

## Research Article

# Protective Effects of *Elaeagnus angustifolia* Leaf Extract against Myocardial Ischemia/Reperfusion Injury in Isolated Rat Heart

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The purpose of this study is to clarify the cardioprotective property of the aqueous extract of *Elaeagnus angustifolia* L. leaf (EA) against myocardial ischemia/reperfusion injury in isolated rat heart. The myocardial ischemia/reperfusion (I/R) injury model of isolated rat heart was set up by the use of improved Langendorff retrograde perfusion technology. Compared with the ischemia/reperfusion (I/R) group, the aqueous extract of *Elaeagnus angustifolia* L. leaf (0.5 mg/mL, 1.0 mg/mL) pretreatment markedly improved the coronary flow (CF) and raised left ventricular developed pressure (LVDP) and maximum rise/down velocity ( $\pm dp/dt_{max}$ ). The infarct size of the EA-treated hearts was smaller than that of I/R group. After treatment with EA, the superoxide dismutase (SOD) activity increased; malondialdehyde (MDA) and protein carbonyl content reduced more obviously ( $P < 0.01$ ) than that of I/R injury myocardial tissue. **Conclusion.** Results from the present study showed that the aqueous extract of *Elaeagnus angustifolia* L. leaf has obvious protective effects on myocardial I/R injury, which may be related to the improvement of myocardial oxidative stress states.

## 1. Introduction

Cardiovascular disease is one of the main causes of mortality of people all over the world [1]; acute myocardial infarction (MI) is one of the leading causes of death in human cardiovascular disease, which is caused by thrombotic occlusion of the coronary artery induced by the death of myocardial cells. Quick return to normal blood supply is the only effective way to reduce heart injury. Paradoxically, reperfusion can cause myocardial injury and further lead to cardiac dysfunction, referred to as “reperfusion injury” [2–4]. Therefore, mitigating myocardial ischemia-reperfusion (I/R) injury is a very important way for the ischemic heart disease treatment [5].

I/R injury is a very complicated process which involves a lot of kinds of possible mechanisms. Oxidative stress damage plays an important role in the progression of I/R injury,

which is one of the most important mechanisms involved in I/R injury [6, 7]. Some studies have shown that increased expression of antioxidant enzymes will protect against I/R injury. A lot of antioxidants, such as vitamin E, catalase (CAT), melatonin, and superoxide dismutase (SOD), have been reported to protect the heart from I/R injury; all the evidences confirm that reduced oxidative stress could decrease the injury induced by I/R [8, 9].

*Elaeagnus angustifolia*, used as a food ingredient or herbal drug for its reputed medicinal properties, commonly called wild olive, silver berry, Russian olive, or oleaster, is a species of *Elaeagnus*, native to western and central Asia, from southern Russia and Kazakhstan to Turkey and Iran [10]. It has been found that *Elaeagnus angustifolia* is used in traditional Turkish medicine as antipyretic, diuretic, tonic, and antidiarrheal and as a medication against kidney disorders (inflammatory or kidney stone) [11, 12] and in Iranian folk medicine for

its anti-inflammatory, antinociceptive, and analgesic effects. Also, decoction and infusion of its fruits are considered to be a good remedy for fever, jaundice, asthma, tetanus, and rheumatoid arthritis [13]; the aqueous and ethanolic extracts of its fruits induced a muscle relaxant effect in a dose dependent manner [14]. In the present study, we investigated the effects of aqueous extract from *Elaeagnus angustifolia* L. leaf on protecting rat heart against I/R injury.

## 2. Material and Methods

**2.1. Plant Material.** The mature leaves of *Elaeagnus angustifolia* L. were collected during the month of October from Shihezi, Xinjiang, China, and the authenticity of the material was verified by one of the authors and later confirmed by a botanist.

**2.2. Chemicals.** Triphenyltetrazolium chloride (TTC) was obtained from the Beijing Biodee Biotechnology Co., Ltd. (Beijing, China). The malondialdehyde (MDA), super oxide dismutase (SOD), and protein carbonyl analyzing reagent kits were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All of the other chemicals used in the study for the biochemical estimation were of analytical grade obtained commercially.

**2.3. Preparation of Extract.** Fresh *Elaeagnus angustifolia* L. leaves were washed in distilled water and air-dried in the shade. Thirty grams of leaves was cut into small pieces and extracted with 70°C distilled water (DW) (DW: material, 10:1, v/w) thrice in an incubator at 85°C for 3 h. The 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 50 mL (amount to 6 × 10<sup>2</sup> mg/mL crude drug).

**2.4. Experimental Groups and Drug Delivery.** Male Wistar rats (250–280 g) were obtained from Xinjiang Medicine University Medical Laboratory Animal Center (SDXK 2011-004). All experimental procedures complied with the Institutional Animal Care and Use Committee of National Institute Pharmaceutical Education and Research.

The rats were divided into four groups randomly: control group (Sham), I/R group, low concentration of aqueous extract of *Elaeagnus angustifolia* L. leaves (EA) treatment group, and high concentration of EA treatment group.

**2.5. Preparation for Isolated Heart.** 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 sublingually venously injected into the rats. Thoracic surgery was performed to remove the heart [15], hearts were excised and mounted on Langendorff's apparatus quickly, and then the hearts were immersed in ice-cold Krebs-Henseleit buffer (1.2 mM KH<sub>2</sub>PO<sub>4</sub>, 1.2 mM CaCl<sub>2</sub>, 25 mM sodium acetate, 120 mM NaCl, 1.2 mM MgSO<sub>4</sub>, and 11 mM glucose; pH 7.4) piped with a gas mixture comprised of 95% O<sub>2</sub>/5% CO<sub>2</sub>; then the hearts were maintained in 37°C in

a water-jacketed organ chamber [16]. The pressure recording was established by a water-filled latex balloon, coupled to a pressure transducer (Statham), which was inserted into the left ventricular cavity via the left auricle.

Control group hearts were stabilized by perfusion for 95 min and the I/R group hearts were stabilized for 30 min, and then, after 20 min global ischemia, the reperfusion was established for 45 min. Hearts in EA treatment group were stabilized for 20 min, instead of K-H buffer with EA (0.5 mg/mL, 1 mg/mL) for 10 min, and then global ischemia for 20 min and reperfusion for 45 min were established.

**2.6. Measurement of Heart Hemodynamic Parameters.** A computer-based data acquisition system was used to continuously monitor the hemodynamic parameters including the following functional parameters: left ventricular developed pressure (LVDP, LVDP = LVSP – LVEDP), left ventricular end-diastolic pressure (LVEDP), left ventricular systolic pressure (LVSP), maximum rise/down velocity of left intraventricular pressure ( $\pm dp/dt_{max}$ ), and heart rate (HR) were monitored continuously using 4S AD Instruments biology polygraph (Powerlab, Australia). The coronary flow (CF) was measured using a flowmeter with an in-line probe.

**2.7. Evaluation of Myocardial Infarct Size.** Frozen hearts were cut into 2 mm thick cross-sectional slices for evaluation of myocardial size. The slices were stained by putting them into the 1% triphenyltetrazolium chloride (TTC) solution for 10 minutes at 37°C. The stained slices were transferred to a formalin solution for 10 min after TTC staining; then we placed them into phosphate buffer (pH 7.4) [17]. The heart slices were then digitally imaged using a digital camera. The ischemic (white) and nonischemic (red) area were measured digitally using Image Pro Plus software. The infarct size was represented as percentage of the ischemic area.

**2.8. Assay of Oxidative Stress.** At the end of the perfusions, the hearts were kept at –70°C for later oxidative stress analysis. Liquid nitrogen-chilled tissue pulverizer was used to crush the frozen hearts to a powder. For tissue analyses, weighed amounts of the frozen tissues were homogenized in appropriate buffer using microcentrifuge tube homogenizer. Then, the SOD activity, malondialdehyde (MDA), and protein carbonyl content were analyzed spectrophotometrically according to the instruction of the assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

**2.9. Statistical Analysis.** Data were presented as mean ± standard deviation from at least six independent experiments. Differences in hemodynamic parameters, infarct size, MDA level, SOD activity, and carbonyl content level were analyzed using the one-way ANOVA with Student's *t*-test. Statistical significance was considered at the probability value of less than 0.05. Analyses were carried out using the Origin 8.0 software (Origin Lab Corporation, Northampton, MA, USA).

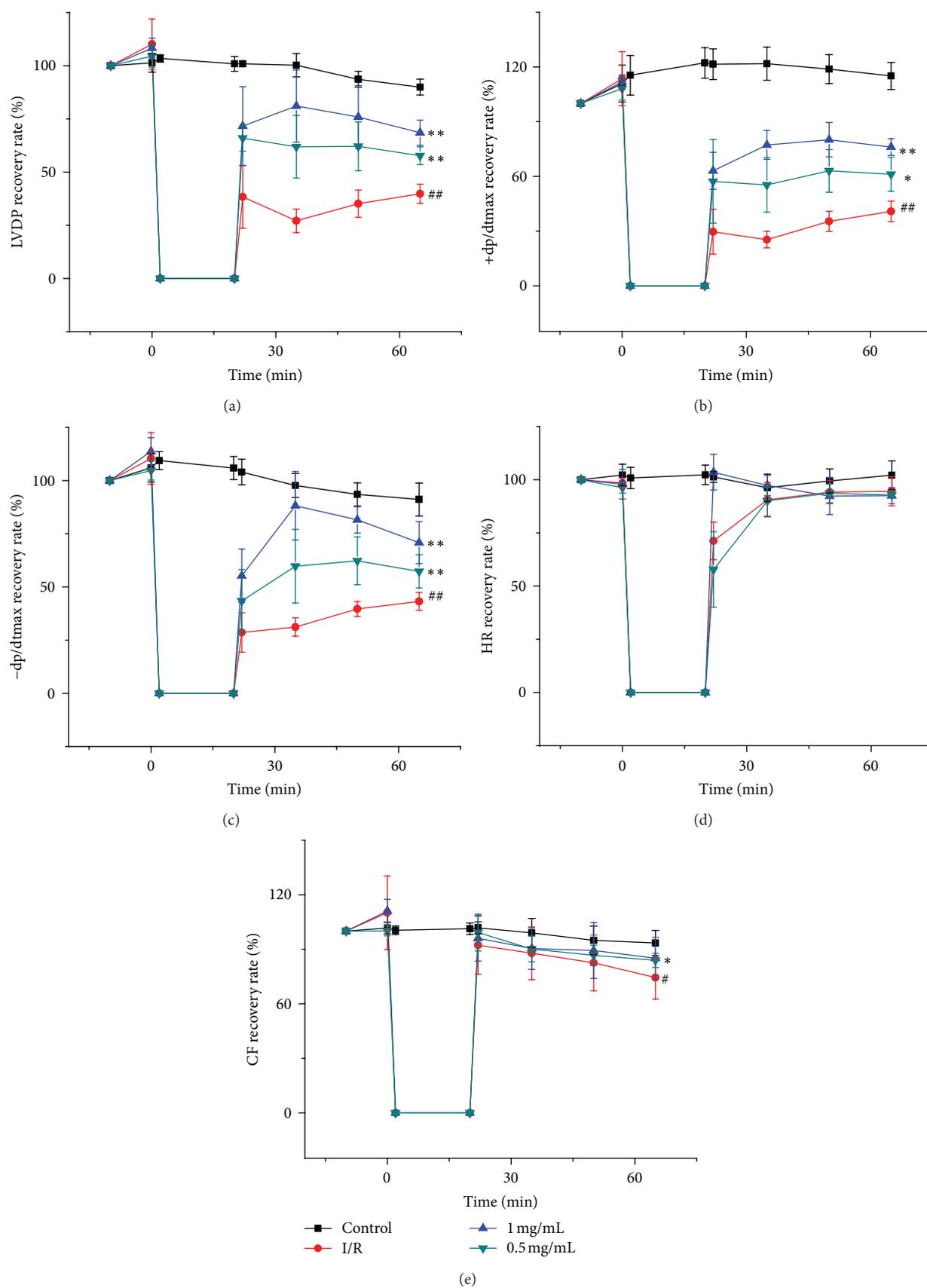


FIGURE 1: Effects of the aqueous extract of *Elaeagnus angustifolia* L. leaves upon hemodynamics in a model of ischemia-reperfusion using isolated rat heart. Data are the mean  $\pm$  SD. Values marked with  $^{##}P < 0.01$  and  $^{\#}P < 0.05$  are significantly different from control group. Values marked with  $^{**}P < 0.01$  and  $^*P < 0.05$  are significantly different from I/R group.

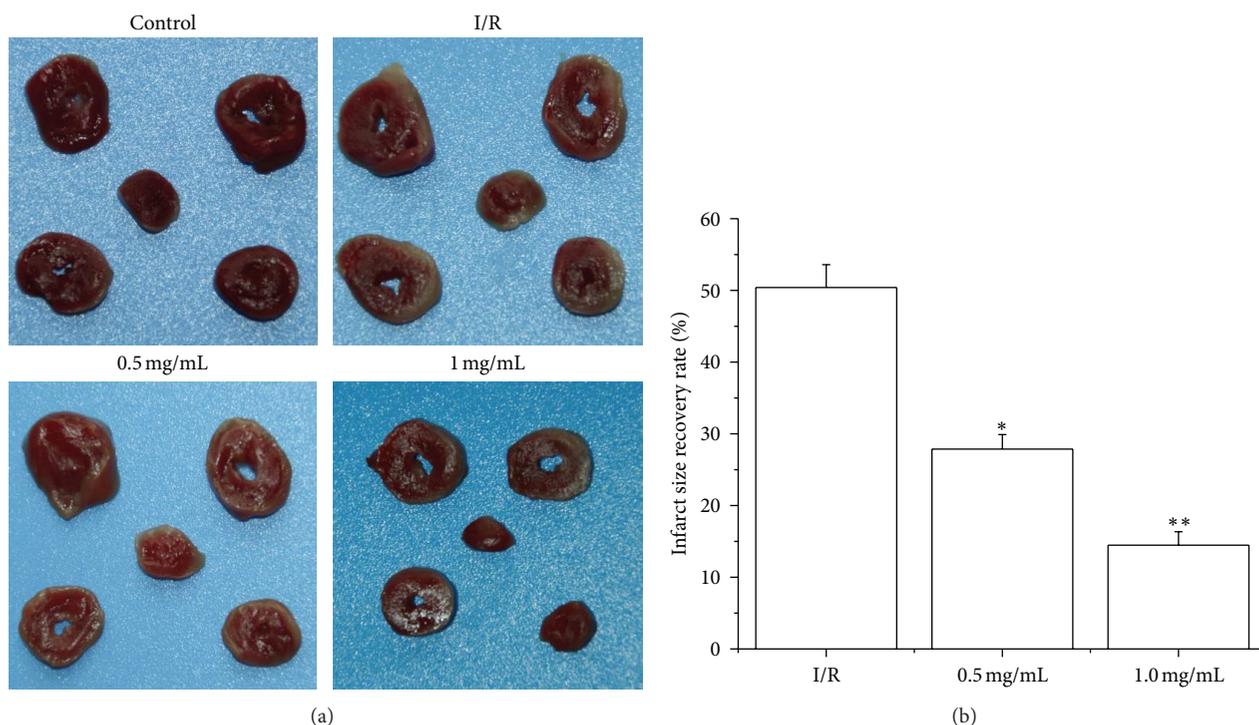


FIGURE 2: Effects of the aqueous extract of *Elaeagnus angustifolia* L. leaves upon myocardial infarct size. Data are the mean  $\pm$  SD. Values marked with \*\* $P < 0.01$  and \* $P < 0.05$  are significantly different from I/R group.

### 3. Result

**3.1. The Aqueous Extract of *Elaeagnus angustifolia* L. Leaves (EA) Enhanced Recovery of I/R Induced Changes in Cardiac Function.** The cardiac function was monitored continuously using 4S AD Instruments biology polygraph. Compared to the sham I/R group, I/R injury caused a significant decrease in left ventricular developed pressure (LVDP), maximum rise/down velocity of left intraventricular pressure ( $\pm dp/dt_{max}$ ), and coronary flow (CF). As shown in Figure 1, the EA administration significantly blunted the reduction of LVDP (Figure 1(a)),  $\pm dp/dt_{max}$  (Figures 1(b) and 1(c)), and CF (Figure 1(d)) caused by I/R injury. However, there were no significant changes in HR (Figure 1(e)) among all of the four groups.

**3.2. The EA Reduced Myocardial Infarct Size following I/R Injury.** At the end of reperfusion, myocardial infarct size was assessed using the TTC staining method. As illustrated in Figure 2, ischemia for 20 min followed by 45 min of reperfusion resulted in development of substantial myocardial infarcts, while the 0.5 mg/mL and 1.0 mg/mL EA preconditioning substantially decreased I/R induced percentage of myocardial infarct size.

**3.3. The EA Improved Oxidative Stress State Induced by I/R.** To identify the possible mechanisms of EA on cardioprotection, the SOD activity and MDA level were determined in myocardial tissue. The SOD activity significantly increased (Figure 3(a)), while MDA level (Figure 3(b)) was significantly

decreased in EA pretreatment groups compared with that of I/R group. Protein carbonyls are formed through oxidation of proteins by a variety of mechanisms which are the sensitive markers of oxidative injury. As shown in Figure 2, the hearts subjected to global myocardial ischemia for 20 min followed by 45 min of reperfusion showed a significant increase of level of carbonyl content, while the EA preconditioning substantially decreased I/R induced rise of level of carbonyl content.

### 4. Discussion

The present work was aimed at studying the cardioprotective activity of the aqueous extract of *Elaeagnus angustifolia* L. leaf (EA) in ischemia/reperfusion (I/R) induced cardiotoxicity in isolated rat heart. The results of this study revealed that EA at the doses of 0.5 mg/mL and 1.0 mg/mL dependently and significantly ameliorated the cardiotoxicity by restoring cardiac function and myocardial biochemical parameters towards the normal values.

After reperfusion of the ischemic myocardium, significant myocardial dysfunction, including LVDP,  $\pm dp/dt_{max}$ , CF and HR, and myocardial infarct, was induced by I/R. Reperfusion is a key role among a number of events leading to myocardial dysfunction associated with I/R injury. In the present study, we found that EA improved recovery of I/R-altered hemodynamic parameters (LVDP,  $\pm dp/dt_{max}$ , and CF and HR) and attenuated infarct size induced by I/R significantly.

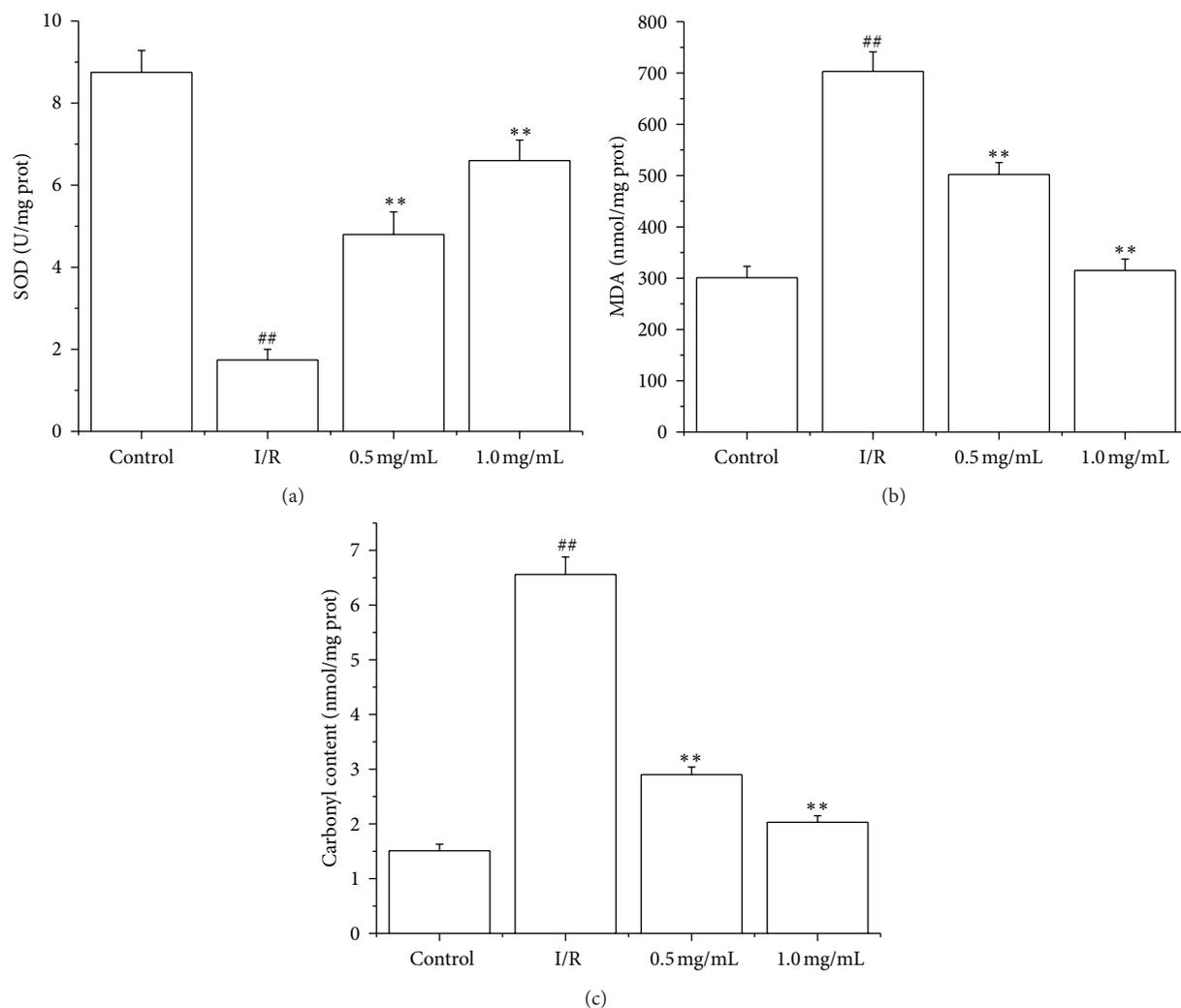


FIGURE 3: Effects of EA on SOD activity (a), MDA level (b), and carbonyl content level (c). Values are means with their standard deviation ( $n = 8$ ); ##  $P < 0.01$  compared with control group; \*\*  $P < 0.01$  compared with I/R group.

The generation of reactive oxygen species (ROS) plays an important role in the I/R induced myocardial injury [18, 19]. The ROS can be diminished by antioxidant systems that include antioxidant enzymes, such as SOD in the normal conditions [20]. However, when the amount of ROS is beyond the capacity of those enzymes and cannot be diminished during reperfusion, oxidative stress occurs. More and more evidence suggested that cardiomyocyte death and myocardial injury occurred during ischemia and reperfusion accompanied with oxidative stress [21–23]. Therefore, reduction of oxidative stress is one of the favorable strategies to alleviate myocardial injury induced by I/R. MDA is a maker of the peroxidation of cell membrane lipids caused by ROS. SOD is one of the most significant antioxidant enzymes, functioning as a superoxide anion scavenger, protein carbonyl is a sensitive biomarker of oxidative injury of proteins, and carbonyls are formed through oxidation of proteins. To further investigate the mechanism of cardioprotective effect of EA, an experiment was performed to examine whether EA affected the levels

of MDA, protein carbonyls, and SOD activity induced by I/R. The present results illuminated that EA protected against myocardial I/R induced injury, accompanied by the attenuation of MDA production and protein carbonyls content and enhancement of SOD activity indicating that one of the mechanisms of the cardioprotection of EA was associated with its antioxidant effects.

In the present investigation, administration of EA significantly enhanced the recovery of I/R-altered cardiac function by blunting the reduction of left ventricular developed pressure (LVDP), maximum rise/down velocity of left intraventricular pressure ( $\pm dp/dt_{max}$ ), and coronary flow (CF) decreased by I/R injury. Also, EA treatment resulted in significant modulation of cardioprotection content, the SOD activity, and MDA level. Therefore, it can be concluded that the aqueous extract of *Elaeagnus angustifolia* L. leaf has obvious protective effects on myocardial I/R injury, which may be related to the improvement of myocardial oxidative stress states.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

## Authors' Contribution

Binsheng Wang and Hengyi Qu contribute equally to this work.

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