

Paeoniflorin Attenuates Myocardial Ischemia / Reperfusion Injury via Up-Regulation of Notch 1 – Mediated Jagged1 Signaling

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ABSTRACT

Ischemic heart disease represents one of the major reasons of death worldwide. Methods and materials: A total number of 48 mice were assigned to one of the following 4 experimental groups (n = 8 in each group); sham; control I/R; I/R + vehicle group and I/R + Paeoniflorin pretreated group treated with (5 mg/kg intraperitoneal dose of PF) 30 min before ischemia and underwent 30 minute of LAD ligation followed by 72 hours reperfusion. After analysis of cardiac function, the heart tissue and blood were collected and prepared for analysis. Results: The % FS was significantly higher at 3 days after surgery in Paeoniflorin treated mice than I/R control and vehicle treated mice. Paeoniflorin treated mice had lower levels of MCP-1 in myocardial tissue and plasma $P < 0.05$. Furthermore, plasma myocardial injury marker (cTn-I) level is associated with over expression of pro-inflammatory mediators after I/R. Over expression of inflammatory

mediators following I/R suppress LV function; Notch-1 and Jagged-1 protein are down-regulated following I/R; and the administration of Paeoniflorin attenuated chemokine and cytokines through up-regulation of Notch-1 and Jagged-1 activation signaling pathway leading to improved left ventricular function

Keywords: Paeoniflorin, myocardial ischemia/reperfusion, Notch1, Jagged1

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INTRODUCTION

Ischemic heart disease represents one of the major reasons of death worldwide. Early blood restoration to the ischemic area considers the most efficient method for the treatment of patients having ischemic heart disease [1]. Ischemic phase for a long time can result in irreversible myocardial injury. Furthermore, the condition does not recuperate but deteriorate after myocardial reperfusion, causing electro physiologic, metabolic, and ultra-structural myocardial damage [2], and the exact mechanisms of IRI are not completely known [3], but the cellular, and tissue alterations like cell death, inflammation, neuro humoral activation, and oxidative stress are regarded to be important for IRI development [4]. Cytokines synthesized by many cell types, but the major producers are macrophages and helper T cells (Th). Cytokine represents a general name; there are other names include monokine (cytokines produced by monocyte), lymphocyte (cytokines produced by lymphocytes), and interleukin (cytokines produced by one leukocyte and acting on other leukocytes) and chemokine (cytokines with chemotactic activities). Cytokines may have autocrine action that means they can act on the cells that secreted by, on nearby cells (paracrine action), or in some situations on distant cells [5]. A kind of cytokines are famous to induce chemo taxis which is a subgroup of structurally related cytokines and known as chemokine. The description chemotactic cytokines usually ascribe to this. These agents indicate a family of low-molecular weight secreted proteins that possess a variety of functions but mainly function in the migration and activation of leukocytes. Chemokine have preserved cysteine residues that permit them to be classified to four groups: C-C

chemokine (monocyte chemoattractant protein or MCP-1, monocyte inflammatory protein or MIP-1 α , and MIP-1 β), C-X-C chemokine (IL-8 also called growth related Oncogene or GRO/KC), C chemokine (lymphotactin), and CXXXC chemokine (fractalkine) [6]. In general, the CC chemokine are strong attractants for mononuclear cells, whereas CXC chemokine are strong Neutrophil chemoattractant [7]. Lately, an increased inflammatory response was recognized as being necessary for the pathophysiology of AMI [7]. Notch indicates a trans-membrane receptor encoded by a gene originally recognized as that responsible for the appearance of a 'notch' in the *Drosophila melanogaster* wings. In the mammalian, there are four Notch receptors, 1-4, and five ligands include jagged 1-2 and Delta-like ligand (DLL) 1, 3 and 4, that have been identified as membrane-bridging proteins. When ligand binding occurs, the surface metalloprotease ADAM10 cuts off the Notch extracellular domain just outside the plasma membrane and liberates an extracellular Notch fraction which stays bound to its ligand until endocytosis by the ligand-bearing cell, that is then undergoes signaling. After wards, the Notch intracellular domain (NICD) cleaved by the inner membrane protease γ -secretase, where NICD represents the active form of Notch that released in the cytoplasm moves to the nucleus where it binds to CSL transcription factors that regulates Notch target gene expression [8]. The expanding interest in Notch pathway as a key regulator of cell differentiation and function has been equaled by the evolution of suitable tools and methods for its investigation in animal and cellular patterns, as comprehensively reported in a recent review [9]. Paeoniflorin is a chemical compound represents one of

the major components of an herbal medicine extracted from *Paeonia lactiflora* [10]. It can also be drawn from the freshwater fern *Salvinia molesta* [11]. It's a monoterpenic glycoside has diverse cellular actions on TRPV1 and NMDA receptors [12]. It also decreases inflammatory signaling by inhibition of p38 MAP kinase and blocks apoptosis of pancreatic cancer cell by arresting MMP-9 and ERK signaling [13]. Moreover, Paeoniflorin inhibits intracellular calcium overload, improves cerebral vasomotor dysfunction induced by ischemia and anoxia and protects against free radicals' effect so it has been shown a significant protective effect in focal cerebral ischemic injury. Furthermore, Paeoniflorin has been indicated for the blood-brain barrier protection after cerebral perfusion during ischemia and enhances the return of cerebral blood flow in the early period of reperfusion. SOD levels increased significantly by Paeoniflorin in rat brain tissue, also decreases malondialdehyde (MDA) levels, and weaken oxidative stress injury in brain tissue resulted from cerebral ischemia [14]. Other studies have reportedly explained that Paeoniflorin may markedly decrease the expression levels of nuclear factor (NF)- κ B [15]. With increasing Paeoniflorin concentrations, NF- κ B and B-cell lymphoma-2 (Bcl-2) expression levels reduced progressively [16]. The current study aimed to study the possible beneficial role of in MIRI through up regulation of Notch 1 mediated Jagged 1 pathway in male mice.

METHODS

Animals and their preparation

Adult (4 - 6 months) male Albino-Webster mice and their weights ranged from 25 to 38 gram obtained from the College of Science, Babylon University. Mice were acclimated for 14 days in a 12:12-hours light-dark cycle with free access to water and regular chow diet before the experiments in animal house of Kufa University and this investigation conforms to the Guide for the Care and Use of Laboratory Animals (National Research Council, revised 1996). The mice could acclimatize in plastic cages in a controlled temperature ($25^{\circ}\pm 1$ C) room the temperature within the cage was monitored and maintained near the thermo neutral zone for mice [17] with 60-65% humidity.

Method of left coronary artery ligation

Induction of myocardial ischemia and reperfusion was performed as described previously [14]. In brief, mice (27 to 38 gm body weight) were anesthetized by intraperitoneal injection with a mixture of ketamine (100 mg/kg) and xylazine 5 mg/kg [5]. Animals were intubated with a 20-gauge polyethylene catheter and were ventilated with a rodent ventilator (Harvard Apparatus). A median sternotomy was performed, the left anterior descending artery was identified anatomically, and a 8-0 silk suture size was passed around the artery and subsequently tied off for about 30 minutes. Infarction was evident from discoloration of the left ventricle (LV). Finally, the chest wall was closed. The animals remained in a supervised setting until fully conscious.

Experimental Protocol

A total number of 48 mice were assigned to one of the following 4 experimental groups (n = 8 in each group):

Sham group a total of eight mice underwent a sham surgery, in which mice were anesthetized and involved the identical surgical procedure without the coronary artery ligation.

Control I/R underwent 30 minute of LAD ligation followed by 72 hours of reperfusion.

I/R + vehicle group received normal saline (the vehicle) and underwent 30 minute of LAD ligation then 72 hours reperfusion. I/R + Paeoniflorin pretreated group treated with (5 mg/kg intraperitoneal dose of PF) 30 min before ischemia and underwent 30 minute of LAD ligation followed by 72 hours reperfusion.

All treatments were performed in the morning and followed for survival for three days as mentioned above. After analysis of cardiac function, the heart tissue and blood were collected and prepared for analysis.

Echocardiography

Transthoracic echocardiography was performed with an FFsonic 8900 (Fukuda Denshi-Japan) with a 10-MHz phased-array transducer at 72h after I/R. The mouse is injected intraperitoneally with ketamine (100 mg/kg). Heart rates are monitored and generally maintained at 400–500 beats per minute and the chest hair is shaved. ECG needle leads are connected to the limbs for electrocardiogram gating. The mouse is then placed on a warm pad to keep the body temperature around 37°C. Warmed echo gel is placed on the shaved chest. The mouse heart is imaged with a 10 MHz linear transducer LV internal dimensions at end systole and end diastole (LVESD and LVEDD) were measured digitally on the M-mode tracings and averaged from 3 cardiac cycles. LV fractional shortening (%FS) was calculated as [(LVEDD-LVESD)/LVEDD] \times 100.

Collection of samples

The blood was drawn using direct needle puncture of the heart. For plasma collection heparin was used as anticoagulant, the samples were stored at 4°C, centrifuged at $4700 \times g$ for 10 min at 4°C. The plasma stored at -20°C until used for further analyses. For cardiac tissue specimens, mice were anaesthetized with ketamine (100 mg/kg), and then killed by injection of 10 % KCl to stop the heart at diastole. The heart was excised and weighed and sectioned transversely into two parts from atrial-ventricular junction. The upper parts (atrial) were rapidly frozen and used for morphometric analysis of infiltration of inflammatory cells by ELISA and lower parts (ventricular) for histological examination through staining with hematoxylin and eosin (H&E).

Assessment of inflammatory cell infiltration into the infarct area, histological sections of the infarcted area was evaluated to assess the degree of accumulated polymorph nuclear Neutrophils and macrophages, because it has been reported that these are important sources for chemokine and cytokine induction or production. The cardiac tissue samples were fixed in 4% paraformaldehyde for 24 h, as described previously [18]. Briefly, cardiac tissue sections (slices) 5 μ m in thickness were paraffin embedded according to the standard procedure. The degree of heart

damage was analyzed by hematoxylin and eosin (H&E) stain, and photographs were obtained from each heart section (n = 3 sections per heart) under optical microscopy. To semi-quantify the difference in cardiac damage, stained histological sections were examined and scored according to the protocol of Zingarelli [19] was used. According to this scoring protocol the following criteria were used: score (0), normal tissue; score (1) mild, interstitial edema and focal necrosis; score (2) moderate, myocardial cell swelling, diffused necrosis; score (3) severe, the presence of ischemia, Neutrophil accumulation; and score (4) highly severe, the presence of contraction bands, leukocyte infiltrate, ischemia, and hemorrhage.

ELISA

The freeze parts of myocardial tissue treated in PBS containing 0.5% Triton X100 with a protease inhibitor cocktail, tissue was homogenized and the supernatant used to quantify the chemokine and cytokines (MCP-1, TNF- α , IL-1 β , and IL-6) in both plasma and myocardial

tissue according to instructions of commercial ELISA kits (Boster, CA), in addition to the plasma cardiac Troponin-I (cTn-I) according to the instruction of commercial ELISA kits (Cloud & Clone, USA). The spectrophotometer of micro plate reader (Bio-Rad Laboratories, USA) was used to determine the absorbance of standards and samples at 450 nm. All obtained data were plotted against the linear portion of a standard curve [20].

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from tissues and cells using the RNA pure Extraction Kit (BioTeke Corp., Beijing, China), according to the manufacturer's instructions. RNA sample concentration was measured by UV spectrophotometer and RNAs were reverse-transcribed using M-MLV Reverse Transcriptase (BioTeke Corp.). Primers were synthesized by Sangon Biotech Co., Ltd., (Shanghai, China) and the primer sequences were as table 2.8.

Table (1): Primer sequences of the different genes with their respective product sizes.

Gene	Primer Sequences	Size of PCR Product (bp)
β -actin	F: 5'-TGGCACCACACCTTCTACAATGAGC-3'	437
	R: 5'-GCACAGCTTCTCCTTAATGTCACGC-3'	
Notch-1	F: 5'-CCGCCTTTGTGCTTCTGTT-3'	490
	R: 5'-TCCTCCTCTTCCTCGCTGTT-3'	
Jagged-1	F: 5'-GATCCTGTCCATGCAGAACG-3'	436

Statistical Analysis

Statistics were performed with the SPSS statistics program (windows version 9.0). To evaluate whether observed differences were significant, paired or non-paired t tests were used when appropriate. A p value (two sided) of less than 0.05 was significant.

RESULTS

Paeoniflorin improved cardiac function following I/R. We determined whether Paeoniflorin improved cardiac function in mice following I/R by using Echo. The measurements of LVEDD, LVESD, and %FS were similar between I/R control and vehicle treated mice. Furthermore, LVEDDs (mm) was 4.020 (P<0.05) in

Paeoniflorin treated mice, While, LVESDs (mm) was 3.020.11 (P>0.05) in Paeoniflorin treated mice. Moreover, %FS was significantly higher at 3 days after surgery in Paeoniflorin treated mice than I/R control and vehicle treated mice, as shown in table 2 (27.9 \pm 1.5% Paeoniflorin, versus 21.2 \pm 2.6% I/R control, 21.5 \pm 2.7% vehicle treated mice P<0.05. Table (2): Echocardiography measures were obtained from a short-axis view at the level of the papillary muscle. Control I/R and vehicle mice displayed significantly reduced left ventricle (LV) function, including LVESDs, LVEDDs (mm), ejection fraction and cardiac output, compared with sham mice. Treatment with Paeoniflorin improved LV function.

Echo Measures	Sham	Cardiac ischemia/reperfusion		
		Control	Vehicle	Paeoniflorin
Heart rate, beats/min	538 \pm 6	459 \pm 3	447 \pm 5	502 \pm 2
LVESDs (mm)	4.5 \pm 0.02	1.6 \pm 0.05*	1.6 \pm 0.14*	3.020.11*#
LVEDDs (mm)	5.1 \pm 0.03	1.9 \pm 0.11*	2.0 \pm 0.01*	4.020.12*#
Ejection fraction, %	63.5 \pm 1.1	24.3 \pm 1.8*	24.2 \pm 1.2*	50.1 \pm 3.1*#
FS %	39 \pm 1	21.2 \pm 2.6%*	21.5 \pm 2.7%*	27.0 \pm 1.5%*#
Cardiac output, ml/min	5.8 \pm 0.3	2.8 \pm 0.5*	2.8 \pm 0.3*	4.0 \pm 0.4*#

The above data are expressed as mean \pm standard error, n = 8 in each group; *P <0.05 versus corresponding sham;

#P <0.05 versus control I/R and vehicle treatment; ¥P>0.05 versus Paeoniflorin treated mice. Our previous

lab study [21] showed that the pro-inflammatory chemokine (MCP-1) elevated after I/R and plays a substantial role in the accumulation of monocyte to the injured myocardium. In our study, we investigated the effects of Paeoniflorin in neutralization of MCP-1 and subsequently, reduced the infiltration of accumulation of monocyte and macrophages in myocardium following I/R.

In comparison to sham greater level of MCP-1 expression found in both control I/R and vehicle treated mice group moreover; following 72hs of reperfusion the levels of MCP-1 expressing in both plasma and myocardium markedly lower in treated mice with Paeoniflorin (Figure 1.)

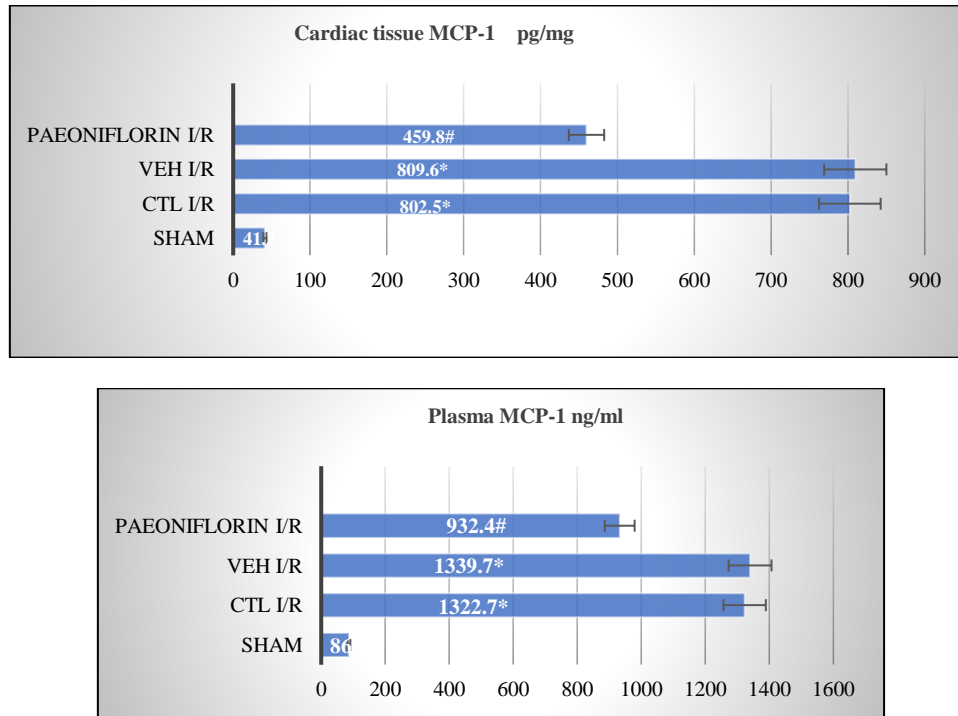
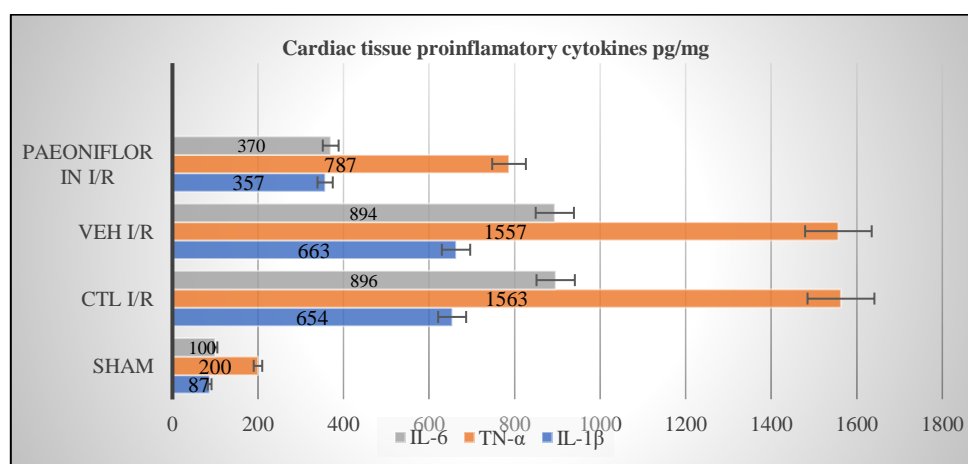


Figure (1): Level of MCP-1 was analyzed by ELISA 72 hrs after reperfusion.

Paeoniflorin treated mice had lower levels of MCP-1 in myocardial tissue and plasma as in figure 1. Data are expressed as mean ± standard error, n = 8 in each group; *P <0.05 versus corresponding sham; #P <0.05 versus control I/R and vehicle treatment. We next investigated the importance effects of Paeoniflorin on the cardiac tissue and systemic pro-inflammatory responses during I/R. At the end of the experiment (72 hrs. after

reperfusion), the levels of pro-inflammatory cytokines IL-1β, IL-6 and TNF-α in myocardial tissue and plasma are measured by ELISA according to manufacture protocol. Comparison with I/R and vehicle treated mice, Paeoniflorin treated mice exhibit greater reduction in the levels of pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) in both myocardium and plasma as in figure 2.



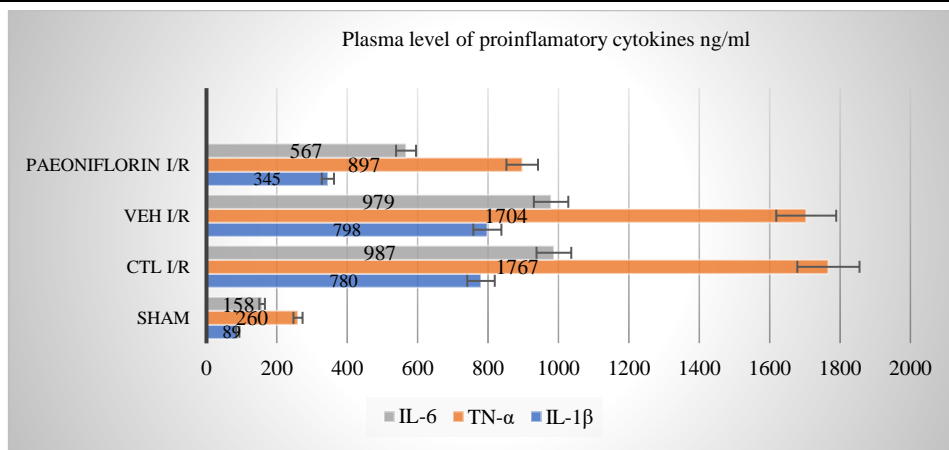


Figure (2): Paeoniflorin reduced the level expression of pro-inflammatory cytokines in both myocardium figure (A) and plasma figure (B).

Data are expressed as mean ± standard error, n = 8 in each group; *P <0.05 versus corresponding sham; #P <0.05 versus control I/R and vehicle treatment. Furthermore, plasma myocardial injury marker (cTn-I) level is associated with over expression of pro-inflammatory

mediators after I/R. The presented data show that the cTn-I is related with elevated of MCP-1 level after I/R corresponding with released of the content of monocyte causing more cardiac injury. Paeoniflorin have effort reduced cTn-I expression after I/R.

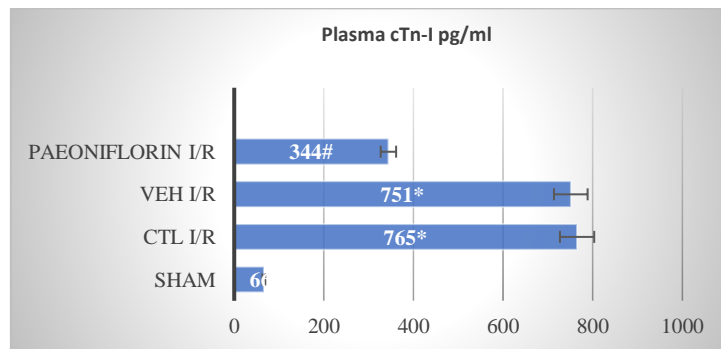
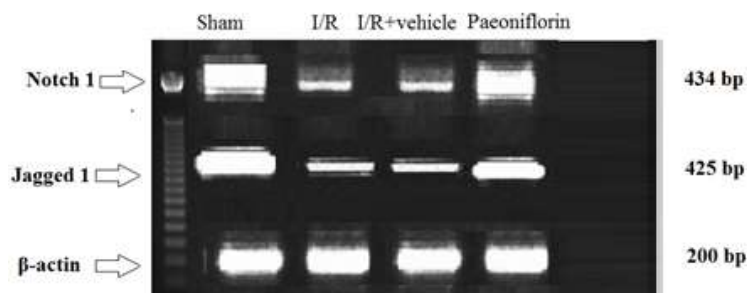


Figure (3): The mean of plasma cTn-I (pg/ml) in the five experimental groups.

Data are expressed as mean ± standard error, n = 8 in each group; *P <0.05 versus corresponding sham; #P <0.05 versus control I/R and vehicle treatment. After cardiac tissue homogenate, the total RNA was extracted, and qRT-PCR experiments were performed using primers recognizing Notch-1 and Jagged-1 while, the RT-PCR was normalized using 26S as manufacture protocol. The presented data showed that the Notch-1/Jagged-1 low expression in the injured myocardial cells after I/R

corresponding with high expression of inflammatory mediators (P<0.05) in control and vehicle I/R treated mice groups as in figure (4). Interestingly the Notch-1/Jagged-1 expression levels are up-regulation in both Paeoniflorin treated groups (P < 0.05) and this result showed in the first time the possible mechanistic pathway of Paeoniflorin in reduced myocardial injury following I/R through up-regulation of Notch-1/Jagged-1 signaling pathway.



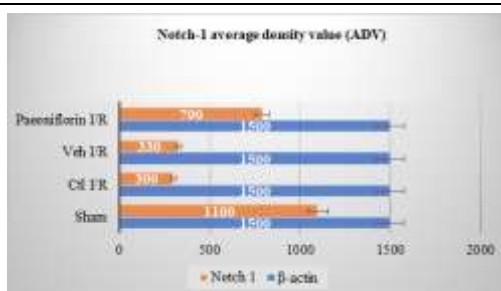


Figure (4): Gel electrophoresis of PCR products. Notch-1 = 434 bp DNA ladder; Jagged-1 = 425 bp; β-actin gene control= 200pb.

Total RNA was isolated from cardiac tissues and mRNA levels of Notch-1 and Jagged-1 were determined by reverse transcription-quantitative polymerase chain reaction. B-actin served as an internal control. Data are expressed as mean ± standard error, n = 8 in each group; *P <0.05 versus corresponding sham; #P <0.05 versus control I/R and vehicle treatment. Histological, myocardial tissue from I/R and vehicle mice after 72 hrs of reperfusion period (Figure 5) revealed a marked myocardial injury with the development of contraction bands and polymorph nuclear leukocytes (PMN) infiltration besides interstitial edema and localized extravasations of red blood cells. While the histological features of the Paeoniflorin treated mice showed mild

architectural alterations. To semi-quantify the difference in cardiac damage, histological sections from all groups were examined and scored according to the protocol of Zingarelli [19] was used. According to this score system the following criteria were used: score 0, no damage; score 1 (mild), interstitial edema and focal necrosis; score 2 (moderate), diffuse myocardial cell swelling; score 3 (severe), the presence of contraction bands and Neutrophil infiltrate; and score 4 (highly severe), the presence of contraction bands, leukocyte infiltrate, and hemorrhage. Eight animals in each group were included, and five sections from each animal were evaluated, figure 5.

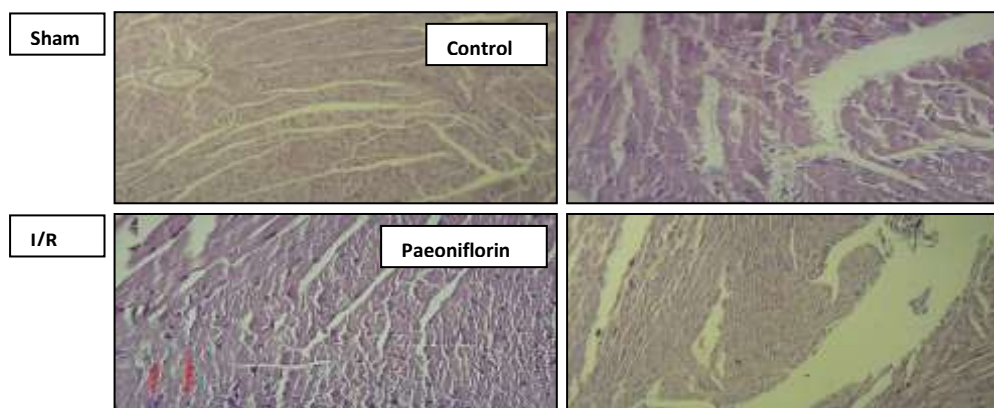


Figure (5): Ischaemia reperfusion in the myocardium

Heart tissues were embedded and cut into sections (5-µm thick). Myocardial ischemia was examined by H&E staining. Scale bar, 100 µm. Magnification: x40 and x100. Demonstrating extensive contraction band change

with margination of poly-morph nuclear leukocytes (PMN) in I/R control and vehicle treated mice. While the histological features of the Paeoniflorin treated mice showed mild architectural alterations



Figure (6): Zingarelli system indicate that the damage score was significantly reduced in Paeoniflorin and Liraglutide treated mice compared with the I/R mice after 72 hrs reperfusion

Data are expressed as mean \pm standard error, n = 8 in each group; *P <0.05 versus corresponding sham; #P <0.05 versus control I/R and vehicle treatment.

DISCUSSION

AMI is a major cause of mortality worldwide. Early and successful myocardial reperfusion with either thrombolytic agents or primary percutaneous coronary intervention is the most effective strategy to reduce infarct size and improve clinical outcome. However, the process of restoring blood flow to the ischemic myocardium can induce myocardial reperfusion injury, which can paradoxically reduce the beneficial effects of myocardial reperfusion. Thus, reperfusion itself may lead to accelerated and additional myocardial injury beyond that generated by ischemia alone [22]. The prompt return of blood to the heart is life-saving, however, reperfusion injury extends myocardial damage beyond that inflicted by the ischemia itself [23]. Although there are clearly multiple mechanisms of tissue damage from ischemia and reperfusion, the blood plays a major role in the inflammatory component of reperfusion injury. Pro-inflammatory mediators, stimulated by ischemia and reperfusion, activate PMNs and the coronary endothelium. Adhesion molecules are then expressed on the surfaces of both cell types, resulting in multiple cell-cell interactions. PMNs adhere to vascular endothelium and generate toxic free radicals, which induce micro vascular dysfunction and blood flow defects, contribute to apoptosis and ultimately extend the myocardial infarction [24]. In the present study, we examined whether Paeoniflorin may reduce myocardial injury following I/R in mice model and possible mechanistic signaling pathway to their pharmacological work. In comparison to sham group, there was significant increase in cardiac tissue and plasma levels of IL-1 β , IL-6 and TNF- α in both control I/R and vehicle I/R group in the present study. In this study, there was greater level of MCP-1 expression found in both control I/R and vehicle I/R treated mice groups in comparison to sham group as it was analyzed by ELISA for both plasma and homogenized cardiac tissue. Many experimental and clinical studies have demonstrated up-regulation of MCP-1 after MI, with recruitment of monocyte/macrophages to the ischemic myocardium [25] reported that an anti-MCP-1 gene therapy improved survival rate of mice as well as attenuated LV cavity dilatation and contractile dysfunction, interstitial fibrosis, recruitment of macrophages, myocardial gene expression of TNF- α , and transforming growth factor- β , while [26] showed a late increase in plasma MCP-1 levels in sub acute phase in AMI patients. In this present study, ELISA analyses for plasma showed that cardiac troponin I (cTn-I) increased obviously in control I/R and vehicle I/R treated groups in comparison to sham group. Several previous studies have shown that cardiac troponin have powerful predictive value for adverse cardiovascular events and death [27] clarified that myocardial damage causes disruption to the membrane integrity of the normal cardiac myocytes and loss of intracellular content into the extracellular space, so that, elevated levels of cytosol and structural proteins, such as cardiac troponin and CK-MB, can be detected in the blood. The measurements of LVEDD, LVESD, heart rate, ejection fraction, cardiac

output and %FS were nearly similar between control I/R and vehicle I/R treated mice and lower than that's appeared in sham group. The development of HF, following AMI, is associated with "ventricular remodeling", i.e., that's changes in cardiac structure and function. Heart rate increased from 450–500 bpm to ~800 bpm in the same mice before myocardial infarction [28] as well as after 8 wk of reperfusion, but in our present study we measure the heart rate after 72 hours of reperfusion show stabilized heart rate of sham group. In this study, the histological examination for myocardial tissue from control I/R and vehicle I/R treated groups after 72 hours of reperfusion period revealed a marked myocardial injury with the development of contraction bands and polymorph nuclear leukocytes (PMN) infiltration besides interstitial edema and localized extravasations of red blood cells indicated that the damage score was significantly increased in comparison to sham group. Other study found that the pathological changes more marked in the myocardial tissues from the rats in control group when compared with the sham group in the rat's model study, and these changes involved inflammatory cell infiltration, necrosis and atrophy of the myocardial fibers [28]. These findings support the present results regarding histopathological changes in the reperfused cardiac tissue and are in relevance with [17] who clarified that myocardial I/R injury showed a significant disruption of the myocardial tissue structure described by the presence of extensive necrosis demonstrated the presence of critical myocardial membrane injury, edema and inflammatory cells infiltrates to the ischemic reperfused cardiac tissue compared to sham group by using microscopic examination regarding histopathology [29]. The present study that showed the Notch-1/Jagged-1 had low expression in the injured myocardial cells after I/R corresponding with high expression of inflammatory mediators (P > 0.05) in both control I/R and vehicle I/R treated mice groups. Notch signaling can assist myocardial regeneration, saves the myocardium from ischemia, stimulates angiogenesis, and prevents cardiac fibroblast to my fibroblast transformation (CMT). Each of these events encourages cardiac repairing after myocardial injury [30], while others demonstrated that cardiac-specific Notch-1 knockdown resulted in significant aggravation of I/R injury, as proved by enlarged infarct size, decreased cardiac function, increased apoptotic evidence in the myocardium and cardiac fibrosis [31]. It clarified that the up-regulation of Notch-1 in the hypertrophic myocardium regulates the adaptive response of the heart to stress conditions, not only by restricting the extent of the hypertrophic response but also by contributing to cell survival in cardiomyocytes [32], and demonstrated that nearly all of the Notch receptors and ligands are found at varying levels in the injured myocytes during post-infarction remodeling, that indicates a regulatory role for Notch signaling in the functional recovery of the ischemic myocardium [33]. In comparison with control I/R and vehicle treated mice, Paeoniflorin treated mice exhibit greater reduction in the levels of pro-inflammatory cytokines (IL-1 β , IL-6 and TNF- α) in both myocardium and plasma in the present study. It is reported that the inflammatory response in macro-phages induced by LPS has been reduced by Paeoniflorin [34]. Also, showed that

Paeoniflorin could inhibit systemic inflammation in rat where [35], clarified that Paeoniflorin arrests the production of inflammatory mediators in arthritic rats. Further experiments showed that Paeoniflorin inhibited lip polysaccharide stimulated tumor necrosis factor- α (TNF- α) and interleukin (IL)-1 β release and facilitated LPS-induced IL-10 production [36]. In rats with experimental colitis, serum levels of IL-6, IL-17, and IL-23 were lowered by treatment with TGP (total glycosides of peony) [37]. In this study, following 72hs of reperfusion the levels of MCP-1 expressing in both plasma and myocardium markedly lower in treated mice with Paeoniflorin in comparison to control I/R and vehicle I/R pretreated groups. It is demonstrated that Paeoniflorin attenuated markedly advanced glycation end products-induced inflammation cytokines IL-6 and monocyte chemo-attractant protein-1 levels in ELISA and western blot analysis in a dose-dependent manner [38], also [39] showed that Mudanpi, which is the root cortex of Paeonia suffruticosa, prevent the secretion of MCP-1, a major mediator of chronic macrophage-mediated inflammation and demonstrated that the inhibition of these chemokine by Mudanpi are due to its suppressions of MCP-1 and IL-8 genes. Thus, the results suggest that one possible anti-inflammatory mechanism of Mudanpi may be due to inhibition of the secretions of inflammatory chemokine. In comparison to control I/R and vehicle I/R treated groups which showed marked increase in plasma myocardial injury marker (cTn-I) level in the present study, Paeoniflorin had effort reduced cTn-I expression after I/R. Proved that pretreatment with paeonol exhibited significant ($P < 0.001$) decrease in the levels of serum cTn-I [19]. Cardiac function in Paeoniflorin I/R treated mice, showed improved LV function, the measurements of LVEDD, LVESD, and %FS was higher than that measured in I/R control and vehicle treated mice in the present study. It is demonstrated that treatment with PF was able to diminish the myocardial infarct size so that suggesting that PF shows cardio-protective effects on AMI [11]. It is found that pretreatment with PF decreased the cardiac systolic dysfunction in LPS-challenged mice, as evidenced by a significant increase in left ventricular EF, FS, LVDd, and LVDs examined with echocardiography and demonstrated that PF could protect mice from LPS-triggered cardiac dysfunction, and latest finding is in according with a previous study [6]. The Notch1/Jagged1 expression levels was up-regulated in Paeoniflorin treated group ($P < 0.05$) and this result showed for the first time the possible mechanistic pathway of Paeoniflorin in reduced myocardial injury following I/R through up-regulation of Notch1/Jagged1 signaling pathway, where the expression level of Notch1/Jagged1 was down-regulated in both I/R control and vehicle treated mice as measured by gel electrophoresis of PCR. In this study, the histological features of Paeoniflorin pretreated mice showed mild architectural alterations and score 2 with mild monocyte infiltration and without hemorrhage ($P < 0.05$). No previous studies examined the effect of PF on heart parenchyma.

CONCLUSIONS

From the findings of the present study, it can be concluded that; over expression of inflammatory

mediators following I/R suppress LV function; Notch-1 and Jagged-1 protein are down-regulated following I/R; the administration of Paeoniflorin attenuated chemokine and cytokines through up-regulation of Notch-1 and Jagged-1 activation signaling pathway leading to improved left ventricular function; and pretreatment with Paeoniflorin decreased infiltration of PMN cells through unclear mechanism.

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Kufa University

CONFLICT OF INTEREST:

None

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