Hindawi Publishing Corporation
Journal of Chemistry
Volume 2014, Article ID 368376, 6 pages
http://dx.doi.org/10.1155/2014/368376

Research Article

Cardioprotective Effect of the Aqueous Extract of Lavender Flower against Myocardial Ischemia/Reperfusion Injury

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Received 6 April 2014; Revised 31 May 2014; Accepted 1 June 2014; Published 29 June 2014

Academic Editor: Wang Chunming

This study was conducted to evaluate the cardioprotective property of the aqueous extract of lavender flower (LFAE). The myocardial ischemia/reperfusion (I/R) injury of rat was prepared by Langendorff retrograde perfusion technology. The heart was preperfused with K-H solution containing LFAE for 10 min before 20 minutes global ischemia, and then the reperfusion with K-H solution was conducted for 45 min. The left ventricular developed pressure (LVDP) and the maximum up/downrate of left ventricular pressure ($\pm dp/dt_{max}$) were recorded by physiological recorder as the myocardial function and the myocardial infarct size was detected by TTC staining. Lactate dehydrogenase (LDH) and creatine kinase (CK) activities in the effluent were measured to determine the myocardial injury degree. The superoxide anion dismutase (SOD) and malondialdehyde (MDA) in myocardial tissue were detected to determine the oxidative stress degree. The results showed that the pretreatment with LFAE significantly decreased the myocardial infarct size and also decreased the LDH, CK activities, and MDA level, while it increased the LVDP, $\pm dp/dt_{max}$, SOD activities, and the coronary artery flow. Our findings indicated that LFAE could provide protection for heart against the I/R injury which may be related to the improvement of myocardial oxidative stress states.

1. Introduction

Cardiovascular disease is common and results in much of mortality of people throughout the world. Acute myocardial infarction is particularly formidable in cardiovascular disease cases, coronary artery stenosis is the initial factor that sets off a chain reaction, and the consequence is myocardial necrosis caused by conditions of acute continuous ischemia and hypoxia. Timely and effective recovery of demand of blood supply is an effective method that is able to minimize the heart injuries. Paradoxically, reperfusion itself can cause myocardial injury and cardiac dysfunction, referred to as "reperfusion injury" [1–3]. As a consequence, relieving the ischemic reperfusion injury can be seen as an additional method to secure the heart against cardiovascular disease [4].

Ischemia-reperfusion injury refers to reducing organ of blood donation for a period of time, there will be damage during reperfusion. Doctors gradually found that the main factors that cause damage to an organ, not ischemia itself, but reperfusion. In the process of reperfusion, tissue cells produce oxygen free radicals in excess. Oxidative stress is an important way involved in I/R injury [5, 6]. Several studies have shown that increased expression of antioxidant enzymes will protect against postischemic injury. It has been reported that antioxidants vitamin E, catalase (CAT), melatonin, and superoxide dismutase (SOD) defend the heart against I/R injury. From another aspect, it confirms that the oxidative stress has been involved in ischemia/reperfusion process [7, 8].

Lavender as a traditional botanical medicine has been applied for many years [9]; people in many countries and regions from ancient times employed lavender as an effective medicine to treat diseases; it has many beneficial effects consisting of anti-inflammatory [10], antioxidant [11],

antibacterial [12], antisenile dementia [13], and anxiolytic, which is particularly evident [14]. The purpose of our study was to detect the protective effect of aqueous extract of lavender flowers on the heart.

2. Material and Methods

2.1. Plant Material. The flowers of *Lavandula angustifolia* Mill were collected in Yili, Xinjiang, China, and the material authenticity was established by one of the authors and later confirmed by a botanist.

2.2. Preparation of Extract. Dried flowers of *Lavandula angustifolia* Mill were washed in distilled water and air-dried in the shade; flowers were extracted with warm distilled water (DW) (DW: material, 10 : 1, v/w) twice in an incubator at 80°C for 1.5 h. The hot-water extract was filtered through a 2 μm pore sterile filter paper. The combined filtrates were concentrated in a vacuum at 60°C, and the resulting filtrates were amounted to 5×10^2 mg/mL crude drug.

2.3. Animals and Experimental Groups. Male Wistar rats (250–280 g) were obtained from Xinjiang Medicine University Medical Laboratory Animal Center (SDXK (new) 2011-004). All experimental procedures were approved by the Institutional Animal Care and Use Committee of National Institute Pharmaceutical Education and Research.

The rats were randomly divided into three groups: control group (Sham), I/R group, and aqueous extract of lavender flower (LFAE) treatment group. Hearts in control group were uninterruptedly perfused with K-H buffer purely for the 95 min. I/R group hearts were perfused firstly for 30 min and then we Suspended the infusion for 20 min and reperfusion for 45 min. Hearts in treatment groups were perfused firstly for 20 min instead of K-H buffer with LFEA (1 mg/mL) for 10 min and then we Suspended the infusion for 20 min and reperfusion for 45 min.

2.4. Isolated Rat Heart Preparation. The male Wistar rats (250–280 g) were anesthetized by an intraperitoneal injection of 60 mmol/L chloral hydrate (0.35 g/kg). To anticoagulate, 250 U/kg of heparin was given as a sublingual venous injection to the rats. After a few minutes, we performed the thoracic surgery on rat to remove heart. we cut the ribs and opened the chest, Then cut off the blood vessels and obtained the heart and then put the heart into pre-cooling K-H solution, squeezed out the blood gently. After that, the heart was excised quickly and immediately mounted on Langendorff's apparatus. The hearts were immersed in ice-cold K-H buffer (120 mM NaCl, 1.2 mM KH_2PO_4, 1.2 mM $CaCl_2$, 1.2 mM $MgSO_4$, 25 mM sodium acetate, and 11 mM glucose, pH7.4) equilibrated with a gas mixture comprised of 95% O_2/5% CO_2. Immediately, the heart was connected to the Langendorff apparatus which was maintained at 37°C [15], and then we cut the left atrial appendage and inserted the latex balloon filled with water into the left ventricle through the left atrial appendage. Hemodynamic parameters will be displayed on the recorder screen, finally.

Table 1: Effect of the aqueous extract of lavender flower (LFAE) on levels of CK and LDH in coronary flow of ischemia/reperfusion injury ($\overline{x} \pm s$, $n = 8$).

Groups	Before ischemia	Reperfusion	
		20 min	45 min
CK (U/L)			
Control	15.59 ± 1.35	17.01 ± 1.37	18.57 ± 1.31
I/R	14.98 ± 1.09	45.25 ± 3.59**	50.36 ± 2.63**
LFAE	15.67 ± 1.05	21.26 ± 2.00##	30.63 ± 2.28##
LDH (U/L)			
Control	12.34 ± 1.13	13.23 ± 1.80	14.48 ± 1.07
I/R	12.63 ± 1.17	30.91 ± 2.62**	37.68 ± 2.01**
LFAE	13.06 ± 0.83	21.39 ± 1.33##	27.60 ± 1.55##

** $P < 0.01$, compared with control group; ## $P < 0.01$, compared with I/R group.

Table 2: Effects of LFAE on SOD activity and MDA level. Values are means with their standard deviation ($\overline{x} \pm s$, $n = 8$).

Group	Dosage (mg·mL^{-1})	After reperfusion	
		SOD (U/mgPr)	MDA (μmol/mgPr)
Control	—	9.29 ± 0.66	190.78 ± 11.93
I/R	—	3.60 ± 0.28**	433.66 ± 29.16**
LFAE	1	7.46 ± 0.51##	231.41 ± 18.93##

** $P < 0.01$, compared with control group; ## $P < 0.01$, compared with I/R group.

2.5. Measurement of Heart Hemodynamic Parameters. The hemodynamic parameters were accurately detected by a signal collecting system (PC Power lab with Chart 5 software, 4S AD instruments). The following functional parameters were measured: left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP, LVDP = LVSP − LVEDP), $\pm dp/dt_{\max}$ (reflecting the important indicators of left ventricular systolic function and diastolic function), and heart rate (HR) were detected uninterruptedly using 4S AD instruments biology polygraph (Power lab, Australia). The coronary flow (CF) was detected using a flow meter with an in-line probe (model T106, Transonic).

2.6. Enzymes Activities Assays. To determine creatine kinase (CK) and lactate dehydrogenase (LDH) activity in the perfusate, samples were collected from the coronary effluent before 20 min ischemia and after 20 min and 45 min of reperfusion. LDH and CK were assayed spectrophotometrically using LDH and CK kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.7. Evaluation of Myocardial Infarct Size. In the wake of frozen, the hearts were cut into five slices along the transverse direction, and every piece was 2 mm thick. We put the sliced hearts in TTC with the concentration of 1% to be incubated for 15 minutes and we turned over the hearts once in this process. When the TTC staining was over, the heart slices were dried with filter paper, and then these slices were covered in formalin solution for 15 minutes, Finally, these

Table 3: Supplemental data for Figure 1.

Group	Reperfusion		
	15 min	30 min	45 min
LVDP			
Control	92.88 ± 4.76	88.13 ± 4.01	85.63 ± 4.81
I/R	46.38 ± 3.20**	52.67 ± 2.83**	44.22 ± 3.60**
LFAE	72.37 ± 3.16##	69.38 ± 2.83##	58.88 ± 3.06##
$+dp/dt_{max}$			
Control	94.63 ± 4.69	89.63 ± 3.96	83.62 ± 3.93
I/R	48.25 ± 2.71**	46.13 ± 2.70**	43.50 ± 2.62**
LFAE	66.75 ± 3.54##	63.38 ± 2.87##	60.63 ± 3.02##
$-dp/dt_{min}$			
Control	91.25 ± 4.10	89.50 ± 3.77	86.13 ± 4.40
I/R	45.75 ± 3.03**	42.38 ± 2.50**	37.38 ± 3.12**
LFAE	64.63 ± 3.29##	61.88 ± 3.27##	58.75 ± 2.66##
CF			
Control	85.75 ± 3.83	83.13 ± 3.87	76.50 ± 2.78
I/R	58.88 ± 3.56**	52.50 ± 2.45**	51.63 ± 3.66**
LFAE	73.75 ± 3.80##	68.38 ± 4.15##	64.63 ± 3.28##
HR			
Control	97.75 ± 4.06	92.63 ± 4.69	90.13 ± 4.64
I/R	81.75 ± 3.37**	74.75 ± 4.71**	73.50 ± 3.25**
LFAE	88.38 ± 4.44#	84.88 ± 4.67#	80.88 ± 5.00#

** $P < 0.01$, compared with control group; ## $P < 0.01$, # $P < 0.05$, compared with I/R group.

slices were immersed in phosphate buffer at 4°C (pH 7.4) [16]. Heart slices were digitally imaged using a Canon camera. The area of infarcted part (pale) and viable part (red) was measured digitally using Image Pro Plus software. Infarct size was represented as percentage of the area of ischemia [17].

2.8. Assay of Oxidative Stress. When the perfusions finished, we froze the hearts under the condition of −70°C to prepare for further testing. The frozen ventricles were crushed to powder by liquid nitrogen-chilled tissue pulverizer. For tissue analyses, weighed amounts of the frozen tissues were homogenized in appropriate buffer using microcentrifuge tube homogenizer. Then the SOD and malondialdehyde (MDA) were analyzed spectrophotometrically according to the instruction of the assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.9. Statistical Analysis. The results were expressed as mean ± S.D. and analyzed by one-way analysis of variance (ANOVA). The values with $P < 0.05$ were considered statistically significant. $P < 0.01$ was considered very statistically significant. The analyses were carried out using the Origin 8.0 software (Origin Lab Corporation, Northampton, MA, USA).

3. Result and Discussion

3.1. The Aqueous Extract of Lavender Flower Improves Resumer of I/R-Induced Cardiac Function. The effects of treatment on LVDP, $\pm dp/dt_{max}$, CF, and HR during reperfusion in control group, I/R group, and treated hearts were shown in Figure 1. The datum was recovery ratio between the value after 15, 30, and 45 min of reperfusion and one minute before stopping irrigation in Table 3. When compared with the unprotected I/R hearts, exposure of 1 mg/mL extract during early reperfusion significantly improved functional recovery. The hearts underwent 20 minutes of ischemia time followed by 45 min of reperfusion and showed a remarkable reduction in the resumer of LVDP, $+dp/dt_{max}$, $-dp/dt_{max}$, CF, and HR. The resumer of LVDP, $\pm dp/dt_{max}$, CF, and HR after 45 min of reperfusion was higher ($^{*}P < 0.05$) in hearts perfused with aqueous extract of lavender flower 10 minutes before ischemia.

3.2. The Aqueous Extract of Lavender Flower (LFAE) Attenuates I/R-Induced Enzyme Release in Rat Heart. Before ischemia, CK and LDH levels in the effluent from control group, I/R group, and LFAE (1 mg/mL) group are fundamentally the same. As shown in Table 1, after 20 min of ischemia followed by 20 min and 45 min of reperfusion, the leakage of CK and LDH markedly increased compared to that of control. The LFAE pretreatment significantly reduced the I/R-induced increase in LDH and CK release in rat heart.

3.3. The Aqueous Extract of Lavender Flower Reduced Myocardial Infarct Size following I/R Injure. At the end of reperfusion,myocardial infarct size was assessed using the TTC staining method. As illustrated in Figure 2(a), there was only a small piece of infarction in control group (Sham) and ischemia for 20 min followed by 45 min of reperfusion resulted in development of substantial myocardial infarcts,

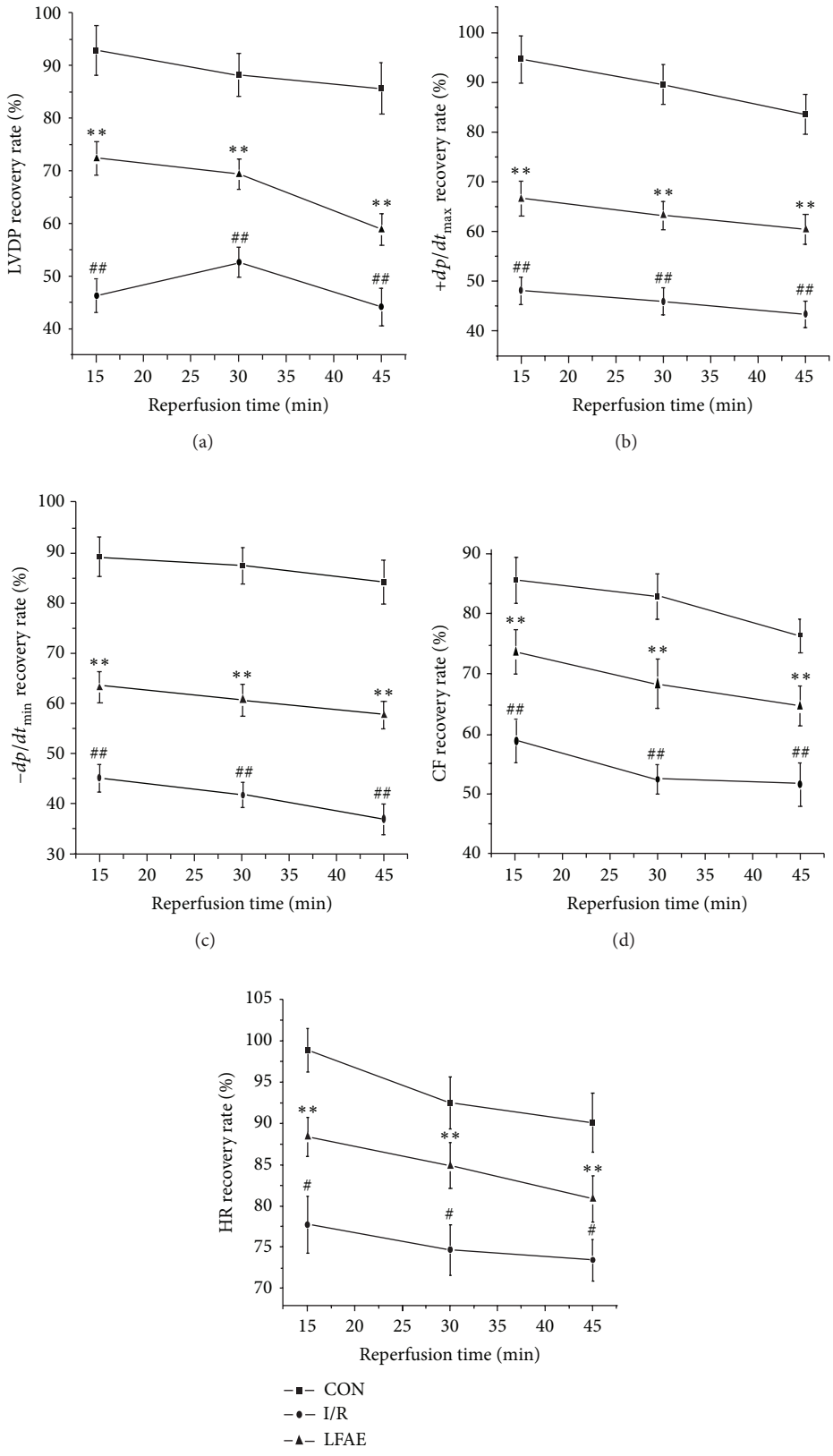

Figure 1: Effect of LFAE on cardiac function (LVDP, $+dp/dt_{max}$, $-dp/dt_{min}$, CF, HR) in rats subjected to I/R ($\overline{x} \pm s$, %, $n = 8$). $^{**}P < 0.01$, compared with control group; $^{\#\#}P < 0.01$, compared with I/R group. CON: control group (Sham), I/R: I/R group, and LFAE: aqueous extract of lavender flower treatment group.

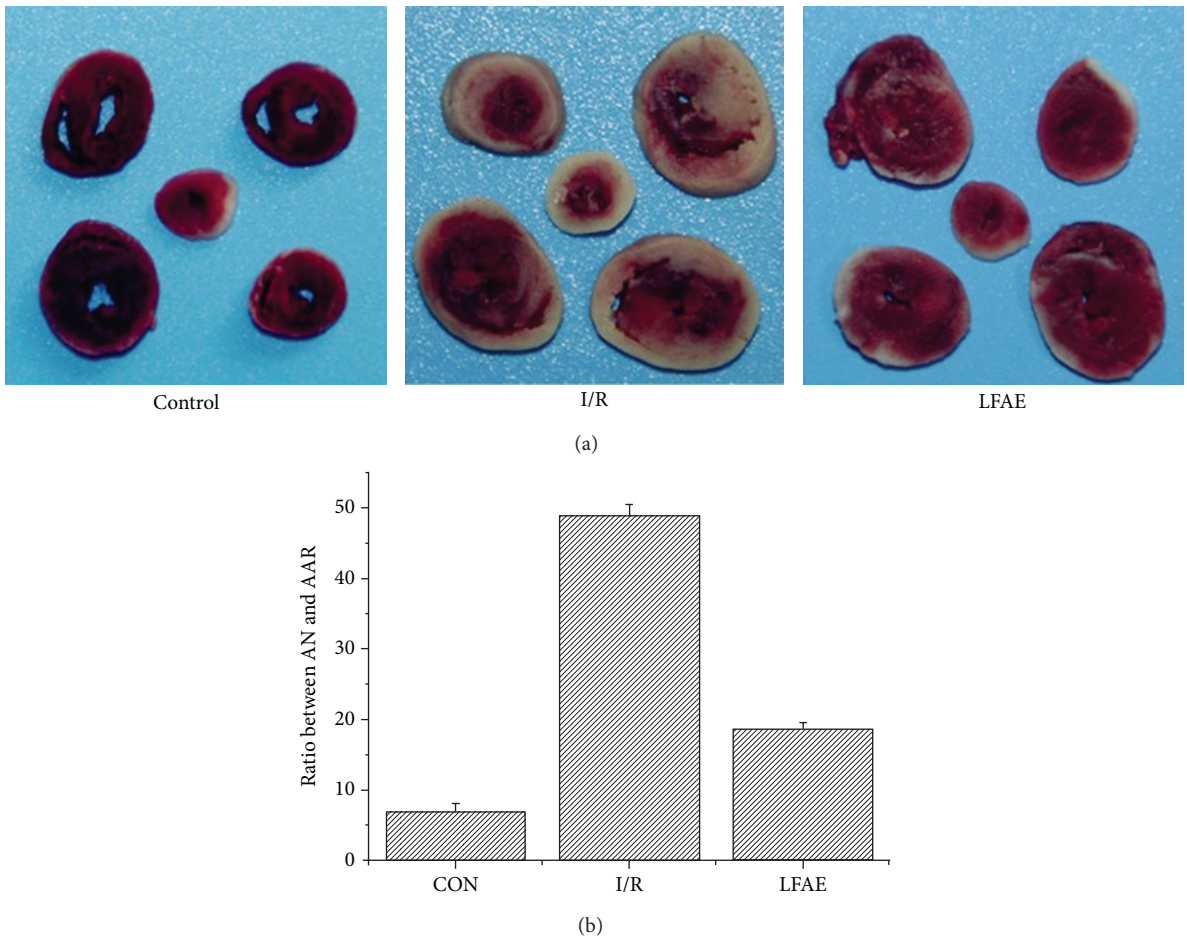

Figure 2: The aqueous extract of lavender flower (LFAE) reduces I/R-induced infarct size. (a) Photos of myocardial tissue sections of control, LFAE and I/R group, respectively, showing infarct (white) zones after TTC staining. (b) Ratio between the AN and AAR, AN is ischemic necrosis area; AAR is ischemic area. Values are means with their standard deviation ($\overline{x} \pm s, n = 8$), $^{**}P < 0.01$ compare with I/R group. CON: control group (Sham), I/R: I/R group, and LFAE: aqueous extract of lavender flower treatment group.

while the 1.0 mg/mL LFAE preconditioning substantially decreased I/R induced percentage of myocardial infarct size. In Figure 2(b), the Ratio between the ischemic necrosis area (AN) and the ischemic area (AAR) is 7% approximately in control group (Sham), the Ratio between the AN and AAR is 49% approximately in I/R group, the Ratio between the AN and AAR is 19% approximately in LFAE group.

3.4. The LFAE Improved Oxidative Stress State Induced by I/R. To identify the possible mechanisms of LFAE on cardioprotection, the SOD activity and MDA level were determined in myocardial tissue. As shown in Table 2, the SOD activity significantly increased, while MDA level was significantly decreased in LFAE (1 mg/mL) pretreatment groups compared with that of I/R group.

The present work was aimed at studing the cardioprotective activity of the aqueous extract of lavender flower (LFAE) in ischemia/reperfusion induced cardiotoxicity in isolated heart. The results of this study revealed that LFAE at the doses of 1.0 mg/mL dependently and significantly ameliorated the cardiotoxicity by restoring cardiac function and myocardial biochemical parameters towards the normal values.

I/R injury leads to heart dysfunction, which is one of the most significant etiological factors [18]. In the present study, we observed significant myocardial dysfunction, including changed hemodynamic parameters (LVDP, $\pm dp/dt_{max}$, CF and HR) and induced myocardial infarct after reperfusion of the ischemic myocardium. This is in agreement with numerous reports, indicating reperfusion as a key trigger of a number of events leading to myocardial dysfunction associated with I/R injury. LFAE markedly improved recovery of I/R-altered hemodynamic parameters (LVDP, $\pm dp/dt_{max}$, CF, and HR) and attenuated infarct size induced by I/R.

Reactive oxygen species (ROS) generation is identified as the major factors of I/R injury [19, 20]. Under normal circumstances, the ROS can be eliminated by antioxidant systems that include antioxidant enzymes, such as SOD [21]. Several studies demonstrated that oxidative stress in the heart caused by ischemia and reperfusion leads to cardiomyocyte death and myocardial injury. To further investigate the mechanism of cardioprotective effect of LFAE, an experiment was

performed to examine whether LFAE affected the changes in MDA level and SOD activity induced by I/R. The present results illuminated that LFAE protected against myocardial I/R-induced injury, accompanied by the attenuation of MDA production and enhancement of SOD activity indicating that one of the mechanisms of the cardioprotection of LFAE was associated with its antioxidant effects.

4. Conclusions

In the present investigation, administration of LFAE significantly enhanced the resumer of I/R-altered cardiac function by blunting the reduction of left ventricular developed pressure (LVDP), maximum up/down rate of left ventricular pressure ($\pm dp/dt_{max}$), and coronary flow (CF) decreased by I/R injury. Also, LFAE treatment resulted in significant modulation of cardioprotection content, the SOD activity and MDA level. Therefore, it can be concluded that the aqueous extract of lavender flower possesses obvious protective effects on myocardial I/R injury, which may be concerned with the improvement of myocardial oxidative stress states.

Conflict of Interests

No conflicts of interests are declared by the authors.

Acknowledgments

This study was supported by the Natural Science Foundation of Shandong Province (no. ZR2011HL013) to Dong Wang and the Xinjiang Production and Construction Corps Fund for Distinguished Young Scientists (2011CD006).

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