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Effects of Oleuropein on Endothelial Functions in Aortas of Rats with Chronic Myocardial Infarction

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ABSTRACT

Background: Rat model of chronic myocardial infarction and heart failure is associated with endothelial dysfunction, which has partly been attributed to increased oxidative stress.

Objectives: This study aimed to examine the effects of oleuropein on vascular endothelial dysfunction in rats with chronic myocardial infarction.

Materials and Methods: The rats were subjected to coronary artery ligation or sham operation. On the next day, they were divided into a sham and a coronary-ligated group receiving distilled water (1 mL/day) and two coronary artery-ligated groups receiving 10 or 20 mg/kg/day oleuropein. Five weeks later, hemodynamic variables were measured, isolated aortic studies were performed, and serum concentrations of superoxide dismutase and malondialdehyde were determined. The data were entered into Sigmastat Statistical Software and were analyzed using one-way ANOVA followed by Duncan's Multiple Range. $P \leq 0.05$ was considered to be statistically significant.

Results: The rats with myocardial infarction receiving vehicle had a significantly lower left ventricular systolic pressure ($P = 0.04$), rate of rise ($P = 0.03$), decrease of left ventricular pressure ($P = 0.03$), relaxation response to acetylcholine ($P = 0.01$), and serum levels of superoxide dismutase ($P = 0.01$). Oleuropein treatment prevented the reduction of these variables. Moreover, the myocardial infarction group receiving vehicle had a significantly higher contraction response to phenylephrine ($P = 0.03$) and serum levels of malondialdehyde ($P = 0.02$) compared to the sham group. Treatment with oleuropein prevented the increase of these variables. There was no significant difference among the study groups regarding heart rate.

Conclusions: The findings indicated that chronic myocardial infarction resulted in heart failure and was associated with endothelial dysfunction. They also demonstrated that oleuropein attenuated endothelial dysfunctions, possibly, by antioxidative effects.

1. Background

Heart failure is associated with endothelial dysfunction in coronary and peripheral arteries (1). Endothelial dysfunction has been described as an imbalance between the release of endothelium-derived relaxing and endothelium-derived contracting factors (2). The dysfunction was associated with reduced endothelium-dependent vasodilation, which has been reported to occur in human (3) and animal models (4) of heart failure. In addition, endothelial dysfunction was associated with increased vasoconstrictor responses in rats (2).

The mechanisms underlying endothelial dysfunction are not completely understood. However, evidences suggested that activation of renin-angiotensin system (5) and increased release of endothelin (5), inflammatory cytokines (6), and markers of oxidative stress (7) might be involved. Considerable evidences have also shown that heart failure was associated with increased oxidative

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stress, indicated by decreased Serum Superoxide Dismutase (SOD) (8) and glutathione peroxidase (8) and increased serum Malondialdehyde (MDA) (8) and reactive oxygen species (9). By reducing Nitric Oxide (NO) bioavailability (10), reactive oxygen species play an important role in endothelial dysfunction (7).

Epidemiological evidence has shown that the incidence of coronary heart disease was lower in Mediterranean countries compared to Western and Northern European countries. In the Mediterranean area, olive products, which are thought to contribute to protection against coronary heart disease (11), are important constituents of the diet. The beneficial effects of Mediterranean diet have been attributed to polyphenol compounds (11), such as oleuropein. Oleuropein has been reported to have cardioprotective (12), antioxidant (13), antihypertensive (14), vasodilatory, and vasoprotective (15) effects.

2. Objectives

Given the beneficial effects of oleuropein, the present study aims to examine the vasoprotective effects of oleuropein in a rat model of heart failure induced by ligation of coronary artery.

3. Materials and Methods

3.1. Materials

Oleuropein was purchased from Serva (Feinbiochemica, Heidelberg, Germany). Ketamine was obtained from Rotexmedica (Trittau, Germany) and xylazine from Alfasan (Woerden, Holland). Thiobutabarbital (Inactin) was bought from ByK Gulden (Konstanz, Germany). Finally, phenylephrine (Phe), acetylcholine (Ach), sodium nitroprusside (SNP), and triphenyltetrazolium chloride (TTC) were obtained from Sigma-Aldrich (Steinheim, Germany).

3.2. Animals

Male Spargue-Dawley rats weighing 200 - 250 g were obtained from Laboratory Animal Breeding Center, Shiraz University of Medical Sciences, Shiraz, Iran. They were maintained under standard conditions (12 h light/dark cycle at 20 - 24 ºC and 25 - 35% humidity) with standard rat chow and water ad libitum. The animals were cared for in accordance with the National Guideline for Care and Use of Laboratory Animals published by National Ethics Committee for Biomedical Research, Ministry of Health, Treatment, and Medical Education, Iran. The study protocol was also approved by the Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran (EC-91-6357).

3.3. Surgical Procedures

In this study, 29 animals were subjected to permanent ligation of coronary artery as described previously (16). Briefly, the animals were anesthetized with intraperitoneal injections of ketamine (60 mg/kg) and xylazine (8 mg/kg). They were then tracheally intubated and connected to rodent respirator (UgoBasile, Comerio, Italy). They were ventilated with room air at a frequency of 70 strokes/min and tidal volume of 1 mL/100 g body weight. The animals' body temperature was maintained at 37 ± 1 °C using a temperature controller (Physitemp Instruments, Clifton, USA) by means of a rectal probe. Afterwards, their chests cavities were opened at the level of the left 4th intercostal space and their hearts were exposed. The pericardial sacs were then opened and the left main coronary arteries were ligated at 2 - 4 mm from their origins using 5 - 0 prolene. In sham-operated rats, the sutures were passed around the coronary arteries, but were not tightened. The chest walls and skin incisions were then closed using absorbable and non-absorbable suture materials, respectively (16). The animals were then recovered from anesthesia and kept in single cages under standard conditions (light cycle 12 hours, temperature 22 - 24 °C) for five weeks during which, they were given vehicle (1 mL distilled water) or oleuropein in the same volume as the vehicle by oral gavages.

3.4. Experimental Design

Starting from the next day after the operation, the animals were divided into 4 groups, including a sham-operated group assigned to receive the vehicle (Sham-V) (n = 8) and 3 coronary artery-ligated groups assigned to receive the vehicle (CHF-V) (n = 6) or oleuropein at 10 mg/kg/day (CHF-Ole10) (n = 7) or 20 mg/kg/day (CHF-Ole20) (n = 8).

3.5. Hemodynamic Measurements

By the 5th week, the animals were anesthetized with single intraperitoneal injections of thiobutabarbital (100 mg/kg). Heparinized saline-filled catheters were inserted into the left carotid arteries for measurement of Systolic Blood Pressure (SBP) and Heart Rate (HR) and were connected to a PowerLab data acquisition system (ML750, AD Instruments PowerLab System, Castle Hill, Australia). The catheters were also placed in the left ventricles through the right carotid arteries for measurement of the Left Ventricular Systolic Pressure (LVSP) and rate of rise of the left ventricular pressure (+dp/dt) using the same PowerLab data acquisition system. The animals were allowed to recover from surgical stress for 30 minutes and then, SBP, HR, LVSP, and + dp/dt were measured.

3.6. Measurements of Biomarkers

After the measurement of hemodynamic variables, blood samples (2 mL) were obtained from the left carotid artery catheters, allowed to clot for 30 min, and centrifuged at 1000 g for 20 min. The samples' sera were then separated and stored at -80 ºC until analysis. Serum levels of MDA and SOD were measured using chemical kits (MDA; Bioassay Technology Laboratory, Shanghai, China, SOD; Biorexfars, Shiraz, Iran).

3.7. Isolated Aortic Ring Studies

After the measurement of hemodynamic parameters and collection of blood samples, the animals' chest cavities were opened. Thoracic aortas were removed, cleaned of surrounding connective tissues, and cut into 4 - 5 mm rings. The rings were mounted on hooks connected to force transducers in isolated tissue organ baths (K30, Hugo Sachs Electronik, Germany) filled with 20 ml physiological solution containing the following composition (mmol/L): NaCl 118, KCl 4.7, KH2PO4 1.2, CaCl2 2.5, MgSO4 1.2, NaHCO3 25,

and D-glucose 11.1, bubbled constantly with 95% O2 and 5% CO2 at a pH of 7.4 and a temperature of 37 ºC. Tension was recorded by a four-channel polygraph (model 705/L, Hugo Sachs Electronik, Germany). The tissues were allowed to stabilize for 60 minutes. Then, a full concentration-response to Phe was performed. After two washes and 30 minutes equilibration, the rings were contracted with Phe concentrations that made similar contraction responses (50% of the maximal response in the Sham-V group) in all groups. Concentration-response curves to Ach or SNP were performed at the plateau of contractile response to Phe. Concentration-responses to Phe were compared using effective concentration 50 (EC50) and maximal response (Emax). Concentration-responses to Ach or SNP were also compared using inhibitory concentration 50 (IC50) and Emax.

3.8. Assessment of the Left Ventricular Infarct Size

Cardiac infarct size was assessed using TTC staining. The hearts were washed in ice-cold saline and were embedded in parafilm. The hearts were kept at 4 ˚C for 1 hour. After that, they were cut into 2-mm-thick slices and incubated in TTC solution (1%) at 37 ˚C for 25 min. The slices were then incubated with 10% formaldehyde for 24 h. Afterwards, the infarct size of each slice was determined as a percentage of the area of that slice. After all, the total left ventricular infarct size was calculated as the sum of percentage of total left ventricular areas using the NIH image software.

3.9. Statistical Analysis

Data, presented as mean ± SEM, were analyzed using One-Way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range test for pairwise comparisons. $P \leq 0.05$ was considered to be statistically significant. Data analysis was performed using Sigmastat statistical software, version 3.0 (San Jose, CA, USA). The illustrations were prepared using SigmaPlot software, version 8.0 (San Jose, CA, USA).

4. Results

4.1. Hemodynamic Variables

The hemodynamic variables have been presented in Table 1. Accordingly, SBP, LVSP, and + dp/dt of the CHF-V group were significantly lower than those of the Sham-V group. In addition, SBP, LVSP, and + dp/dt of CHF-Ole10 and CHF-Ole20 groups were significantly higher compared to the CHF-V group. However, no significant difference was found among Sham-V, CHF-V, CHF-Ole10, and CHF-Ole20 groups with respect to heart rate (Table 1).

4.2. Isolated Aortic Study

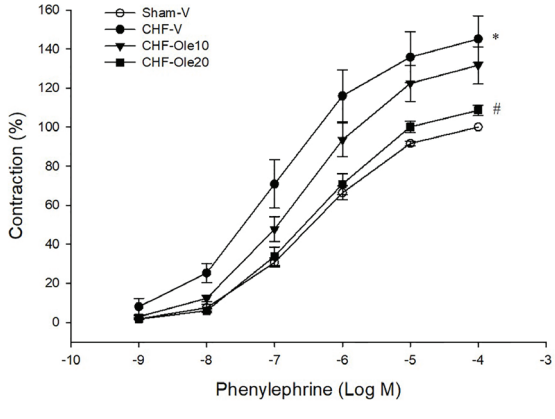
The Emax of contraction response to Phe was significantly higher in the CHF-V group compared to the Sham-V group. There was no significant difference between the CHF-Ole10 group and the CHF-V group regarding the Emax of contraction responses to Phe. However, the Emax of Phe contraction response was significantly lower in the CHF-

Table 1. The Values of Hemodynamic Parameters in All Groups

	Sham-V	CHF-V	CHF-Ole10	CHF-Ole20
SBP (mmHg)	125 ± 2	110 ± 2 *	121 ± 2 #	124 ± 4 #
HR (beats/min)	420 ± 8	387 ± 9	412 ± 15	388 ± 14
LVSP (mmHg)	134 ± 2	113 ±2 *	126 ± 2 #	128 ± 1 #
+dp/dt (mmHg/sec)	5249 ± 167	3795 ± 95 *	4426 ± 116 #	5180 ± 125 #

Abbreviations: SBP, systolic blood pressure; HR, heart rate; LVSP, left ventricular systolic pressure; +dp/dt, rate of rise of left ventricular pressure; Sham-V, sham group receiving vehicle (1 mL distilled water/day); CHF-V, coronary artery-ligated group receiving vehicle; CHF-Ole10, coronary artery-ligated receiving oleuropein at 10 mg/kg/day; CHF-Ole20, coronary artery-ligated group receiving oleuropein at 20 mg/kg/day. Data are shown as mean ± SEM, n = 6 - 8 each group. * indicates significant ($P \leq 0.05$) difference from the Sham-V group. # indicates significant ($P \leq 0.05$) difference from the CHF-V group.

Figure 1. Phenylephrine Concentration-Response Curves in Isolated Aortic Rings of All Groups after Five Weeks of Treatment with Vehicle or Oleuropein



The response (contraction %) was calculated as the percentage of phenylephrine maximal response in the control group.
Abbreviations: Sham-V, sham group receiving vehicle (distilled water, 1 mL/day); CHF-V, coronary artery-ligated group treated with vehicle (distilled water, 1 mL/day); CHF-Ole10, coronary artery-ligated group treated with oleuropein at 10 mg/kg/day; CHF-Ole20, coronary artery-ligated group treated with oleuropein at 20 mg/kg/day. Data are shown as mean ± SEM, n = 6 - 8 each group. *indicates significant difference from the Sham-V group. #indicates significant difference from the CHF-V group.

Ole20 group in comparison to the CHF-V group (Figure 1).

The EC50 of contraction response to Phe was significantly lower in the CHF-V group (-7.01 ± 0.094 Log M) compared to the Sham-V group (-6.44 ± 0.084 Log M). In addition, the EC50 of contraction response to Phe was significantly higher in CHF-Ole10 (-6.61 ± 0.0879 Log M) and CHF-Ole20 (-6.46 ± 0.110 Log M) groups in comparison to the CHF-V group (-7.01 ± 0.094 Log M).

The Emax of Ach relaxation response of the CHF-V group was significantly less than that of the Sham-V group (Figure 2A). Additionally, the Emax of Ach concentration-response was significantly higher in CHF-Ole10 and CHF-Ole20 groups than in the CHF-V group (Figure 2A).

The IC50 of relaxation response to Ach was significantly higher in the CHF-V group (-5.96 ± 0.048 Log M) compared to the Sham-V group (-6.35 ± 0.045 Log M). Indeed, the IC50 of relaxation response to Ach was significantly lower in CHF-Ole10 (-6.39 ± 0.148 Log M) and CHF-Ole20 (-6.307 ± 0.055 Log M) groups in comparison to the CHF-V group.

The results showed no significant differences among Sham-V, CHF-V, CHF-Ole10, and CHF-Ole20 groups concerning IC50 or Emax of SNP relaxation responses (Figure 2B).

4.3. Serum Biomarkers

Serum level of SOD was significantly lower in the CHF-V group compared to the Sham-V group. On the other hand, serum SOD concentrations of CHF-Ole10 and CHF-Ole20 groups were significantly higher than that of the CHF-V group (Table 2).

Serum MDA concentration of the CHF-V group was significantly higher than that of the Sham-V group. In addition, serum MDA concentration was significantly lower in CHF-Ole10 and CHF-Ole20 groups in comparison to the CHF-V group (Table 2).

4.4. Body and Organ Weights

There was no significant difference among the study groups regarding body weight on the first and last days of the study (Table 3). Accordingly, the CHF-V group showed significantly higher weight gain compared to the Sham-V group. However, CHF-Ole10 and CHF-Ole20 groups showed significantly lower weight gain in comparison to the CHF-V group (Table 3).

The results revealed no significant difference among the study groups regarding heart weight (calculated as the percentage of body weight) (Table 3). The results also indicated that lung weight was significantly higher in the CHF-V group (calculated as the percentage of body weight) compared to the Sham-V group. On the other hand, CHF-Ole10 and CHF-Ole20 groups showed significantly lower lung weights in comparison to the CHF-V group (Table 3).

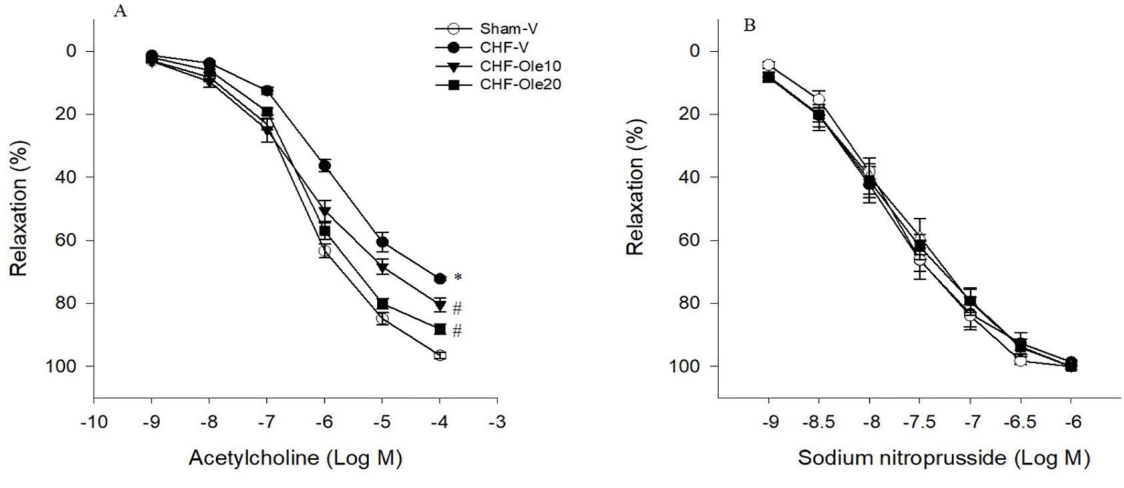
4.5. Left Ventricular Infarct Size

The Sham-V group had no left ventricular infarct. The infarct size (calculated as the percentage of the left ventricular volume) was significantly lower in CHF-Ole10 (30.9 ± 1.9%) and CHF-Ole20 (27.4 ± 1.6%) groups compared to the CHF-V group (44.5 ± 2.8%).

5. Discussion

The main objective of the present study was to examine the protective effects of oleuropein on endothelial dysfunction in rats with chronic myocardial infarction induced by permanent ligation of the coronary artery. The study findings demonstrated that chronic myocardial infarction resulted in heart failure associated with impaired cardiac systolic and diastolic functions and endothelial dysfunction,

Figure 2. Acetylcholine and Sodium Nitroprusside Concentration-Response Curves in Isolated Aortic Rings of All Groups after Five Weeks of Treatment with Vehicle or Oleuropein.



The response (relaxation %) was calculated as the percentage of acetylcholine or sodium nitroprusside maximal response in the control group.

Abbreviations: Sham-V, sham group receiving vehicle (distilled water, 1mL/day); CHF-V, coronary artery-ligated group treated with vehicle (distilled water, 1 mL/day); CHF-Ole10, coronary artery-ligated group treated with oleuropein at 10 mg/kg/day; CHF-Ole20, coronary artery-ligated group treated with oleuropein at 20 mg/kg/day. Data are shown as mean ± SEM, n = 6 - 8 each group. * indicates significant difference from the Sham-V group. # indicates significant difference from the CHF-V group.

Table 2. The Values of Serum Markers of Oxidative Stress

	Sham-V	CHF-V	CHF-Ole10	CHF-Ole20
Superoxide dismutase (U/mL)	241.6 ± 43.0	113.5 ± 25.8 *	210.2 ± 22.1 #	223.1 ± 40.5 #
Malondialdehyde (nmol/mL)	4.55 ± 0.32	12.48 ± 0.85 *	9.17 ± 0.89 #	8.4 ± 0.70 #

Abbreviations: Sham-V, sham group receiving vehicle (1 mL distilled water/day); CHF-V, coronary artery-ligated group receiving vehicle; CHF-Ole10, coronary artery-ligated group receiving oleuropein at 10 mg/kg/day; CHF-Ole20, coronary artery-ligated group receiving oleuropein at 20 mg/kg/day. Data are shown as mean ± SEM, n = 6 - 8 each group. * indicates significant ($P \leq 0.05$) difference from the Sham-V group. # indicates significant ($P \leq 0.05$) difference from the CHF-V group.

Table 3. The Values of Body and Organ Weights in All Groups

	Sham-V	CHF-V	CHF-Ole10	CHF-Ole20
Weight 1 (g)	231 ± 11	236 ± 3	227 ± 10	241 ± 7
Weight 2 (g)	267 ± 13	302 ± 12	280 ± 7	275 ± 11
Weight gain (g)	36.5 ± 3.4	72.1 ± 9.8 *	44.1 ± 5.8 #	38.8 ± 10.5 #
HW(% BW)	0.30 ± 0.01	0.33 ± 0.02	0.31 ± 0.01	0.34 ± 0.02
LW (% BW)	0.53 ± 0.03	0.73 ± 0.09 *	0.51 ± 0.03 #	0.56 ± 0.02 #

Abbreviations: Sham-V, sham group receiving vehicle (1 mL distilled water/day); CHF-V, coronary artery-ligated group receiving vehicle; CHF-Ole10, coronary artery-ligated group receiving oleuropein at 10 mg/kg/day; CHF-Ole20, coronary artery-ligated group receiving oleuropein at 20 mg/kg/day; weight 1, weights of the animals at the beginning of the study; weight 2, weights of the animals at the end of the study; HW, heart weights of the animals as a percent of body weights at the end of the study; LW, lung weights of the animals as a percent of body weights at the end of the study. Data are shown as mean ± SEM, n = 6 - 8 each group. * indicates significant ($P \leq 0.05$) difference from the Sham-V group. # indicates significant ($P \leq 0.05$) difference from the CHF-V group.

which were attenuated by oleuropein treatment. The cardiovascular protection offered by oleuropein might be related to its ability to ameliorate oxidative stress.

The present study results showed that SBP, LVSP, and + dp/dt were significantly lower in the vehicle-treated coronary artery-ligated group in comparison to the sham-operated group. Generally, heart failure is induced in animals by coronary artery ligation (17) or administration of doxorubicin (18) or isoproternol (19). Such models were associated with decreased SBP, LVSP, and + dp/dt (17, 19). Moreover, human heart failure was associated with decreased Ejection Fraction (EF) and increased left ventricular end systolic and diastolic dimensions and volumes (20). Cardiac Output (CO) was not measured in the present study. However, we showed in an earlier study (16) that this model was associated with decreased CO, EF, and other cardiac echocardiographic characteristics of heart failure. Therefore, it might be concluded that ligation of the coronary artery resulted in heart failure.

The study findings showed that weight gain and lung weight, but not heart weight, were significantly higher in the vehicle-treated coronary artery-ligated group compared to the sham-operated group. The lack of a significant difference between heart weights might result from the fact that decreased cardiac weight due to necrosis and thinning of the infarct area could counter the hypertrophy-induced increase in heart weight (21). On the other hand, increased lung weight in the CHF-V group was indicative of fluid accumulation and congestion in the lungs. When left ventricular dysfunction develops, lung circulation and distal airway spaces become susceptible to hemodynamic backward effects caused by elevated Left Ventricular End-Diastolic Pressure (LVEDP) and pulmonary capillary stasis (22).

In the current study, the model of heart failure was associated with increased contraction response to Phe. This finding is similar to those of the previous studies (23, 24). The increased responsiveness to Phe might be related to increased calcium release and sensitivity (23), decreased basal release of NO (24), overabundance of oxygen free radicals resulting in increased NO degradation, increased vasoconstrictors release from endothelium, or oversensitivity to vasoconstrictors (25).

The present study findings revealed that heart failure was associated with decreased relaxation response to Ach, but with no change in the relaxation response to SNP. Such changes are similar to those observed in earlier studies using experimental models of coronary ligation-induced (10) and doxorubicin-induced (18) heart failure. The findings also indicated that the model was associated with decreased endothelium-dependent relaxation, but with no change in the endothelium-independent one. Decreased endothelium-dependent relaxation response to Ach, but not to endothelium-independent vasodilator SNP, has been taken as an indication of endothelial dysfunction (26).

Earlier studies showed that chronic heart failure was associated with increased oxidative stress (27) and that increased oxidative stress played an important role in endothelial dysfunction by reduction of NO bioavailability (28). In agreement with the previous reports, our findings showed that the present model of heart failure was associated with increased MDA serum levels (29) and decreased SOD serum levels (8). Therefore, it could be concluded that, in line with previous suggestions, increased oxidative stress (30) contributed to endothelial dysfunction in the present model of chronic heart failure.

The findings of the current study indicated that oleuropein had cardioprotective effects characterized by preventing decrease in SBP, LVSP, and + dp/dt. Oleuropein was also shown to have cardioprotective effects in acute and chronic doxorubicin-induced cardiotoxicities (31, 32) and ischemia-reperfusion injuries (13) by reducing infarct size (13), creatine kinase-MB (CK-MB), and lactate dehydrogenase

(32) and preserving left ventricular contractility (31).

The study findings showed that oleuropein attenuated the increase in lung weight and body weight. The decrease in lung weight might be due to increase in cardiac output (16), which increased unloading of pulmonary interstitial fluid and edema. The decrease in body weight gain might also be related to a similar mechanism although other mechanisms might be involved, as well.

The current study findings showed that oleuropein attenuated the contraction response to Phe, which is similar to those reported in earlier studies (33). This finding might be attributed to the reduction of sympathetic nervous system activity, enhancement of basal release of NO, and antioxidant mechanisms (33).

The present study results showed that oleuropein prevented the impairment of endothelial function characterized by preventing the impairment of endothelial-dependent relaxation to Ach. This is consistent with the results of previous studies showing that oleuropein preserved endothelial-dependent relaxation to Ach in diabetic/hypertensive (33), hypertensive (34), and high carbohydrate-, high fat-fed rats (15). The previous studies attributed most of cardiac and vascular protective effects of oleuropein to attenuation of oxidative stress (12, 13, 31, 32, 34). Therefore, serum levels of MDA and SOD were measured in the current study. The findings showed that administration of oleuropein was associated with decreased MDA serum levels and increased SOD serum levels, indicating an antioxidant effect. Oleuropein was shown to decrease oxidative stress by decreasing MDA (13) and increasing SOD (13), glutathione peroxidase, and glutathione reductase and catalase (35) in cardiac ischemia and reperfusion (12, 13) and diabetes (35). Overall, the previous studies suggested that, by virtue of antioxidative effect, oleuropein did have beneficial vascular effects, such as improvement of vascular function (33, 34). Therefore, it may not be illegitimate to suggest that the beneficial effect of oleuropein in attenuating the impairment of endothelial function is partly attributed to its antioxidant effects.

5.1. Conclusion

In conclusion, the findings of the present study showed that ligation of the coronary artery in rats resulted in chronic heart failure characterized by cardiac and endothelial dysfunctions as well as increased oxidative stress. They also indicated that oleuropein decreased cardiac and endothelial dysfunctions possibly by decreasing oxidative stress.

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Authors' Contribution

Study concept and design: Ali Akbar Nekooeian, Zeinab Janahmadi, Ali Reza Moaref, Masoumeh Emamghorieshi; Data acquisition: Zeinab Janahmadi; Data analysis and interpretation: Ali Akbar Nekooeian, Zeinab Janahmadi, Ali Reza Moaref; Manuscript drafting: Ali Akbar Nekooeian, Zeinab Janahmadi; Study supervision: Ali Akbar Nekooeian; Critical revision of article: Ali Akbar Nekooeian, Zeinab Janahmadi, Ali Reza Moaref, Masoumeh Emamghorieshi

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