

# Effects of puerarin on lipopolysaccharide-induced myocardial dysfunction in isolated rat hearts

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**Abstract:** Myocardial dysfunction is a serious complication induced by sepsis. Puerarin is an oriental medicine that possesses therapeutic benefits for cardiovascular diseases. The aim of this study was to evaluate the anti-myocardial dysfunction effects of puerarin in isolated rat hearts induced by lipopolysaccharide- and compare the myocardial protective effects between the different concentrations of puerarin. Isolated hearts were attached to a Langendorff apparatus and perfused with lipopolysaccharide (LPS) and different concentrations of puerarin. The hemodynamic parameters of heart rate (HR), left ventricular end systolic pressure [LVESP],  $+dp/dt_{max}$ , and  $-dp/dt_{max}$  were recorded. The biochemical indexes of lactic dehydrogenase (LDH), tumor necrosis factor alpha (TNF- $\alpha$ ), and creatine kinase (CK) in the coronary effluent were measured at 40, 90, and 120 min of perfusion. TNF- $\alpha$  in myocardial tissues was measured after perfusion was completed. As a result, puerarin (0.24 mmol/L–0.48 mmol/L) significantly increased LVESP,  $+dp/dt_{max}$ ,  $-dp/dt_{max}$ , and HR in isolated rat hearts that were declined by LPS during perfusion periods. Puerarin could protect against increased LDH, CK, and TNF- $\alpha$  in coronary effluent of isolated rat hearts by LPS during perfusion periods. Treatment of 0.48 mmol/L puerarin significantly decreased the TNF- $\alpha$  in coronary effluent of isolated rat hearts compared with the treatment of 0.12 and 0.24 mmol/L puerarin, but the TNF- $\alpha$  values were not reverted to baseline levels. However, the difference of TNF- $\alpha$  in myocardial tissue in the three puerarin-combined groups was statistically significant. This study confirms that puerarin can improve LPS-induced contractile dysfunction in isolated heart and inhibit LPS-stimulated myocardial TNF- $\alpha$  production.

**Keywords:** Puerarin, Myocardial dysfunction, Lipopolysaccharide, TNF- $\alpha$ .

## INTRODUCTION

Sepsis is a systemic inflammatory reaction syndrome caused by infection and is accompanied by multiple organ failure (MOF) (Wu, *et al.*, 2015). Over the past few decades, sepsis has a mortality rate of 20%-40% (Wiersinga, *et al.*, 2010; Bhatia M *et al.*, 2009). As one of the major complications, myocardial dysfunction occurs during severe sepsis and septic shock which contributes to mortality and morbidity in critically ill patients. Most of sepsis cases are caused by Gram-negative bacterial infection. Endotoxins or lipopolysaccharides (LPS) from Gram-negative bacteria play a major role in the pathogenesis by inducing an over-production of inflammatory cytokines, which usually trigger beneficial inflammatory responses but cause tissue injury and lethal MOF in excessive amounts (Wang, *et al.*, 2012). Though the myocardial dysfunction mechanism induced by LPS is incompletely described, cytokine-mediated production of inducible nitric oxide (NO), intracellular calcium currents, oxidation–reduction imbalance, and disrupted respiratory chain activities are used to explain the physiology of septic cardiomyopathy (Flynn, *et al.*, 2010). Therefore, the process of LPS-induced myocardial dysfunction is

complex and the myocardial dysfunction induced by severe sepsis or septic shock is severe resulting in difficult treatment.

Puerarin is isoflavone monomer composition extracted from the dried root of *Pueraria lobata* Ohwi, and its chemical name is 4',7-dihydroxy-8-beta-D-glucose isoflavone. Puerarin is one of the earliest and most important herbs in oriental medicine; it possesses anti-inflammatory (Lim, *et al.*, 2013) anti-hypertensive (Guerra, *et al.*, 2000), anti-oxidative (Chung, *et al.*, 2008), anti-thrombotic (Chen *et al.*, 2013), neuroprotective (Zhu, *et al.*, 2014), hepatoprotective (Yu, *et al.*, 1997) and cancer chemopreventive properties (Yu, *et al.*, 2006). Therefore, puerarin is widely used in China for various medical purposes, especially for the treatment of cardiovascular diseases. However, whether puerarin has a protective effect against sepsis-induced myocardial dysfunction remains unknown.

In this study, we aimed to evaluate the protective effects of puerarin on LPS-induced myocardial dysfunction according to hemodynamic parameters and biochemical indicators measured in Langendorff perfused rat isolated

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hearts. These parameters include left ventricular end systolic pressure (LVESP), maximum rate of intraventricular pressure development ( $+dP/dt_{max}$ ), maximum rate of intraventricular pressure relaxation ( $-dP/dt_{max}$ ), heart rate (HR), and the levels of lactic dehydrogenase (LDH), creatine kinase (CK) and tumor necrosis factor alpha (TNF- $\alpha$ ).

## MATERIALS AND METHODS

### Materials and reagents

This experimental protocol was approved by the Animal Care and Use Committee of Zhejiang University, and all procedures were carefully conducted following their guidelines of animal research.

Sprague-Dawley rats were purchased from Slac Laboratory Animal Corporation (Shanghai, China). Puerarin (purity 99.4%) was purchased from Han Yin HEYEMAIDISEN Plant Pharmaceutical Co., Ltd. (China). LPS (LPS L2880, LPS from *E. coli* 055:B5) was obtained from Sigma-Aldrich (USA). Enzyme-linked immunosorbent assay (ELISA) kits for LDH, CK, and TNF- $\alpha$  were purchased from Shanghai Westang Biotechnology Co., Ltd. (Shanghai, China). Modified Krebs-Henseleit (KH) buffer was prepared in our laboratory according to (Yu, *et al.*, 2006) containing 118 mmol/L NaCl, 25.2mmol/L NaHCO<sub>3</sub>, 4.7mmol/L KCl, 1.5mmol/L CaCl<sub>2</sub>, 1.2mmol/L MgSO<sub>4</sub>, 1.2mmol/L KH<sub>2</sub>PO<sub>4</sub>, and 11.1mmol/L glucose. Other reagents and chemicals used were of analytical grade.

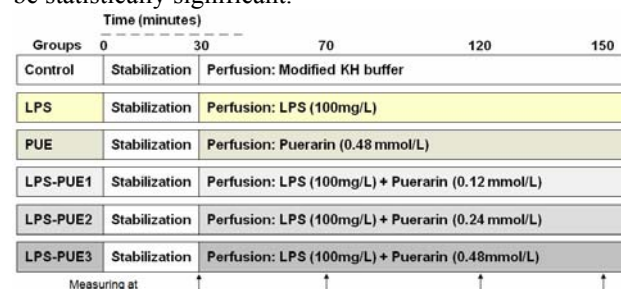
### Experimental protocol

The method of isolated hearts obtained was referred to the preparation of ischemia and reperfusion injury model in isolated rat heart by Zheng *et al.* (Zheng, *et al.*, 2006). Sprague-Dawley rats, male sex, body mass 250g-350g, were allowed free diet for food and water till the surgery day. Rats were anesthetized by pentobarbital (60mg/kg) intraperitoneally injected, and sodium heparin (250 IU) was then intraperitoneally injected for anti-freezing. Hearts were rapidly isolated and they were retrograde perfused with spontaneous heart beat rate in the nonrecirculating Langendorff apparatus. Modified KH buffer was kept at 37°C and pH 7.4 that bubbled with 95% O<sub>2</sub>/5% CO<sub>2</sub>. A latex balloon filled with water was placed in the left ventricle through left atrium, and connected to a computer with MedLab software (Nanjing MedEase Science & Technology, Nanjing, China) via a pressure sensor. After equilibration of the heart, balloon volume was adjusted to maintain a constant diastolic pressure between 6 and 8 mmHg through injecting water. All hearts were maintained on the Langendorff system for approximately 30 min to stabilize for baseline values, and then treated for 120 min. All isolated hearts were divided into control, PUE, LPS, LPS-PUE1, LPS-PUE2, and LPS-PUE3 groups, respectively (fig. 1). During the perfusion

periods, isolated hearts were continuously infused with modified KH buffer in the control group, and were treated with 100mg/L LPS in the LPS group. In the PUE group, hearts were treated with 0.48mmol/L puerarin during the perfusion periods. In the LPS-PUE1 group, hearts were treated with perfusate containing 100mg/L LPS as well as 0.12mmol/L puerarin during the perfusion periods. In the LPS-PUE2 group, hearts were treated with perfusate containing 100 mg/L LPS as well as 0.24mmol/L puerarin during the perfusion periods. In the LPS-PUE3 group, hearts were treated with perfusate containing 100 mg/L LPS as well as 0.48mmol/L puerarin during the perfusion periods. Measurements for statistical analysis were directly obtained after stabilization and treatment as well as after 40, 90 and 120 min of perfusion. LVESP, maximum rate of intraventricular pressure development and relaxation ( $\pm dP/dt_{max}$ ) and HR were recorded for analysis. The concentrations of LDH, CK, and TNF- $\alpha$  in the coronary effluent were measured by ELISA method during the perfusion periods. After perfusion was completed, each heart was immediately homogenized with 2mL physiological saline and homogenates were centrifuged (3,000g, 15min). The concentrations of TNF- $\alpha$  in the supernatant were measured by ELISA to evaluate the levels of TNF- $\alpha$  in myocardial tissues.

## STATISTICAL ANALYSIS

Values for cardiac function, HR, and biochemical indicators are expressed as mean  $\pm$  SD. After normal testing, ANOVA was used in statistical analysis for values at the corresponding measure time points among the groups by SPSS 16.0 software.  $P < 0.05$  was considered to be statistically significant.

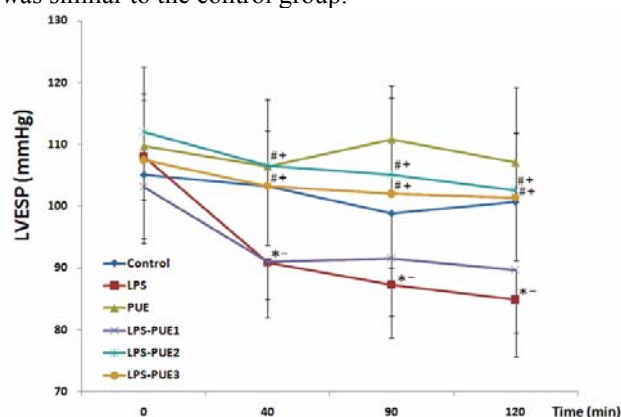


**Fig. 1:** Experimental protocols. All experimental groups began with a 30min perfusion period in order to allow for stabilization of the isolated hearts. Then the hearts were divided into six groups. The treatment of each group as follows: perfusate modified KH buffer for the control group; perfusate 100mg/L LPS for LPS group; 0.48 mmol/L puerarin for PUE group; mixed 100mg/L LPS with 0.12mmol/L puerarin for LPS-PUE1 group; 100mg/L LPS and 0.24mmol/L puerarin for LPS-PUE2 group; 100mg/L LPS and 0.48mmol/L puerarin for LPS-PUE3 group. Hemodynamic variables and biochemical indicators were measured after stabilization and 40, 90, 120 minutes after the treatment of perfusion started (Arrows). TNF- $\alpha$  in myocardial tissues after perfusion was completed.

## RESULTS

### LVESP (fig. 2)

After 40, 90, and 120 min of perfusion, LVESP in the LPS group was significantly lower than baseline. LVESP in the LPS group was significantly decreased after 40, 90, and 120min of LPS perfusion versus those in control group ( $P<0.05$ ). By contrast, the groups infused with LPS combined with puerarin showed that this herb could protect against decreased LVESP induced by LPS. In the LPS-PUE2 and LPS-PUE3 groups, LVESP was significantly increased compared with the LPS-PUE1 and LPS groups ( $P<0.05$ ). LVESP in the LPS-PUE1 group were increased at 90 and 120 min of perfusion compared with the LPS group, but the difference between these two groups has no statistically significant ( $P>0.05$ ). LVESP values in PUE group were close to the baseline, which was similar to the control group.

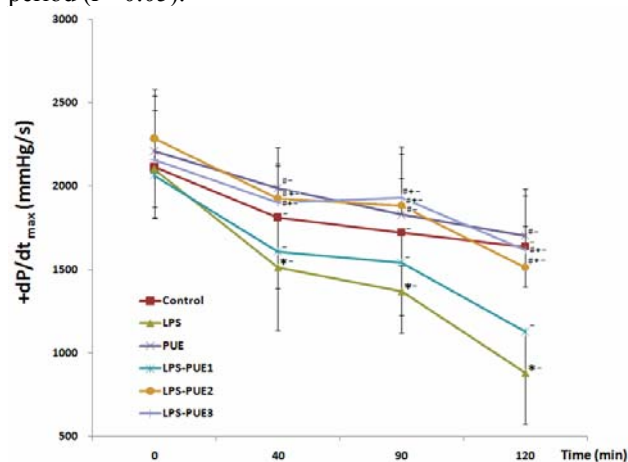


**Fig. 2:** The changes in the left ventricular end systolic pressure (LVESP). Values are expressed as mean  $\pm$  SD. Control group: no treatment during perfusion period, LPS, PUE, LPS-PUE1, LPS-PUE2 and LPS-PUE3 groups: lipopolysaccharide (100mg/L), puerarin (0.48mmol/L), lipopolysaccharide (100mg/L) combined with 0.12mmol/L puerarin, lipopolysaccharide (100mg/L) combined with 0.24mmol/L puerarin, lipopolysaccharide (100mg/L) combined with 0.48mmol/L puerarin were infused during perfusion period, respectively. 0: immediately after stabilization, 40: at 40 minutes after perfusion, 90: at 90 minutes after perfusion, 120: at 120 minutes after perfusion. Probability value was indicated as follows; \*  $P<0.05$  vs. control group at the same time point, #  $P<0.05$  vs. LPS group at the same time point, +  $P<0.05$  vs. LPS-PUE1 group at the same time point, -  $P<0.05$  vs. time 0. In the LPS group and in the LPS-PUE1 group, LVESP significantly decreased during the perfusion period compared with baseline, however in the LPS-PUE2 and LPS-PUE3 groups there were no significant decrease in LVESP.

### Maximal rise rate of left ventricular pressure (+dP/dt<sub>max</sub>, fig. 3)

At 120 min of perfusion, +dP/dt<sub>max</sub> values of all groups were significantly lower versus baseline values ( $P<0.05$ ).

At 40, 90, and 120 min of perfusion, +dP/dt<sub>max</sub> in the LPS group was significantly decreased versus the baseline values and the values in control group ( $P<0.05$ ). However, +dP/dt<sub>max</sub> values were significantly higher in the PUE, LPS-PUE2, and LPS-PUE3 groups than those in the LPS group ( $P<0.01$ ). Compared with the LPS-PUE1 group, +dP/dt<sub>max</sub> values were significantly higher in the LPS-PUE2 and LPS-PUE3 groups during the perfusion period ( $P<0.05$ ).

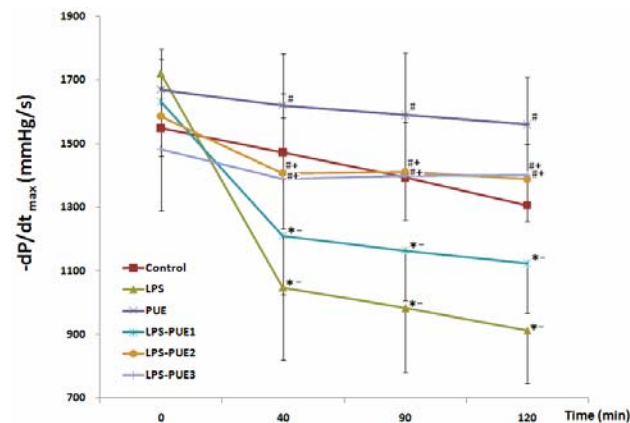


**Fig. 3:** The changes in the maximal rise rate of left ventricular pressure (+dP/dt<sub>max</sub>). Values are expressed as mean  $\pm$  SD. Control group: no treatment during perfusion period, LPS, PUE, LPS-PUE1, LPS-PUE2 and LPS-PUE3 groups: lipopolysaccharide (100mg/L), puerarin (0.48mmol/L), lipopolysaccharide (100mg/L) combined with 0.12mmol/L puerarin, lipopolysaccharide (100mg/L) combined with 0.24mmol/L puerarin, lipopolysaccharide (100mg/L) combined with 0.48mmol/L puerarin were infused during perfusion period, respectively. 0: immediately after stabilization, 40: at 40 minutes after perfusion, 90: at 90 minutes after perfusion, 120: at 120 minutes after perfusion. Probability value was indicated as follows; \*  $P<0.05$  vs. control group at the same time point, #  $P<0.05$  vs. LPS group at the same time point, +  $P<0.05$  vs. LPS-PUE1 group at the same time point, -  $P<0.05$  vs. time 0. All groups demonstrated decreased values after perfusion compared with the baseline. But the +dP/dt<sub>max</sub> values in LPS-PUE2 and LPS-PUE3 groups were with significant difference compared with LPS and LPS-PUE1 groups.

### Maximal fall rate of left ventricular pressure (-dP/dt<sub>max</sub>, fig. 4)

DP/dt<sub>max</sub> changes trend was similar to +dP/dt<sub>max</sub>. After perfusion for 40, 90 and 120min, -dP/dt<sub>max</sub> values in the LPS group were significantly decreased versus the baseline values and values in the control group ( $P<0.01$ ). -dP/dt<sub>max</sub> values were significantly higher in PUE, LPS-PUE2, and LPS-PUE3 groups than those in LPS and LPS-PUE1 groups ( $P<0.05$ ). Compared with the LPS and LPS-PUE1 groups, -dP/dt<sub>max</sub> values in LPS-PUE1 group were higher than those in LPS group at 40, 90, and 120 min of perfusion with no statistical significant difference

( $P > 0.05$ ). During the perfusion period, the values of  $-dp/dt_{max}$  in the LPS and LPS-PUE1 groups were significantly decreased versus the baseline values ( $P < 0.05$ ); in contrast, these values were not markedly decreased in the PUE, LPS-PUE2, and LPS-PUE3 groups compared with the baseline values.

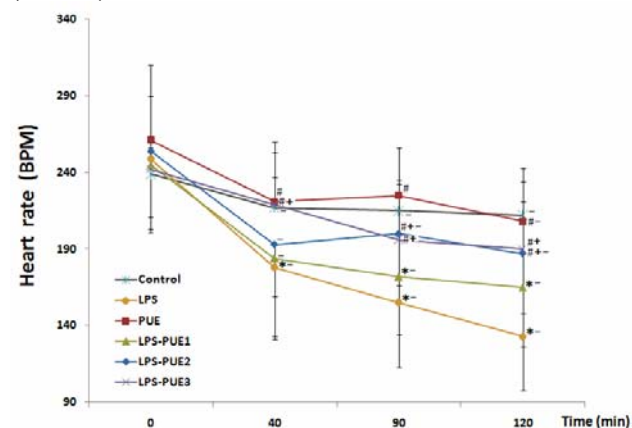


**Fig. 4:** The changes in the maximal rise rate of left ventricular pressure ( $-dp/dt_{max}$ ). Values are expressed as mean  $\pm$  SD. Control group: no treatment during perfusion period, LPS, PUE, LPS-PUE1, LPS-PUE2 and LPS-PUE3 groups: lipopolysaccharide (100mg/L), puerarin (0.48mmol/L), lipopolysaccharide (100mg/L) combined with 0.12mmol/L puerarin, lipopolysaccharide (100mg/L) combined with 0.24mmol/L puerarin, lipopolysaccharide (100mg/L) combined with 0.48mmol/L puerarin were infused during perfusion period, respectively. 0: immediately after stabilization, 40: at 40minutes after perfusion, 90: at 90 minutes after perfusion, 120: at 120 minutes after perfusion. Probability value was indicated as follows; \*  $P < 0.05$  vs. control group at the same time point, #  $P < 0.05$  vs. LPS group at the same time point, +  $P < 0.05$  vs. LPS-PUE1 group at the same time point, -  $P < 0.05$  vs. time 0. The  $-dp/dt_{max}$  values in LPS-PUE2 and LPS-PUE3 groups demonstrated significantly increased values compared with LPS and LPS-PUE1 groups during the perfusion periods.

**HR (fig. 5)**

HR of isolated rat hearts in the LPS group was significantly decreased from  $249 \pm 48$  beats/min to  $133 \pm 35$  beats/min at 120 min of perfusion ( $P < 0.05$ ), but this effect of HR reduction was not observed in the control group. The HR values in the PUE, LPS-PUE2, and LPS-PUE3 groups were significantly higher than those in the LPS and LPS-PUE1 groups ( $P < 0.05$ ), but the difference of the HR values between the LPS-PUE2 and LPS-PUE1 groups was not statistically significant at 40 min of perfusion ( $P > 0.05$ ). HR in the LPS-PUE2 group was decreased from  $254 \pm 43$  beats/min to  $193 \pm 35$  beats/min at 40 min of perfusion, and then the HR value ( $200 \pm 32$  beats/min) was slightly increased, which was nearly the value of LPS-PUE3 group ( $196 \pm 39$  beats/min) at the corresponding time point of 90 min after perfusion. The HR values in the

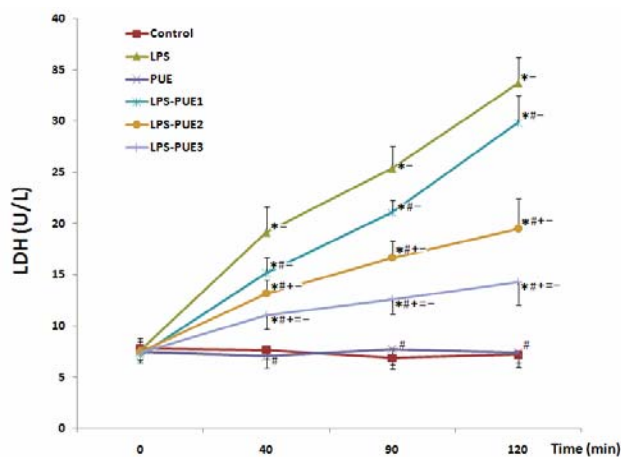
LPS-PUE2 group with  $187 \pm 38$  beats/min were close to those in the LPS-PUE3 group ( $190 \pm 35$  beats/min) at 120 min of perfusion, with no significant statistical difference ( $P > 0.05$ ).



**Fig. 5:** The changes in the heart rate (HR). Values are expressed as mean  $\pm$  SD. Control group: no treatment during perfusion period, LPS, PUE, LPS-PUE1, LPS-PUE2 and LPS-PUE3 groups: lipopolysaccharide (100mg/L), puerarin (0.48mmol/L), lipopolysaccharide (100mg/L) combined with 0.12mmol/L puerarin, lipopolysaccharide (100mg/L) combined with 0.24mmol/L puerarin, lipopolysaccharide (100mg/L) combined with 0.48mmol/L puerarin were infused during perfusion period, respectively. 0: immediately after stabilization, 40: at 40 minutes after perfusion, 90: at 90 minutes after perfusion, 120: at 120 minutes after perfusion. Probability value was indicated as follows; \*  $P < 0.05$  vs. control group at the same time point, #  $P < 0.05$  vs. LPS group at the same time point, +  $P < 0.05$  vs. LPS-PUE1 group at the same time point, -  $P < 0.05$  vs. time 0. In the LPS group, HR was significantly reduced after perfusion compared with that at the baseline. HR in the puerarin- contained groups were higher than that of LPS group.

**Lactate LDH (fig. 6)**

In the LPS group, LDH in coronary effluent was gradually increased after perfusion; the values at 40, 60, and 120 min of perfusion were lower than the baseline values ( $P < 0.001$ ). During perfusion periods, LDH values in the LPS-contained groups were higher than those in the PUE and control groups ( $P < 0.05$ ). However, LDH was lower in puerarin-contained groups than that in the LPS group ( $P < 0.05$ ). Moreover, LDH values in the LPS-PUE2 and LPS-PUE3 groups were lower than those in the LPS-PUE1 group ( $P < 0.05$ ), and the difference between the LPS-PUE2 and LPS-PUE3 groups was also statistically significant during perfusion periods. LDH gradually increased during perfusion periods in the LPS, LPS-PUE1, LPS-PUE2, and LPS-PUE3 groups than the baseline ( $P < 0.001$ ). LDH level in the LPS-PUE3 group was higher than the baseline level at the 120 min of perfusion ( $P < 0.05$ ).



**Fig. 6:** The changes of lactate dehydrogenase levels in coronary effluent. Values are expressed as mean  $\pm$  SD. Control group: no treatment during perfusion period, LPS, PUE, LPS-PUE1, LPS-PUE2 and LPS-PUE3 groups: lipopolysaccharide (100mg/L), puerarin (0.48mmol/L), lipopolysaccharide (100mg/L) combined with 0.12mmol/L puerarin, lipopolysaccharide (100mg/L) combined with 0.24mmol/L puerarin, lipopolysaccharide (100mg/L) combined with 0.48mmol/L puerarin were infused during perfusion period, respectively. 0: immediately after stabilization, 40: at 40 minutes after perfusion, 90: at 90 minutes after perfusion, 120: at 120 minutes after perfusion. Probability value was indicated as follows; \*  $P < 0.05$  vs. control group at the same time point, #  $P < 0.05$  vs. LPS group at the same time point, +  $P < 0.05$  vs. LPS-PUE1 group at the same time point, =  $P < 0.05$  vs. LPS-PUE2 group at the same time point, ^  $P < 0.05$  vs. time 0. LDH in the puerarin-contained groups were significantly reduced after perfusion compared with that the LPS group.

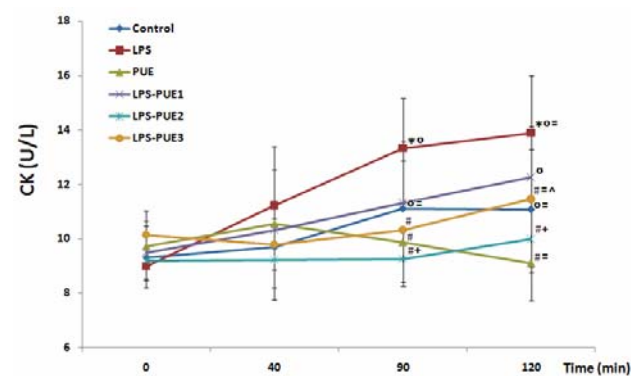
#### CK (fig. 7)

During perfusion periods, CK values of LPS group were gradually increased, and the values at 90 and 120 min time points were significantly increased than the baseline values ( $P < 0.05$ ). CK values were lower in PUE, LPS-PUE2, and LPS-PUE3 groups than those in LPS group at 90 and 120 min of perfusion ( $P < 0.05$ ). Moreover, the CK values exhibited no statistical difference between the PUE, LPS-PUE2, and LPS-PUE3 groups, and the control group. CK values in LPS-PUE2 group was also lower than that in LPS-PUE1 group at 90 and 120 min of perfusion ( $P < 0.05$ ). CK values in LPS-PUE3 were lower than those in LPS-PUE1 group during the perfusion periods, which had no statistical significant difference between the two groups ( $P > 0.05$ ). The CK values in the LPS-PUE3 group were markedly increased at 120 min of perfusion than those at 90 min of perfusion ( $P < 0.05$ ).

#### TNF- $\alpha$ (figs. 8-9)

During the perfusion periods, TNF- $\alpha$  in the PUE group was maintained in a low level, which was similar to that in the control group. However, TNF- $\alpha$  in the LPS group were gradually increased after perfusion, the values at 40, 90, and 120min of perfusion were higher than the baseline

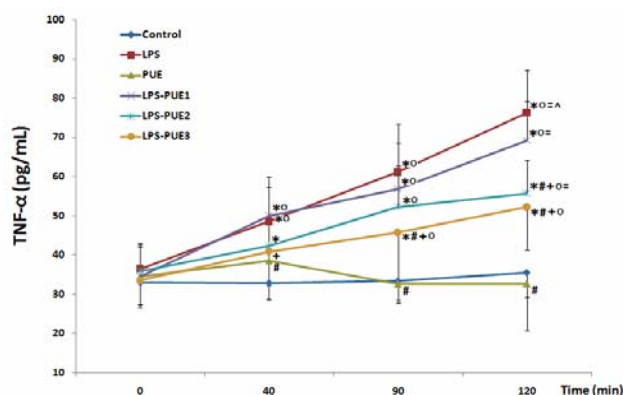
values ( $P < 0.05$ ). The level of TNF- $\alpha$  in the LPS-PUE1 group was decreased than that in the LPS group at 90 and 120min of perfusion, but the TNF- $\alpha$  values exhibited no statistical difference between these groups. TNF- $\alpha$  values were also lower in the LPS-PUE2 and LPS-PUE3 groups than that in the LPS group (at 120 min of perfusion in the LPS-PUE2 group:  $P < 0.05$ , at 90 and 120 min of perfusion in the LPS-PUE3 group:  $P < 0.05$ ). TNF- $\alpha$  values were reduced in the LPS-PUE3 group than those in the LPS-PUE1 group during the perfusion periods ( $P < 0.05$ ). TNF- $\alpha$  values were lower in the LPS-PUE2 group than those in the LPS-PUE1 group at 120 min of perfusion ( $P < 0.05$ ). However, in all the puerarin-contained LPS groups, TNF- $\alpha$  values at 90 and 120 min of perfusion were higher than those at 0 min (baseline values).



**Fig. 7:** The changes of Creatine kinase levels in coronary effluent. Values are expressed as mean  $\pm$  SD. Control group: no treatment during perfusion period, LPS, PUE, LPS-PUE1, LPS-PUE2 and LPS-PUE3 groups: lipopolysaccharide (100mg/L), puerarin (0.48mmol/L), lipopolysaccharide (100mg/L) combined with 0.12mmol/L puerarin, lipopolysaccharide (100mg/L) combined with 0.24mmol/L puerarin, lipopolysaccharide (100mg/L) combined with 0.48mmol/L puerarin were infused during perfusion period, respectively. 0: immediately after stabilization, 40: at 40 minutes after perfusion, 90: at 90 minutes after perfusion, 120: at 120 minutes after perfusion. Probability value was indicated as follows; \*  $P < 0.05$  vs. control group at the same time point, # vs. LPS group, + vs. LPS-PUE1 group, ° vs. time 0, ^ vs. time 40, ^ vs. time 90. In the LPS group, CK was gradually increased after perfusion compared with that at the baseline, and was higher than that in the puerarin-contained groups during the perfusion periods.

After perfusion, TNF- $\alpha$  in myocardial tissue of each group was measured. fig. 9 shows that TNF- $\alpha$  values in LPS, LPS-PUE1, LPS-PUE2 and LPS-PUE3 groups were higher than those in the control and PUE groups with statistical difference ( $P < 0.05$ ). TNF- $\alpha$  was lower in the LPS-PUE1, LPS-PUE2 and LPS-PUE3 groups than that in the LPS group. However, there was no statistical significant difference between LPS-PUE1 and LPS groups ( $P > 0.05$ ). TNF- $\alpha$  was also lower in the LPS-PUE3

group than those in the LPS-PUE1 and LPS-PUE2 groups with statistical significant difference between these groups ( $P < 0.05$ ). No significant differences between control and PUE groups were observed.



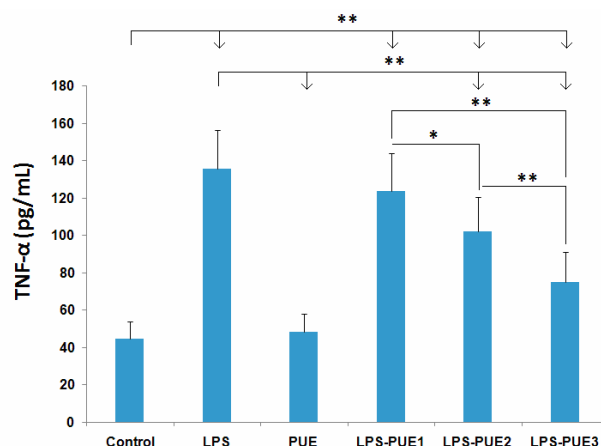
**Fig. 8:** The changes of TNF- $\alpha$  levels in coronary effluent. Values are expressed as mean  $\pm$  SD. Control group: no treatment during perfusion periods, LPS, PUE, LPS-PUE1, LPS-PUE2 and LPS-PUE3 groups: lipopolysaccharide (100mg/L), puerarin (0.48mmol/L), lipopolysaccharide (100mg/L) combined with 0.12mmol/L puerarin, lipopolysaccharide (100mg/L) combined with 0.24mmol/L puerarin, lipopolysaccharide (100mg/L) combined with 0.48mmol/L puerarin were infused during perfusion period, respectively. 0: immediately after stabilization, 40: at 40 minutes after perfusion, 90: at 90 minutes after perfusion, 120: at 120 minutes after perfusion. Probability value was indicated as follows; \*  $P < 0.05$  vs. control group at the same time point, # vs. LPS group, + vs. LPS-PUE1 group, ° vs. time 0, = vs. time 40, ^ vs. time 90. In the LPS group, TNF- $\alpha$  was gradually increased after perfusion compared with that at the baseline, and was higher than that in the puerarin-contained groups during the perfusion periods.

## DISCUSSION

In the current study, we checked on the protective effects of puerarin on LPS-induced myocardial dysfunction in isolated rat heart model, and compared the different concentrations of puerarin against the myocardial dysfunction. According to the hemodynamic index changes in this study, LPS produced lower LVESP, HR, and  $\pm dP/dt_{max}$  than baseline. LVESP, HR, and  $\pm dP/dt_{max}$  were higher in the puerarin-contained LPS groups than those in the LPS group during perfusion periods. Moreover, the laboratory indexes of HDL, CK and TNF- $\alpha$  were higher in LPS group than those in puerarin-contained LPS groups. These results demonstrate that puerarin exhibited myocardial protective effects and protected against LPS-induced myocardial dysfunction.

LPS, a high molecular material with high biological activity, is a major factor that causes pathophysiologic changes that are similar to those observed in human

patients with sepsis (Kumar, *et al.*, 2010). Intravenous injection of LPS can cause serious myocardial dysfunction that is similar to the septic shock patients complicated with myocardial dysfunction, which is manifested as decrease in myocardial contractility, decrease in myocardial compliance, and ventricular dilation (Karima *et al.*, 1999; Rudiger, *et al.*, 2007). Therefore, the LVESP,  $\pm dP/dt_{max}$  and HR in the LPS group declined with time during the perfusion procedure, whereas those values in the control and puerarin groups were maintained in the baseline. One of the potential mechanisms of LPS that cause serious myocardial dysfunction is that LPS can stimulate proinflammatory cytokines produced by local circulation and myocardium, such as cytokines, NO, and eicosanoids (Lin, *et al.*, 2005; López-Bojórquez, *et al.*, 2004). These mediators cause the disorder of energy metabolism, lipid peroxidation, and increased production of oxygen free radicals in myocardial cells, thereby causing the damage of vascular endothelial cells and the structure and function of myocardial cells (Maitra, *et al.*, 2009; Drosatos, *et al.*, 2013). These molecular pathogenesis processes appear to be critical determinants in the development of heart failure.



**Fig. 9:** The levels of TNF- $\alpha$  in myocardial tissues. Values are expressed as mean  $\pm$  SD. Control group: no treatment during perfusion period, LPS, PUE, LPS-PUE1, LPS-PUE2 and LPS-PUE3 groups: lipopolysaccharide (100mg/L), puerarin (0.48mmol/L), lipopolysaccharide (100mg/L) combined with 0.12mmol/L puerarin, lipopolysaccharide (100mg/L) combined with 0.24mmol/L puerarin, lipopolysaccharide (100mg/L) combined with 0.48mmol/L puerarin were infused during perfusion period, respectively. \*\* represents  $P < 0.01$  when control group compared with LPS, LPS-PUE1, LPS-PUE2 or LPS-PUE3 groups, LPS group compared with PUE, LPS-PUE2 or LPS-PUE3 groups, and LPS-PUE3 group compared with LPS-PUE1 or LPS-PUE2 groups. \* represents  $P < 0.05$  when LPS-PUE1 group compared with LPS-PUE2 group. There were no significant differences between control group and PUE group, and LPS group and LPS-PUE1 group.

To compare the cardioprotective effect in different concentrations of puerarin, we used low (0.12 mmol/L, LPS-PUE1), middle (0.24mmol/L, LPS-PUE2), and high (0.48mmol/L, LPS-PUE3) concentrations of puerarin. These concentrations were determined according to a previous study which suggested that 0.24 mmol/L puerarin is the effective concentration in cardioprotective effect against injury of myocardial ischemia reperfusion (Pan, *et al.*, 2006). The perfusion periods of 120 min were employed based on previous studies (Yang, *et al.*, 2006). The findings showed that puerarin-contained group can protect against the abnormal values of hemodynamic parameters and laboratory indexes caused by LPS. The cardioprotective effect of puerarin was concentration dependent. In the LPS-PUE1 group, the changes of LVESP,  $\pm dp/dt_{max}$ , and HR were similar to the LPS group after initial stabilization during 120min of perfusion. In the LPS-PUE2 group, LVESP,  $\pm dp/dt_{max}$ , and HR were increased toward the baseline value after perfusion. In the LPS-PUE3 group, LVESP,  $\pm dp/dt_{max}$ , and HR were increased the near baseline in contrast to those in the LPS-PUE1 and LPS groups. The near-baseline values of hemodynamic indexes of LVESP,  $\pm dp/dt_{max}$  and HR helped reduce the LPS-induced myocardial injury and enabled other vital organs to maintain blood flow in septic shock.

LDH and CK are two important markers of myocardial injuries and disease (Yang, *et al.*, 2014; Brien, *et al.*, 2008). According to the current study, the changes of LDH and CK were gradually increased caused by the perfusion of LPS. The levels of LDH were different on the basis of different concentrations of puerarin, and there were statistical significant difference between these puerarin-contained groups at the corresponding perfusion times. CK values in the LPS-PUE2 and LPS-PUE3 groups were close to those values in control group. CK values in LPS-PUE3 group were slightly higher than those values in LPS-PUE2 group during the perfusion periods. Because the baseline CK values in LPS-PUE3 group were higher than those values in LPS-PUE2 group, the high concentration of 0.48mmol/L did not significantly affect the heart function. These findings also show that the cardioprotective effect was concentration dependent, and the cardioprotective effect occurred with puerarin at concentrations of 0.24mmol/L. The cardioprotective effect was better on higher concentration of puerarin (0.48 mmol/L). However, the generalization of the cardioprotective effect and safety dosage range are still not confirmed to date, and further investigation is necessary.

Given that the first study conducted by Kapadia *et al.* (Kapadia, *et al.*, 1995) reported that biologically active TNF- $\alpha$  can synthesize in adult mammalian myocardium, Kalra *et al.* (Kalra, *et al.*, 2002) investigated that PKC-dependent pathway maybe the possible process of TNF biosynthesis. When the LPS combined with the LPS

receptor-CD14 that exists on the myocardial cell membrane, the myocardial cells could be stimulated with large synthetic TNF- $\alpha$ . TNF- $\alpha$  has a negative inotropic effect; it can inhibit myocardial contractility and induce myocardial cell apoptosis, thus reducing the normal heart muscle cells (Comstock, *et al.*, 1998). An experiment shows the higher content of TNF- $\alpha$  in the body with greater injury on cardiac function (Haudek, *et al.*, 2001). Our experiment also showed that the levels of TNF- $\alpha$  in coronary effluent and myocardial tissues were significantly higher than those in the control group and baseline values. These results corresponded to previous reports (Comstock, *et al.*, 1998; Haudek, *et al.*, 2001). Several studies suggest that the negative inotropic effect of TNF- $\alpha$  is related to the altered  $Ca^{2+}$  concentration of myocardial cells (Yang, *et al.*, 2006; Stamm *et al.*, 2001). Intracellular calcium concentration decreases early and calcium overload occurs later in myocardial cells, thus reducing the sensitivity of myocardial fibers with calcium ions; this phenomenon is considered an important mechanism of LPS-induced cardiac dysfunction (Yang, *et al.*, 2006). This study presents supplementary data on the cardioprotective effect against LPS damage of puerarin treatment through the decrease of TNF- $\alpha$  level.

## CONCLUSION

In summary, the present study showed that puerarin had direct cardioprotection to isolated hearts against LPS-induced damage. The results of this study widened the range of favorable effects of puerarin. The findings also demonstrated that puerarin can improve LPS-induced contractile dysfunction in isolated heart and inhibit LPS-stimulated myocardial TNF- $\alpha$  production. Puerarin maybe a potential therapeutic drug for treating myocardial dysfunction induced by LPS, but its cardioprotection effect of research on *in vivo* endotoxemia model remains unverified, and further investigation is necessary.

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