiac function in streptozotocin-induced diabetic cardiomyopathy. *J Cell Physiol.* 2019;234(2):1925-1936. [\[CrossRef\]](#)
48. Liao HH, Zhu JX, Feng H, et al. Myricetin possesses potential protective effects on diabetic cardiomyopathy through inhibiting I $\kappa$ B $\alpha$ /NF $\kappa$ B and enhancing Nrf2/HO-1. *Oxid Med Cell Longev.* 2017;8370593. [\[CrossRef\]](#) [published correction appears in *Oxid Med Cell Longev.* 2021 May 4;2021:9812928. (<https://doi.org/10.1155/2021/9812928>)
49. Arafah A, Rehman MU, Ahmad A, et al. Myricetin (3,3',4',5,5',7-hexahydroxyflavone) prevents 5-fluorouracil-induced cardiotoxicity. *ACS Omega.* 2022;7(5):4514-4524. [\[CrossRef\]](#)