

## The effects of calcium dobesilate and micronized purified flavonoid fractions on myocardial protection

*Kalsiyum dobesilat ve mikronize pürifiye flavonoid fraksiyonlarının miyokardın korunması üzerine etkileri*

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**Background:** We aimed to investigate the effects of calcium dobesilate (DOBE) and micronized purified flavonoid fractions (MPFF) on the functions of rat hearts following ischemia and reperfusion.

**Methods:** Eighteen male Wistar albino rats were randomly divided into three groups: the DOBE group (100 mg/kg/day), the MPFF group (100 mg/kg/day), and the control group. The hearts were perfused twice for 25 minutes before and after 15 minutes of ischemia. Creatine kinase, lactate dehydrogenase, troponin I and myoglobin levels in the coronary perfusates, and malondialdehyde and glutathione levels in the myocardial tissue samples were analyzed. Mean *P* (mean pressure perfusing coronary arteries), PP (peak systolic pressure), (+)dp/dt<sub>max</sub> (contraction strength change over time), (-)dp/dt<sub>max</sub> (relaxation strength change over time), time to reach PP, ejection time, and contraction time were calculated.

**Results:** The mean '*p*' values of the DOBE group were significantly lower than those of the control group at the first and fifth minutes of reperfusion; moreover, PP, (+)dp/dt<sub>max</sub>, and (-)dp/dt<sub>max</sub> values were found to be significantly higher than the measurements made at the 15<sup>th</sup>, 20<sup>th</sup> and 25<sup>th</sup> minutes in the control group. In the MPFF group, the (-)dp/dt<sub>max</sub> value was significantly higher than that of the control group at the fifth minute of reperfusion.

**Conclusion:** DOBE is helpful in improving myocardial functions following ischemia and reperfusion.

**Key words:** Calcium dobesilate; micronized purified flavonoid fractions; myocardial protection.

**Amaç:** Kalsiyum dobesilat (DOBE) ve mikronize pürifiye flavonoid fraksiyonlarının (MPFF) iskemi ve reperfüzyon sonrası sıçan kalplerinin fonksiyonları üzerindeki etkileri araştırıldı.

**Çalışma planı:** On sekiz adet Wistar albino cinsi erkek sıçan randomize olarak üç gruba ayrıldı: DOBE (100 mg/kg/gün), MPFF (100 mg/kg/gün) ve kontrol grubu. On beş dakikalık iskemiden önce ve sonra kalpler iki kez 25 dakika perfüze edildi. Koroner perfüzlarda kreatin kinaz, laktat dehidrogenaz, troponin I ve miyogloblin seviyeleri, miyokard doku örneklerinde ise malondialdehid ve glutatyon düzeyleri analiz edildi. Ortalama *P* (koroner arterleri perfüze eden ortalama basınç), PP (pik sistolik basınç), (+)dp/dt<sub>max</sub> (kontraksiyon kuvvetinin zamanla değişimi), (-)dp/dt<sub>max</sub> (relaksasyon kuvvetinin zamanla değişimi), PP'ye ulaşılan kadar geçen süre, ejeksiyon zamanı ve kontraksiyon zamanı hesaplandı.

**Bulgular:** DOBE grubunda reperfüzyonun 1. ve 5. dakikalarındaki ortalama '*p*' değerleri kontrol grubundakilerden anlamlı derecede düşük idi; ayrıca 15., 20. ve 25. dakikalarda PP, (+)dp/dt<sub>max</sub> ve (-)dp/dt<sub>max</sub> ölçümleri ise kontrol grubundakilerden anlamlı derecede yüksek bulundu. MPFF grubunda ise (-)dp/dt<sub>max</sub> değeri reperfüzyonun 5. dakikasında kontrol grubundakilere göre anlamlı ölçüde daha yüksek idi.

**Sonuç:** DOBE iskemi ve reperfüzyon sonrası miyokard fonksiyonlarını iyileştirmede yararlıdır.

**Anahtar sözcükler:** Kalsiyum dobesilat; mikronize pürifiye flavonoid fraksiyon; miyokard korunması.

Ischemia-reperfusion injury (IRI) after cardiac surgery is composed of myocardial edema, reversible functional disorder (stunning) without structural tissue injury, and/or cell death.<sup>[1]</sup> Stunning is caused by insufficient

myocardial protection in the ischemic period and reperfusion injury following this period.<sup>[2]</sup> For this reason, investigations are under way to develop better myocardial protection techniques.

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The term “myocardial protection” refers to strategies and methods used to inhibit or decrease post-ischemic myocardial dysfunction during or following cardiac surgery.<sup>[3]</sup> The clinical reflection of the prolonged ischemic period is increased by postoperative inotropic requirements, prolonged ventilatory support, and connection to assistive devices.<sup>[4,5]</sup>

It is thought that free oxygen radicals have an important role in the decrease of myocardial function. In recent years, the use of free oxygen radical scavengers or antioxidants have become the center of focus in order to remove or decrease the damage caused by free oxygen radicals.<sup>[6-10]</sup>

Calcium dobesilate (DOBE) is a pharmacological agent frequently used in the treatment of pain, edema, paresthesia, and cramps consequent to chronic venous insufficiency, venous stasis ulcers, and superficial thrombophlebitis. This occurs particularly in microangiopathies, such as diabetic retinopathy, hemorrhoids, and microcirculation disorders of arteriovenous origin.

Micronized purified flavonoid fractions (MPFF) are the active parts of a vasculoprotective phlebotonic agent.<sup>[11]</sup> Flavonoids also have analgesic and antiaggregant effects.

Aside from the positive effects of DOBE and MPFF on vasculoprotective and hemorrheologic parameters, antioxidant effects and enhancement of the release of vasodilator substances suggest that they may have positive effects on cardiac mechanical dysfunction by decreasing the endothelial dysfunction that has an important role on the physiopathology of myocardial IRI.

In this study, using the Langendorff model, we planned to balance the increased oxidant load with antioxidant agents by oral application of DOBE and MPFF and to demonstrate their effects on cardiac IRI.

## MATERIALS AND METHODS

### Preparations of materials

In the present study, 18 healthy male Wistar albino rats weighing 150-200 gr provided by the İstanbul University Cerrahpaşa Medical Faculty Experimental Research Laboratory were used. Subjects were randomly selected and divided into three groups with six rats in each. All rats were cared for, fed, and followed up by veterinary physicians in special cages in a climate-controlled environment with sunlight at 20±2 °C throughout the experiment. This study complies with the Declaration of Helsinki, and the

Ethics Committee of the Cerrahpaşa Medical Faculty approved the research protocol.

One group received 100 mg/kg/day DOBE as gavage fluid, and the other group received 100 mg/kg/day MPFF orally for 15 days. The control group received no medications.

The study began following all preparations and preliminary studies. The subjects were decapitated, and the abdominal and mediastinal cavities were opened by laparosternotomy. Cardiectomy was performed by dissecting the ascending aorta and other vessels without any mechanical damage to the heart. The extracted hearts were placed in an iced (4 °C) Tyrode solution containing 1000 U/L heparin (Table 1).

Preparation of the ascending aorta was completed by cleansing the surrounding tissues. Then the ascending aorta was cannulated by a No. 18 cannula using the Langendorff method for perfusion and fixed using 4/0 silk. Immediately afterward, perfusion was begun under constant flow with 10 ml/min of modified Tyrode solution via Miniplus 3 peristaltic pump (Gilson Medical Electronics Middleton WI USA). The Tyrode solution was gassed with 1L/min carbogen (95% O<sub>2</sub> and 5% CO<sub>2</sub>) to provide an oxygen partial pressure of over 500 mmHg. The perfused heart was placed in a glass chamber at 37 °C constant temperature in order to fix the heart and to prevent desiccation.

The chamber and solution temperatures were fixed at 37 °C by passing the fluid through the double-walled glass apparatus throughout the study.

### Protocol of experiments

Computer records were begun simultaneously with decapitation. All hearts were perfused with the modified Tyrode solution for 25 minutes by 10 ml/min. In the 15<sup>th</sup> minute of the perfusion period, a coronary perfusate sample was obtained for pre-ischemic measurements and biochemical analyses. At the end of this period, perfusion was stopped, and all hearts were submitted to global ischemia for 15 minutes. The hearts were

**Table 1. Contents of modified tyrode solution**

	Modified tyrode solution (mmol/L)
NaCl	128
KCl	4.7
CaCl <sub>2</sub>	1.36
NaHCO <sub>3</sub>	20
NaH <sub>2</sub> PO <sub>4</sub>	0.36
MgCl <sub>2</sub>	1
Glucose	10

kept in the modified tyrode solution during ischemia. Post-ischemic measurements were made from the hearts reperfused with the modified tyrode solution for 25 minutes following ischemia. In addition to the first and 25<sup>th</sup> minutes of reperfusion, samples were obtained from the coronary perfusate for biochemical analyses. At the end of the experiment, the hearts were weighed, and appropriate tissue samples were obtained for histopathological and biochemical investigations.

### Measured and calculated parameters

#### *Parameters related to cardiac mechanics*

A latex balloon was inserted into the left ventricle to measure the parameters related to cardiac contractility (Latex balloon No: 3, volume: 0.03, Hugo-Sachs Electronics, Berlin, Germany), and 5 mmHg pressure was exerted onto the balloon. Intraventricular pressure was measured by a Biopac Systems TSD 104A (Santa Barbara, California, USA) pressure transducer. The data underwent analogue-to-digital conversion by a Biopac Systems MP 100 data collection system. Then, the "AcqKnowledge Software Version 3.2.6" program was used to record 100 samples per second on the computer.

Bipolar electrocardiogram recordings were obtained by two electrodes inserted simultaneously, one on the metallic aorta cannula and the other on the apex of the heart. Recordings were followed simultaneously on a different channel with an ECG 100B electrocardiogram amplifier added to the modular MP 100 system.

All measurements were made by analysis of files recorded after the experiments.

#### *Hemodynamic measurements*

Calculations of the mean P (mean perfusion; mean pressure perfusing coronary arteries), PP (peak systolic pressure), (+)dp/dt<sub>max</sub> (alteration of contraction strength with time), (-)dp/dt<sub>max</sub> (alteration of relaxation strength with time), time to reach peak systolic pressure, ejection time, and contraction time were made using the intraventricular pressure and electrocardiogram records.

#### *Biochemical measurements*

*Enzyme analysis:* In the coronary perfusate, creatine kinase (CK) (Olympus AU 800, Olympus Diagnostica, Hamburg, Germany), lactate dehydrogenase (LDH) (Olympus AU 800, Olympus Diagnostica, Hamburg, Germany), troponin I (Trp I) (Beckman Coulter, CA, USA), and myoglobin (Myglb), (Boehringer-Mannheim Hitachi 717 autoanalyser, Tokyo, Japan) enzymes were measured as markers of myocardial injury. For both subjects, samples were obtained first in the stabilization period (preischemic value), and the

other two in the first and 25<sup>th</sup> minutes of reperfusion. Samples were kept in the refrigerator, and at the end of the study, these samples were studied at the İstanbul University Cerrahpaşa Medical Faculty Central Research Laboratory.

*Tissue analysis:* At the end of all experiments, the hearts were weighed. For myocardial malondialdehyde (MDA) analysis, the most important marker for IRI (lipid peroxidation), one proper tissue sample was obtained. The samples taken were frozen in liquid nitrogen at -80 °C (RUA instruments, Cryosystem®) and kept in a deep freezer.

Tissue samples were weighed and homogenized with 0.09% NaCl and 10% (W/V). The homogenate was centrifuged for 15 minutes at 3000 cycles. Tissue MDA analysis depends on the principle of evaluating MDA produced by thiobarbituric acid in an acid medium at 535 nm wavelength using an extinction coefficient.<sup>[12]</sup> Tissue glutathione (GSH) determination depends on the principle of evaluating sulfhydryl groups by making a compound with 5-5' dithiobis-2-nitrobenzoic acid (DTNB) at 412 nm wavelength and using an extinction coefficient.<sup>[13]</sup> Protein assay was also performed in the tissue.<sup>[14]</sup>

#### Statistical analysis

All data was expressed as mean ± standard deviation. Statistical evaluations were performed using one-way ANOVA between groups, and the initial and reperfusion values in the same group were evaluated by a repetitive ANOVA test. Post-hoc comparison was performed by Tukey's HSD (Honestly Significance Test). A p value of <0.05 was accepted as significant.

## RESULTS

#### Parameters related with cardiac mechanics

1- Mean perfusion: The values at the first and fifth minutes of reperfusion in the DOBE group were significantly lower than those of the control group (p=0.031 and 0.023, respectively; Table 2).

2- Peak systolic pressure: In the DOBE group, the values at the 15<sup>th</sup>, 20<sup>th</sup>, and 25<sup>th</sup> minutes of reperfusion were significantly higher when compared to those of the control group (p=0.016, p=0.005 and p=0.002), and it was also higher than the value in the MPFF (p=0.045) groups at the 25<sup>th</sup> minute of reperfusion (Table 3).

3- (+)dp/dt<sub>max</sub>: The values of the DOBE group were significantly high compared to those of the control group at the 15<sup>th</sup>, 20<sup>th</sup>, and 25<sup>th</sup> minute reperfusion (p=0.028; p=0.011; p=0.020; Table 4).

4- (-)dp/dt<sub>max</sub>: The 15<sup>th</sup>, 20<sup>th</sup>, and 25<sup>th</sup> minute reperfusion values of the DOBE group were significantly

**Table 2. Mean perfusion pressure values of all groups**

	Initial	Reperfusion period (minutes)						<i>p</i>
	Mean±SD	1 Mean±SD	5 Mean±SD	10 Mean±SD	15 Mean±SD	20 Mean±SD	25 (End) Mean±SD	
Control	123.1±24.7	95.3±17.9**	76.2±15.9**	76.9±16.0**	86.1±18.7**	89.6±16.7**	91.9±15.3**	<0.0001
MPFF	100.3±30.7	75.8±13.3**	62.1±11.1**	69.8±19.1**	79.8±28.7**	82.2±28.8*	75.5±17.0**	<0.0001
DOBE	105.9±35.6	71.0±13.4**	54.8±7.70**	57.8±11.2**	66.7±20.7**	72.4±28.3**	75.4±31.6**	<0.0001
<i>p</i>	0.428	0.031†	0.023†	0.142	0.356	0.514	0.369	

SD: Standard deviation; MPFF: Micronized purified flavonoid fractions; DOBE: Dobesilate; †: DOBE compared to control *p*<0.05; \*: compared to initial *p*<0.05; \*\*: Compared to initial *p*<0.01. Note: The *p* values on the last line of the tables demonstrate the comparison between groups (one-way ANOVA and Tukey HSD test), and the *p* values in the last column on the right show the comparison with initial values in the same group.

high compared to those of the control group (*p*=0.013; 0.005; 0.002, respectively), and fifth minute reperfusion value of the MPFF group was significantly higher compared to the control group (*p*=0.029; Table 5).

5- Time to reach peak systolic pressure: No significant differences were detected between groups at the time of measurement (no data was shown).

6- Ejection time: There was no significant difference on any of the measurement times (no data was shown).

7- Contraction time: There was no significant difference between groups at any measurement time (no data was shown).

**Biochemical parameters**

1- Troponin I (Trp-I in coronary perfusate): Although there was an increase in all three groups compared to the initial at the first minute of reperfusion, this was significant in the control and MPFF groups (*p*=0.0340 and 0.0332). There was no significant difference between groups at any measurement time (no data was shown).

2- Myoglobin (Mygb in coronary perfusate): Although there was no significant difference in any of the three groups compared to the initial, the value of the DOBE group was significantly higher than that of the control at the 25th minute of reperfusion (*p*=0.041) (no data was shown).

3- Creatinine (CK kinase in coronary perfusate): While there was an increase at the first minute of reperfusion compared to the control and MPFF groups, no change was observed in the DOBE group. Although there was no change at the 25<sup>th</sup> minute of reperfusion in the control and MPFF groups compared to control, the DOBE group demonstrated a decrease. However, these changes were not significant. There was no significant difference between groups at any measurement time (no data was shown).

4- Lactate dehydrogenase (LDH in coronary perfusate): Although there was an increase at the first minute of reperfusion in the control and MPFF groups compared to the initial, the DOBE group demonstrated a decrease. A decrease at the 25<sup>th</sup> minute of reperfusion compared to the initial was seen. However, these changes were not statistically significant. There was no significant difference between groups at any measurement time (no data was shown).

5- Malondialdehyde: Although the DOBE group compared to control and MPFF groups and the MPFF group compared to the control group had lower MDA values, a comparison between the groups control-MPFF, control-DOBE, and MPFF-DOBE showed no significant difference (*p*=0.699, 0.310, and 0.337, respectively; no data was shown).

6- Glutathion: Although lower GSH values were detected in DOBE and MPFF groups compared to the

**Table 3. Mean peak to peak values of all groups**

	Initial	Reperfusion period (minutes)						<i>p</i>
	Mean±SD	1 Mean±SD	5 Mean±SD	10 Mean±SD	15 Mean±SD	20 Mean±SD	25 (End) Mean±SD	
Control	118.4±17.7	9.60±8.40**	25.8±19.5**	38.3±21.2**	45.7±24.6**	51.0±23.6**	57.5±24.4**	<0.0001
MPFF	89.90±28.6	53.0±54.0	52.5±15.5	60.3±26.8	61.4±24.2	66.0±18.2	73.0±11.7	0.174
DOBE	107.3±17.4	47.6±50.3**	53.4±27.4**	69.7±34.0*	87.2±15.6	92.3±11.1	98.4±9.4	<0.0001
<i>p</i>	0.108	0.196	0.069	0.169	0.016†	0.005††	0.002†† / 0.045‡	

SD: Standard deviation; MPFF: Micronized purified flavonoid fractions; DOBE: Dobesilate; †: DOBE compared to control *p*<0.05; ††: DOBE compared to control *p*<0.01; ‡: DOBE compared to MPFF *p*<0.05; \*: Compared to initial *p*<0.05; \*\*: Compared to initial *p*<0.01.

Note: The *p* values on the last line of the tables demonstrate the comparison between groups (one-way ANOVA and Tukey HSD test), and the *p* values in the last column on the right show the comparison with initial values in the same group.

**Table 4. Mean (+)dp/dt<sub>max</sub> values of all groups**

	Initial		Reperfusion period (minutes)					<i>p</i>
		1	5	10	15	20	25 (End)	
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Control	1805.6±187.4	170.7±161.6**	320.0±186.3**	500.7±285.4**	622.9±327.3**	716.1±316**	829.4±324.8**	<0.0001
MPFF	1402.9±457.6	843.2±718.1	731.8±250.3	848.8±354.3	903.6±336.4	1014.7±278.5	1114.9±205.8	0.085
DOBE	1616.8±273.9	757.8±724.9**	695.8±345.1**	952.2±427.8**	1216.5±221.6	1312.9±161.3	1416.0±74.7	<0.0001
<i>p</i>	0.986	0.148	0.909	0.138	0.028†	0.011†	0.020†	

SD: Standard deviation; MPFF: Micronized purified flavonoid fractions; DOBE: Dobesilate; †: DOBE compared to control group *p*<0.05; \*\*: Compared to initial *p*<0.01.

Note: The *p* values on the last line of the tables demonstrate the comparison between groups (one-way ANOVA and Tukey HSD test), and the *p* values in the last column on the right show the comparison with initial values in the same group.

control group, comparison between the groups control-MPFF, control-DOBE, and MPFF-DOBE showed no significant difference (*p*=0.485, 0.394, and 0.262, respectively; no data was shown).

## DISCUSSION

Insufficient protection of the heart during cardiopulmonary bypass (CPB) and cardiac arrest may cause postoperative long-term cardiac insufficiency. The cause of impaired cardiac function is not always cell death. Transient cardiac dysfunction, referred to as stunning, may occur due to IRI. Secondary to decreased contraction, systemic blood pressure falls, and vital organ perfusion is endangered. This condition may risk separation of the patient from CPB. In order to solve this problem, it may be necessary for the patient to undergo CPB again and receive secondary cardioplegia, be put on medical (+) inotropic support, apply an intraaortic balloon pump, or use right/left ventricular assist devices. Overcoming myocardial stunning will support the postoperative recovery of patients and decrease cardiac morbidity and mortality.

Myocardial stunning has been thought to be caused by free oxygen radicals and intracellular calcium retention. Free oxygen radicals cause tissue injury in macromolecules, like enzymes, proteins, and nucleic acids. They initiate lipid peroxidation and increase cellular membrane permeability.<sup>[15]</sup>

Tissues are paradoxically damaged to a higher extent in the reperfusion period than in ischemia. Excessive formation of reactive oxygen radicals and excessive calcium load play a role in the development of IRI. Evidence of a relationship between neutrophils and endothelial cells has recently begun to be investigated. In formation of IRI, increased adhesiveness of neutrophils to endothelial cells was noted.<sup>[16]</sup>

Granulocytes, primarily neutrophils, accumulate in the myocardium during acute myocardial ischemia. Dense neutrophil reaction during reperfusion and release of hazardous oxidant products and proteolytic enzymes play an important role in the production of myocardial injury during reperfusion.<sup>[17]</sup>

Cardiopulmonary bypass activates leukocytes. In a study on isolated rabbit hearts based on the fact that this activation damages the coronary endothelium and myocardium during reperfusion, it was demonstrated that reperfusion performed by blood with suppressed leukocytes decreased the coronary endothelial injury and had a positive effect on functional recovery following prolonged cardioplegic arrest.<sup>[18]</sup>

Having antioxidant characteristics, N-acetyl cysteine has been shown to maintain systolic cardiac functions following cardiopulmonary bypass and cardioplegic arrest along with decreasing myocardial edema and oxidative stress.<sup>[19]</sup>

**Table 5. Mean (-)dp/dt<sub>max</sub> values of all groups**

	Initial		Reperfusion period (minutes)					<i>p</i>
		1	5	10	15	20	25 (End)	
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Control	1056.0±147	142.6±126.3**	216.4±98.5**	351.7±225.9**	447.8±235.3**	500.6±222.3**	582.2±250.2**	<0.0001
MPFF	1082.6±470.9	484.2±383.5**	473.5±143.1**	582.2±257.2*	636.3±260.0*	727.5±217.9	795.4±221	0.0081
DOBE	1056.0±222.2	474.0±386.9**	413.5±204.0**	640.4±261.3**	828.2±136.1	872.4±79.3	942.2±65.4	<0.0001
<i>p</i>	0.136	0.139	0.029‡	0.109	0.013†	0.005††	0.002††	

SD: Standard deviation; MPFF: Micronized purified flavonoid fractions; DOBE: Dobesilate; †: DOBE compared to control group *p*<0.05; ††: DOBE compared to control *p*<0.01; ‡: MPFF compared to control *p*<0.05; \*: Compared to initial *p*<0.05; \*\*: Compared to initial *p*<0.01.

Note: The *p* values on the last line of the tables demonstrate the comparison between groups (one-way ANOVA and Tukey HSD test), and the *p* values in the last column on the right show the comparison with initial values in the same group.

Calcium dobesilate has been shown in various studies to prevent platelet adhesion and aggregation by decreasing Platelet Activating Factor (PAF) synthesis which decreases erythrocyte aggregation, increases erythrocyte flexibility, regulates microcirculation by decreasing blood viscosity, normalizes the disrupted tissue oxygen consumption, serves as a free radical scavenger and an antioxidant, and stimulates nitric oxide (NO) synthesis by increasing NO synthetase activity.<sup>[20-26]</sup> In experimental ischemia-reperfusion studies in rats, DOBE has been shown to decrease oxidative stress in the lungs and the retina following ischemia-reperfusion.<sup>[27,28]</sup> In a study by Del Maestro reactive oxygen radicals were shown to increase microvascular permeability.<sup>[29]</sup> In 1997, Ruiz et al.<sup>[30]</sup> and in 1998, Brunet et al.<sup>[31]</sup> demonstrated that DOBE has an antioxidant effect by clearing hydroxyl radicals and superoxide anions *in vitro*.

In an *in vivo* microvascular permeabilization model developed in rats, DOBE was shown to decrease microvascular permeability produced in the peritoneal cavity by free oxygen radicals.<sup>[32]</sup> In another study performed by the same investigators, in endothelial cell lines of cattle, DOBE was found to decrease the build-up of cytosolic free calcium caused by hydrogen peroxide and decrease the level of cytosolic potassium due to phenazine methosulfate. In conclusion, DOBE was shown to decrease cellular injury produced by free oxygen radicals.<sup>[26]</sup>

In a study where the effects of DOBE on mortality and infarct area were investigated by producing acute myocardial infarction which induced coronary occlusion in rats, mortality at the first six hours in the DOBE group was significantly lower than that in the placebo group. In a similar study, the myocardial infarct area produced by coronary artery occlusion in dogs proved to be decreased by DOBE.<sup>[32]</sup>

These results showed that DOBE provides slow calcium passage through the cellular membrane by activating in the plateau phase and becoming effective on myocardial cells.<sup>[33]</sup>

Micronized purified flavonoid fractions is effective in the normal venous system and postphlebotic syndrome, venous insufficiency related with pregnancy, and functional venous insufficiency.<sup>[34,35]</sup> The presence of anti-inflammatory and gastro-protective effects of flavonoids at the same time may be explained by their interference with oxygenated free radical production. Vasodilatation begins with bradykinin and histamine while PGE<sub>2</sub>, histamine, and bradykinin are the main agents responsible for the increased permeability of microcapillaries.<sup>[11,36,37]</sup> Free oxygen radicals, particularly those formed by phagocytes

in the extracellular area, contribute to inflammatory lesions in different forms. They initiate cytolysis, increase blood vessel permeability, and maintain the inflammatory process. Flavonoids, which inhibit PGE<sub>2</sub> and TxA<sub>2</sub> synthesis, decrease histamine release from mast cells and also decrease edema by inhibiting the increase in vascular permeability triggered by bradykinin or free oxygen radicals.<sup>[38]</sup>

In an ischemia-reperfusion model study performed on rat cremaster and mesenterium by administering oral MPFF 500 mg before the procedure, Korhuis et al.<sup>[39]</sup> investigated leukocyte endothelial cell interaction and venular proteins. They concluded that MPFF decreased leukocyte adhesion and migration and venular protein leak as much as antiadhesive monoclonal antibodies.

In the present study, we aimed to test the roles of DOBE and MPFF, two agents with antioxidant properties, by demonstrating their effectiveness through oral consumption via *in vivo* and *in vitro* studies.

We can conclude that DOBE is helpful in improving the disrupted myocardial functions following ischemia and reperfusion. Peak systolic pressure, (+)dp/dt<sub>max</sub> and (-)dp/dt<sub>max</sub> measurements were significantly higher in the DOBE group in comparison to the main control group at certain periods of reperfusion.

#### Declaration of conflicting interests

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