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In vivo and *in vitro* cardioprotective effects of *Panax quinquefolium* 20*(S)*-protopanaxadiol saponins (PQDS), isolated from *Panax quinquefolium*

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In this study, we investigated the cardioprotective effect of *Panax quinquefolium* 20(*S*)-protopanaxadiol saponins (PQDS) both *in vivo* and *in vitro*. An animal model of acute myocardial infarction was induced by permanent ligation of the left anterior descending coronary artery in Sparague Dawley rats. Neonatal rat cardiomycocytes were used to examine the cytoprotective effect of PQDS against H_2O_2 exposure. Pretreatment with PQDS (25 and 50 mg/kg) could significantly improve the heart function, remarkably decrease infarct size from 20.87% to 14.87% ($p < 0.01$), decrease the levels of creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), alanine aminotransferase (ALT) and cardiac troponin T (cTnT) content in serum ($p < 0.05$). Meanwhile, pretreatment with PQDS (25 and 50 mg/kg) significantly increased the activities of superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px) in the heart, and decreased the level of malondiadehyde (MDA) in the myocardium ($p < 0.05$). Histopathological results demonstrated the same protective effect of PQDS. Pretreatment with PQDS (200 and 400 μg/ml) prior to H_2O_2 exposure could increase cell viability of neonatal rat cardiomycocytes. Pretreatment PQDS (200 and 400 μg/ml) also increased the activity of SOD, decreased level of LDH in the cultured supernatant and the MDA level in cardiomyocytes. These results indicated that PQDS had a cardioprotective effect proven *in vivo* and *in vitro*. The mechanisms might be due to its scavenging lipid peroxidation products, increasing endogenous antioxidant defense enzymes.

1. Introduction

Although clinical care is improved, public awareness is raised and health innovations are widely used, acute myocardial infarction (AMI) remains a leading cause of death in developed countries (Aronow 2006; Whellan 2005). People who experienced AMI would be disability or die (Alla et al. 2007). The infarct size is an important determinant of the prognosis of myocardial infarction. So, reduction the infarction size would be the major therapeutical goal. Although modern drugs, such as angiotensin-converting enzyme inhibitors (ACEI), calcium channel blockers have been shown to have cardioprotective effect in both preclinical and clinical studies. In recent years, chinese medical herbs and their extracts have received great attention for effective synergy and few side-effects (An and Yang 2006; Wu et al. 2007).

Panax quinquefolium L. (American ginseng) which is native to North America, was introduced into China several years ago. Now, *Panax quinquefolium L.* is used as a traditional medicine in China and other countries for its various pharmacological activities, against stress, cancer, diabetes mellitus and as an immunostimulant (Li et al. 2006; Nishijo et al. 2004; Shin et al. 2002; Wang and Lee 2000). Numerous studies showed that ginsenosides were the principal components responsible for the pharmacological activities of ginseng. Ginsenosides were generally divided into three types based on the chemical structure of their aglycones, the protopanaxadiol, the protopanaxatriol and the oleanoic acid ginsensides. *Panax quinquefolium L.* 20(*S*)-protopanaxadiol saponins (PQDS) were extracted from the stems and leaf of *Panax quinquefolium*. PQDS contained ginsenoside Rd, Rb2, Rb3, Rc, Rg3, pF11 and so on (Beveridge et al. 2002).

Our laboratory previously showed that PQDS could reduce the infarct size in canine models with coronary occlusion (Sui et al. 2001). However the mechanism responsible for the infarct size limiting effect of PQDS is unknown so far. The aim of this study was to explore the cardioprotective effects of PQDS on animal model of myocardial infarction *in vivo* and in cultured cardiomyocytes incubated with H_2O_2 *in vitro*, as well as to investigate the mechanisms involved in the protective effect of PQDS.

2. Investigations and results

2.1. Effect of PQDS on electrocardiograph parameters and survival rate

As shown in Fig. 1, the ST segment was markedly increased in rats of the AMI group compared with the rats of the

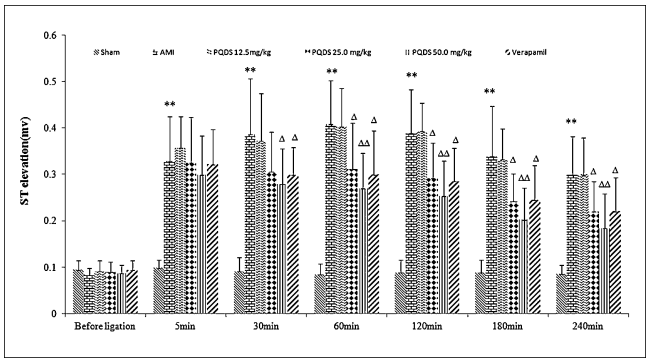

Fig. 1: Effects of PQDS on $\sum$-ST in rats. Treatment with PQDS significantly attenuated the elevation in ST segments with the passage of time in 240 min. Values were expressed as mean ± S.D. Significance were determined by ANOVA followed by Dunnett's test. $^{**}P<0.01$ vs sham group; $^{\Delta}P<0.05$, $^{\Delta\Delta}P<0.01$ vs AMI group

sham group. Pretreatment with PQDS (25 and 50 mg/kg) significantly attenuated the elevation in ST segment compared with AMI group ($P<0.05$, or $P<0.01$). The survival rates at the end of the observation period in the sham group, AMI group, PQDS groups(12.5, 25 and 50 mg/kg) and verapamil group were 100%(20/20), 60%(12/20), 60%(12/20), 70%(14/20), 80%(16/20) and 70%(14/20), respectively. Rats in PQDS groups had higher survival rates than rats in the AMI group, but the difference was not statistically significant.

2.2. Effect of PQDS on cardiac function and infarct size

Compared with rats in the sham group, the systolic function (LVSP and LV + dp/dt max) was decreased and the diastolic function (LVEDP and LV –dp/dt min) was compromised in rats of the AMI group. Pretreatment with PQDS (25 and 50 mg/kg) could ameliorate the changes of systolic and diastolic function compared with the AMI group ($P<0.05$, or $P<0.01$) (Table 1). Based on the examination of NBT hearts, a typical myocardial ischaemic zone was observed in AMI rats, white for ischemic myocardium and dark red for non-ischemic myocardium (Fig. 2). The infarct size presented as a ratio of the weight of ischemic zone over ventricular mass being 20.87 ± 3.95% in the AMI rats. Pretreatment with PQDS (12.5, 25 and 50 mg/kg) resulted in dose-dependent reduction in the infarct size, 17.97 ± 3.15, 16.39 ± 4.00 and 14.87 ± 3.71%, respectively. There were significant differences between the AMI rats and rats treated with PQDS (25 and 50 mg/kg) (Fig. 3).

2.3. Effect of PQDS on the levels of CK-MB, ALT, LDH and cTnT in serum

Compared with the rats in the sham group, the activities of all myocardial enzymes CK-MB, ALT, LDH and the cTnT content

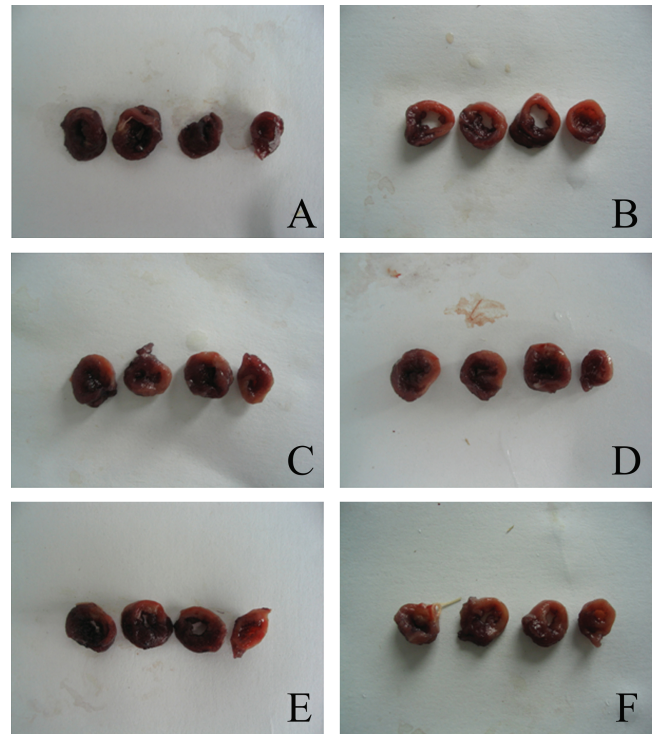

Fig. 2: The representative heart morphological photographs of PQDS on infarct zone in rats stained by NBT. The tissues of ventricles were excised and were cut into transverse slices and incubated for 15 min in 0.025% nitrotetrazolium blue chloride (NBT). White color means ischemic myocardium and dark red color means non-ischemic myocardium. A: Sham group; B: AMI group; C: PQDS 12.5 mg/kg; D: PQDS 25 mg/kg; E: PQDS 50 mg/kg; F: Verapamil 5 mg/kg

were increased in AMI rats. Pretreatment with PQDS (25 and 50 mg/kg) could inhibit the AMI-induced the increase in CK-MB, ALT, LDH and the cTnT content compared with AMI group ($P<0.05$, or $P<0.01$) (Table 2). PQDS had a dose-dependent effect because the high dose of PQDS showed a more significant inhibition than the low dose.

2.4. Effect of PQDS on the levels of catalase, GSH-Px, SOD and MDA in heart homogenate

As shown in Fig. 4, the activities of antioxidant enzyme catalase, GSH-Px and SOD were significantly decreased, while the content of MDA, an index of lipid peroxidation, was increased significantly in the AMI group compared with the sham group. Pretreatment with PQDS (12.5, 25 and 50 mg/kg) could increase the homogenate level of catalase (6.28 ± 2.38, 8.81 ± 2.37, 10.21 ± 2.51U/ml, Fig. 4A), and that of GSH-Px (67.6 ± 19.76, 73.70 ± 17.33, 96.7 ± 13.40U/ml, Fig. 4B). In addition, PQDS also increased the homogenate level of

Table 1: Effect of PQDS on hemodynamic parameters

Group	LVSP (mmHg)	LVEDP (mmHg)	LV + dp/dtmax (mmHg/s)	LV-dp/dtmax (mmHg/s)
Sham	130.7 ± 10.9	2.06 ± 0.59	5984 ± 1099	4321 ± 922
AMI	110.5 ± 8.7**	6.93 ± 1.06**	4399 ± 782**	3243 ± 648**
12.5 mg/kg	117.0 ± 7.6	6.12 ± 1.42	4567 ± 818	3548 ± 724
25.0 mg/kg	120.3 ± 11.2$^{\Delta}$	5.91 ± 0.93$^{\Delta}$	4900 ± 694	3810 ± 643
50.0 mg/kg	124.2 ± 7.3$^{\Delta\Delta}$	5.30 ± 0.75$^{\Delta\Delta}$	5220 ± 849$^{\Delta}$	4010 ± 704$^{\Delta}$
Verapamil	120.9 ± 11.0$^{\Delta}$	5.72 ± 1.24$^{\Delta}$	5161 ± 785$^{\Delta}$	3955 ± 741$^{\Delta}$

** $P<0.01$ vs. sham group
$^{\Delta}$ $P<0.05$, $^{\Delta\Delta}P<0.01$ vs AMI group

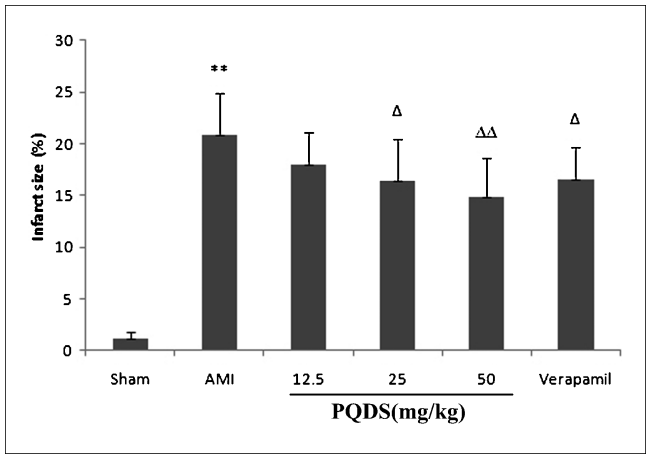

Fig. 3: Effects of PQDS on myocardial infarct size. The tissues of ventricles were excised and were cut into transverse slices and incubated for 15 min in 0.025% nitrotetrazolium blue chloride (NBT). White color means ischemic myocardium and dark red color means non-ischemic myocardium. The ischemic myocardium was cut and weighed. The infarct size as a percent of the ventricular mass was calculated as: weight of ischemia zone/total weight of ventricular mass × 100%. Myocardial infarct size is expressed as percentage of region of ventricle. Data are expressed mean ± S.D for each group. $^{**}P < 0.01$ vs sham group; $\Delta P < 0.05$, $\Delta\Delta P < 0.01$ vs AMI group

SOD (2.98 ± 0.57, 3.44 ± 0.88, 4.14 ± 0.93 U/ml, Fig. 4C), but decreased the homogenate level of MDA (10.37 ± 3.04, 9.35 ± 2.00, 8.75 ± 1.81 nmol/ml, Fig. 4D).

2.5. Histopathological examination of cardiac tissues

As shown in Fig. 5, the heart tissues in AMI group showed myocardial cell loss, widespread myocardial structure disorder, myocardium fragment, hyperaemia and leukocyte infiltration. Pretreatment with PQDS (25 and 50 mg/kg) significantly attenuated the pathophysiological changes in the cardiac muscle fiber. PQDS (12.5 mg/kg) had no effect.

2.6. Effect of PQDS against H_2O_2 induced injury in cultured cardiomyocyte of neonatal rat

Cardiomyocyte viability was decreased after exposure to H_2O_2. Pretreatment with PQDS (200 and 400 μg/ml) significantly increased the viability of H_2O_2-exposed cells (Fig. 6). In addition, the activity of SOD in the cardiomyocytes was decreased while the activity of LDH in the culture supernatant and the MDA level in cardiomyocytes were increased compared with normal culture. Pretreatment with PQDS significantly increased the activity of SOD, but decreased the level of LDH and MDA (Table 3).

3. Discussion

In the present study, we demonstrated that PQDS elicited a significant cardioprotective effect on the myocardial infarction induced by permanent ligation of the left anterior descending coronary artery *in vivo* and in cultured cardiomyocytes incubated with H_2O_2 *in vitro*.

Survival rates and infarct size are important parameters for evaluation the effectiveness of cardiovascular drugs in the treatment of ischemic heart disease (Sun et al. 2005). Probably due to a small sample size, we could not get a statistical significance in terms of survival rates. Rats treated with PQDS tended to have a longer survival rate after AMI. Meanwhile, PQDS (25 and 50 mg/kg) significantly decreased the infarct size of the left ventricle after AMI in rats (Fig. 3).

Electrocardiograph abnormalities are important criteria used for the diagnosis of myocardial ischemia and infarction (Zhou et al. 2008). ST segment elevation is observed in patients with myocardial ischemia. These change due to the consecutive loss of cell membrane in the injured myocardium. In the present study, we found a marked elevation of ST segment in AMI rats, but pretreatment with PQDS (25 and 50 mg/kg) markedly inhibited the elevation suggestive of its cell membrane protective effects (Fig. 1).

To better understand the protective effect of PQDS on cardial dysfunction, hemodynamic parameters were incorporated into the present experiment design. The present study demonstrated that PQDS significantly prevented the left ventricular systolic dysfunction by improvement LV + dp/dt max and LVSP, as well as left ventricular diastolic dysfunction by improvement LV-dp/dt min and LVEDP.

Injury due to myocardial ischemia occurs following inhibition of the aerobic oxidation of glucose, augmentation of anaerobic glycolysis and accumulation of lactic acid dehydrogenase. Meanwhile, reduction of ATP production, disruption of ionic gradients and degradation of membrane stability can lead to leakage of enzymes normally residing within cardiomyocytes. Consequently, the content of myocardial enzymes in blood serum increases, so changes in serum myocardial enzymes are considered to be a measure of impairment produced by myocardial ischemia (Mo et al. 2011). Our results showed a significant increase in the activities of CK-MB, ALT and LDH in AMI rats, which was in line with previous reports (Li et al. 2010; Yang et al. 2010). Preatment with PQDS (40 and 80 mg/kg) significantly lowered those marker enzymes. Recently, cTnT was considered more significant in diagnosis of AMI (Fishbein et al. 2003). In the present study, an increase of cTnT was observed in AMI rats. Pretreatment with PQDS (25 and 50 mg/kg) significantly decreased the content of cTnT in serum. These results suggest that the protective effects of PQDS against AMI may be produced by elevating cardiomyocyte membrane stability, to decrease the leakage of

Table 2: Effect of PQDS on the levels of creatine-MB(CK-MB), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and cardiac troponin T (cTnT) in serum

Group	Infarct size (%)	CK-MB (U/L)	ALT (U/L)	LDH (U/ml)	cTnT (ng/ml)
Sham	1.69 ± 0.79	619.7 ± 126.4	56.0 ± 11.9	491.3 ± 94.7	0.29 ± 0.09
AMI	$20.87 \pm 3.95^{**}$	$2944 \pm 768.8^{**}$	$412.9 \pm 129.7^{**}$	$3451.9 \pm 888.1^{**}$	$4.29 \pm 0.97^{**}$
12.5 mg/kg	17.97 ± 3.15	$2315.7 \pm 550.7^{\Delta}$	329.2 ± 76.2	2946.9 ± 619.3	3.79 ± 0.95
25.0 mg/kg	$16.39 \pm 4.00^{\Delta}$	$2159.0 \pm 481.7^{\Delta}$	$289.8 \pm 79.2^{\Delta}$	$2424.4 \pm 947.5^{\Delta}$	$3.31 \pm 0.80^{\Delta}$
50.0 mg/kg	$15.57 \pm 3.33^{\Delta\Delta}$	$1975.7 \pm 458.1^{\Delta\Delta}$	$218.3 \pm 80.9^{\Delta\Delta}$	$1986.7 \pm 658.9^{\Delta\Delta}$	$2.67 \pm 0.84^{\Delta\Delta}$
Verapamil	$16.47 \pm 3.23^{\Delta}$	$2162.8 \pm 370.2^{\Delta\Delta}$	$275.1 \pm 78.8^{\Delta}$	$2302.3 \pm 679.0^{\Delta\Delta}$	$3.12 \pm 0.86^{\Delta}$

** $P < 0.01$ vs. sham group

$^{\Delta}$ $P < 0.05$, $\Delta\Delta P < 0.01$ vs AMI group

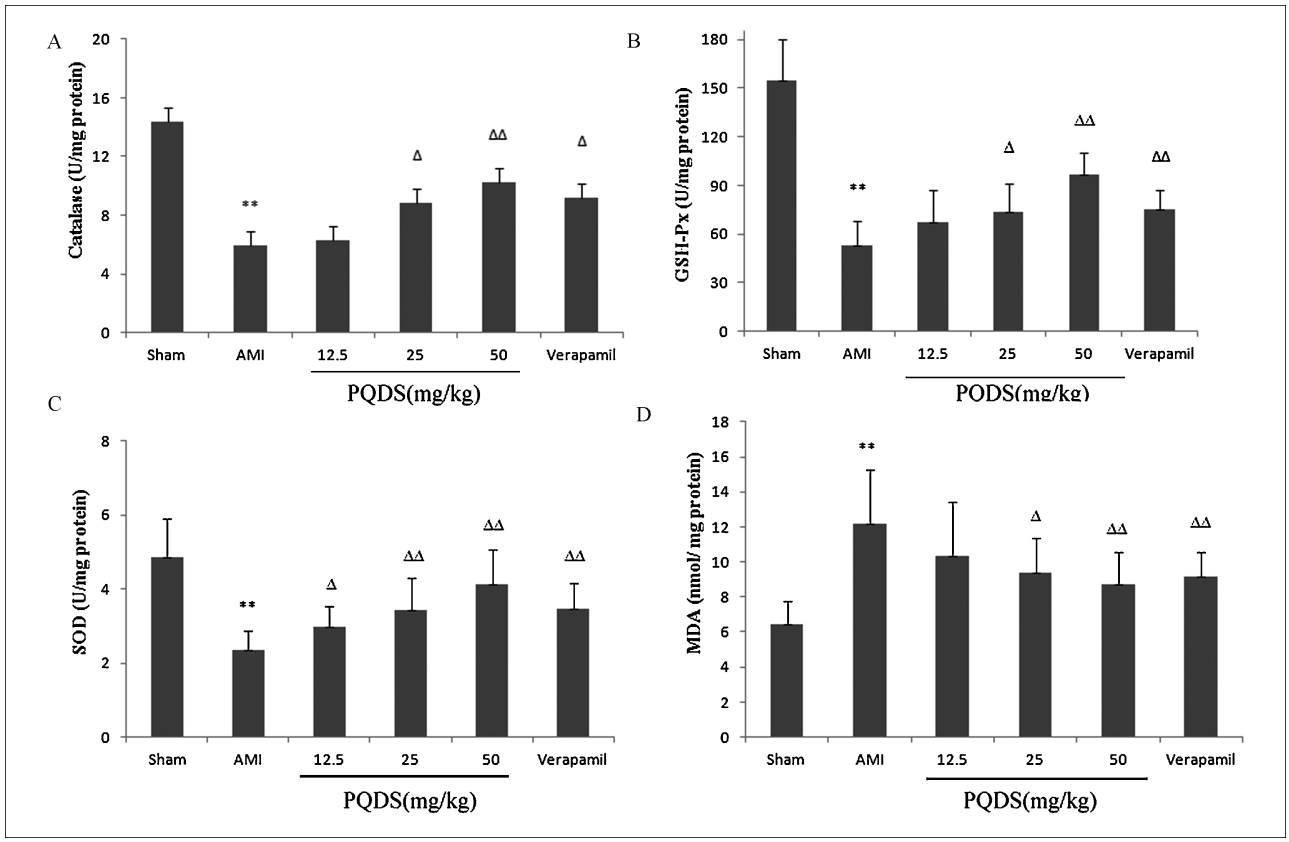

Fig. 4: Effects of PQDS on the levels of catalase (A), glutathione peroxidase (GSH-Px)(B), superoxide dismutase (SOD) (C), and malondialdehyde (MDA) (D) in heart homogenate. The tissues of ventricles were excised and homogenized with ice-cold buffer. The homogenate was centrifuged and the supernatant was taken to measure the activities of superoxide dismutase (SOD), catalase(CAT) and glutathione peroxidase (GSH-Px) and the content of malondialdehyde (MDA)by using diagnostic kits. Data are expressed mean ± S.D for each group. $^{**}P < 0.01$ vs sham group; $\Delta P < 0.05$, $\Delta\Delta P < 0.01$ vs AMI group

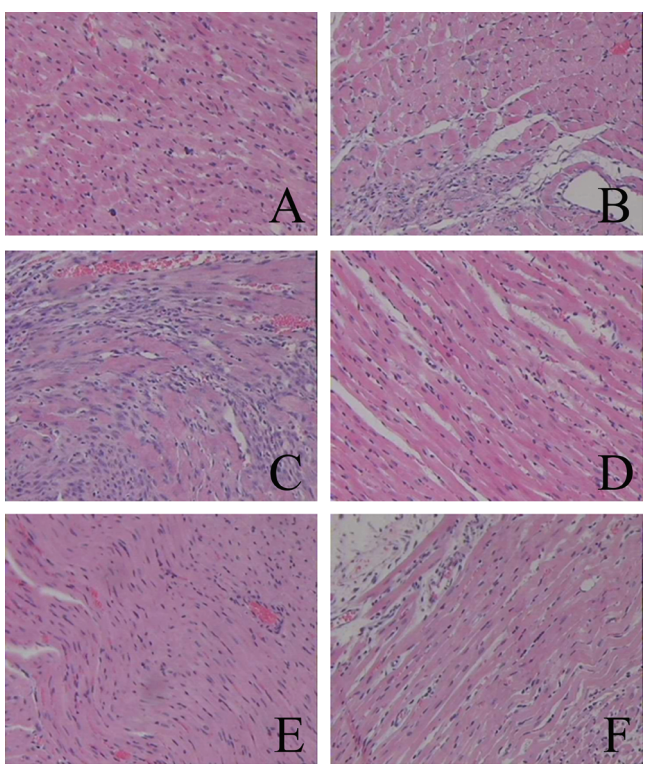

Fig. 5: Representative haematoxylin-eosin pathological photomicrographs of left ventricular tissue. The cardiac apex was excised and fixed with 10% formalin for subsequent haematoxylin-eosin (H&E) staining. The sections were examined under light microscope, and then photomicrographs were taken. Treatment with PQDS significantly attenuated the pathological changes in the cardiac muscle fiber. A: Sham group; B: AMI group; C: PQDS 12.5 mg/kg; D: PQDS 25 mg/kg; E: PQDS 50 mg/kg; F: Verapamil 5 mg/kg

enzymes and by reducing the activities of these enzymes. In a sense, our results suggested that PQDS offered protection to the myocardium by abating ventricular dysfunction through maintaining the ECG-patterns, cardiac marker enzymes, and anti-oxidant enzymes.

Acute myocardial infarction could induce severe oxidative stress (Ji et al. 2003). The increased generation of reactive oxygen species and/or the depletion of the antioxidants in the defense system may contribute to oxidative stress and affect the pathogenesis of myocardial infarction (Sawyer et al. 2002). Free radical scavenging enzymes such as superoxide dismutase, catalase and glutathione peroxides are the first line cellular defense against oxidative stress, eliminating reactive oxygen radicals.

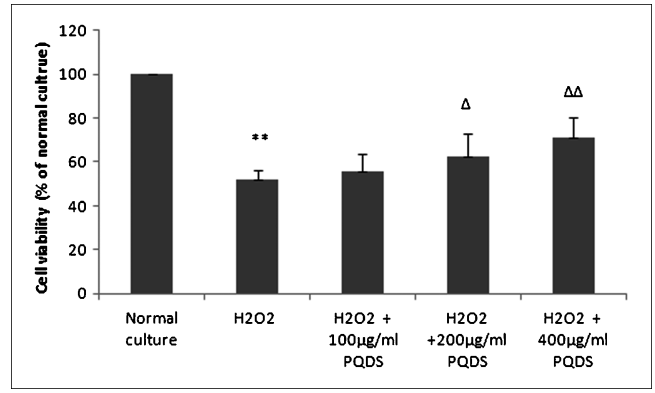

Fig. 6: Effect of PQDS on cardiomyocytes viability determined by MTT assay. The cardiomyocytes were pretreated with PQDS (100, 200 and 400 μg/ml) for 24 h, and then treated with H_2O_2. Cell viability was assessed by MTT assay. Data are expressed mean ± S.D for each group. $^{**}P < 0.01$ vs normal culture; $\Delta P < 0.05$, $\Delta\Delta P < 0.01$ vs H_2O_2 group

Table 3: Effect of PQDS against H_2O_2 induced injury in cultured cardiomyocytes of neonatal rats

Group	LDH (U/L)	SOD (U/mg protein)	MDA (nmol/mg protein)
Normal culture	1117.8 ± 105.1	83.0 ± 5.59	12.5 ± 3.94
H_2O_2(100 μM)	1942.2 ± 197.1**	51.8 ± 6.01**	27.2 ± 5.95**
H_2O_2(100 μM) + PQDS(100 μg/ml)	1829.2 ± 218.7	$59.2 \pm 4.84^{\Delta}$	22.5 ± 4.50
H_2O_2(100 μM) + PQDS(200 μg/ml)	$1599.8 \pm 91.8^{\Delta\Delta}$	$63.2 \pm 6.05^{\Delta\Delta}$	$19.2 \pm 4.71^{\Delta}$
H_2O_2(100 μM) + PQDS(400 μg/ml)	$1470.3 \pm 93.8^{\Delta\Delta}$	$67.3 \pm 5.32^{\Delta\Delta}$	$15.2 \pm 3.06^{\Delta\Delta}$

** $P < 0.01$ vs. normal culture group
$^{\Delta}$ $P < 0.05$, $\Delta\Delta P < 0.01$ vs H_2O_2(100 μM) group

Malondialdehyde is a major lipid peroxidant end product, and increased malondialdehyde content indicates activation of lipid peroxidative process. The results presented in this study demonstrated that PQDS pretreatment could reduce malondialdehyde content elevation and increase the activities of superoxide dismutase, catalase and glutathione peroxides in myocardial infarcted rats. These findings suggest that PQDS could considerably improve cellular antioxidative defense against oxidative stress.

In summary, PQDS could provide significant cardiopretective effects against AMI injury in rats. The mechanisms might be attributed to increasing endogenous antioxidant defense enzymes and scavenging lipid peroxidation products. These results indicated that PQDS might be effective in preventing and treating AMI and other cardiovascular diseases.

4. Experimental

4.1. Chemicals and reagents

Malondialdehyde (MDA), superoxide dismutase (SOD), creatine kinase-MB (CK), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), catalase and glutathione peroxidase (GSH-Px) assay kits were purchased from Nanjing Jiancheng Biotechnology (Najing, china). Cardiac troponin T assay kit was purchased from Wuhan USCN sciences Co. Ltd (Wuhan, China). The PQDS were obtained from Prof. Yanping Chen. PQDS was dissolved in physical saline for use. Other chemicals and reagents were obtained from local vendors in the highest available quality.

4.2. Animals treatment

Male and female Sprague Dawley rats (60 of each gender), 250 ± 20 g, were obtained from Experimental Animal Center of Jilin University, were housed in conventional cages with free access to water and rodent chow for 7 days before ligation. Experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals of Jilin University, and approved by the ethics committee. Those rats were randomly assigned to six groups: (1) sham group (n = 20), rats were given physical saline by intraperitoneal injection at a dose of 2 ml/kg; (2) AMI group (n = 20), rats were given physical saline by intraperitoneal injection at a dose of 2 ml/kg; (3) PQDS 12.5 mg/kg group (n = 20), rats were given PQDS by intraperitoneal injection at a dose of 12.5 mg/kg; (4) PQDS 25 mg/kg group (n = 20), rats were given PQDS by intraperitoneal injection at a dose of 25 mg/kg; (5) PQDS 50 mg/kg group (n = 20), rats were given PQDS by intraperitoneal injection at a dose of 50 mg/kg; (6) verapamil group (n = 20), rats were given verapamil by intraperitoneal injection at a dose of 5 mg/kg. The compounds were administesed once a day for 7 days continually. Thirty minutes after the last dose, the rats were anesthetized for acute myocardial infarction.

4.3. Surgical preparation

The acute myocardial infarction animal model was established by ligation of the left anterior descending (LAD) coronary artery, as described previously (Yang et al. 2010; Zhou et al. 2012). Briefly, rats were anaesthetized with urethane (1.0 g/kg, i.p.), and artificially ventilated using a volume-regulated respirator. The thorax was opened, the heart was exteriorized, and the left coronary artery was ligated 2–3 mm between the pulmonary artery conus and left atrium using a 6–0 Prolene suture. The heart was returned and the thorax was closed. Sham group underwent the same surgical procedure without LAD ligation. The hearts were collected at the end of a 4 h ischemic period.

4.4. Measurement of electrocardiograph and hemodynamic parameters

An electrocardiogram was recorded before and 5, 30, 60, 120, 180 and 240 min after coronary ligation. Lead II ECG was monitored and ST-segment elevation was taken into consideration.

Four hours after ligation, the rats were anesthetized with urethane (1 g/kg, ip). To evaluate the cardiac left ventricular function, a catheter filled with heparin saline was inserted into the left ventricle, and left ventricular systolic pressure (LVSP), the left ventricular end diastolic pressure (LVEDP), the maximal rate of left ventricle systolic pressure (LV + dp/dt max) and the minimum rate of left end diastolic pressure (LV-dp/dt min) were recorded by BL-420E + Biologic Function Experiment System. After measuring hemodynamic parameters, blood samples were collected from the abdominal aorta and the hearts were also removed.

4.5. Determination of the serum enzymes

The collected blood samples were clotted for 2 h at room temperature, and centrifuged at 2500 rpm for 15 min. The supernatant serums were separated and stored at $-80\,^{\circ}$C. The activities of creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), alanine aminotransferase (ALT) and cardiac troponin T (cTnT) were measured using diagnostic kits according to the manufacturer's instructions.

4.6. Determination of infarct size

After measuring hemodynamic parameters, the hearts were removed quickly. Hearts in each group were selected. The determination of infarct size was performed according to the method of Ji et al. (2003) with minor modifications. The tissues of ventricles were cut into 4 transverse slices and the slices were incubated in 0.025% nitrotetrazolium blue chloride (NBT) for 15 min. Myocardial infarction was distinguished by the different color tone (white for ischemic myocardium and dark red for non-ischemic myocardium). The ischemic myocardium was cut and weighed. The infarct size as a percent of the ventricular mass was calculated as: weight of ischemia zone / total weight of ventricular mass × 100%.

4.7. Biochemical analysis

The tissues of ventricles were excised and homogenized with ice-cold buffer in homogenizer. The homogenate was centrifuged and the supernatant was taken to measure the activities of SOD, CAT and GSH-Px and the content of MDA by using diagnostic kits according to the manufacturer's instructions.

4.8. Histopathological examination

The hearts were excised and fixed with 10% formalin for subsequent haematoxylin-osin (H&E) staining. The sections were examined under light microscope, and then photomicrographs were taken.

4.9. In vitro study

Neonatal rat cardiomyocytes were cultured as described previously (Kessler-Icekson et al. 1984; Suzuki et al. 1997). For subsequent experiments, cells at 3-day to 5-day culture were used. To approach the protection of PQDS, the cardiomyocytes were pretreated with PQDS (100, 200 and 400 μg/ml) for 24 h, and then treated with H_2O_2. Cell viability was assessed by the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. After exposure of H_2O_2, the MTT solution (5 mg/ml) was added for 4 h. The formed formazan blue crystals remaining in the cells were dissolved in 150 μl DMSO for 10 min. The absorbance at 570 nm was measured spectrophotometrically. The level of LDH in the cultured supernatant, the activity of SOD and the content of MDA in cardiomyoctyes were measured using diagnostic kits according to manufacturer's instruction.

4.10. Statistical analysis

All data were reported as mean ± S.D. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Dunnett's test. $P < 0.05$ was considered statistical significant.

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