

Effect of Gallic Acid Pretreatment and SGK1 Enzyme Inhibition on Cardiac Function and Inflammation in a Rat Model of Ischemia-Reperfusion Injury

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Abstract

Background: Serum and glucocorticoid-induced kinase 1 (SGK1) is an enzyme that may play an important role in ischemic-reperfusion (I/R) injury and myocardial dysfunction. Although many studies have been conducted on individual antioxidants, little attention has been paid to the effects of co-administration of an antioxidant with an SGK1 inhibitor on cardiac function after I/R.

Methods: This study aimed to determine the effects of gallic acid (as an antioxidant) combined with an SGK1 inhibitor on I/R-induced cardiac dysfunction and inflammation. Sixty male Wistar rats were randomized into 6 groups, pretreated with gallic acid or vehicle for 10 days. Subsequently, the heart was isolated and exposed to I/R. In groups that received the SGK1 inhibitor, the heart was perfused with the SGK1 inhibitor GSK650394, 5 min before induction of ischemia. After that, cardiac function, inflammatory factors, and myocardial damage were evaluated.

Results: The combination of these two compounds improved cardiac contractility, heart rate, rate pressure product, left ventricular developed pressure, left ventricular systolic pressure, perfusion pressure, and QRS voltage significantly ($P < 0.05$). In addition, concomitant therapy of these two agents reduced tumor necrosis factor- α and interleukin-6, and the activity of creatine kinase-MB, lactate dehydrogenase, and troponin-I ($P < 0.05$).

Conclusions: The results indicated that administration of gallic acid with the SGK1 inhibitor may have a potentiating effect on the improvement of cardiac dysfunction and I/R-induced inflammation.

Keywords: Gallic Acid, Inflammation, Ischemic-Reperfusion Injury, Rat, Serum-glucocorticoid regulated kinase 1 (SGK1).

Introduction

Myocardial ischemia is the leading cause of death worldwide and has many destructive consequences for the cardiomyocytes, which left untreated, can lead to cell death (1). Damage to cardiomyocytes or disruption of their functions can lead to osmotic imbalance and leakage of cellular metabolites into the extracellular space. In addition, lysosomes' membrane damage could result in the release

of destructive enzymes that can digest cellular elements and lead to cell necrosis (2).

Inflammatory reactions induced by ischemia-reperfusion (I/R) lead to release of cytokines that are important components of I/R-induced injury to the myocardium. In this process, the production of potential proinflammatory cytokines including interleukin 6 (IL-6) and tumor necrosis factor-

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alpha (TNF- α) (3) lead to inflammation and immune cell activation (4).

Myocardial injury in acute coronary syndrome is a primary and direct cause of hemodynamic changes that can result in elevated cardiac biomarkers and the electrocardiogram (ECG) changes (5). Elevated cardiac biomarkers, particularly cardiac troponin-I (cTnI) and creatine kinase-MB (CK-MB), indicate myocardial cell damage and necrosis (6, 7), and ECG changes in PR interval, QRS complex voltage, and ST-segment potentially reflect myocardial ischemia (8). However, cardiac biomarkers and ECG changes alone are not sufficient to diagnose acute myocardial infarction (9).

Serum and glucocorticoid-induced kinase 1 (SGK1) is an important enzyme that plays a role in ischemic-reperfusion injury and myocardial infarction. This enzyme belongs to a subfamily of serine/threonine kinases (10). SGK1 expression is high in the heart (11). Recent research has shown that SGK1 performs a vital function in cardiac apoptosis, inflammatory cytokine expression, and vascular remodeling. In addition, SGK1 is dynamically regulated during acute biological stress in the heart and controls the survival and hypertrophic response of cardiomyocytes (12). Stress-induced stimuli have been shown to activate SGK1 (13). Thus, SGK1 is an important factor involved in the pathophysiological features of various cardiovascular diseases (14), including inflammatory response regulation (15).

Gallic acid is found in numerous plants and commonly used in medicine as an antioxidant (16) and anti-inflammatory factor and can also protect against those changes (17). Furthermore, SGK1 may be involved in inflammation and cardiac dysfunction in the isolated heart due to ischemic-reperfusion injury. Therefore, the present study aimed to co-administer gallic acid and SGK1 inhibitor to investigate their possible protective and potentiating effect on inflammation and

cardiac dysfunction due to ischemic-reperfusion injury.

Materials and Methods

Materials

Xylazine (2%) and ketamine HCl (10%) were received from Alfasan Co. (Netherlands). Krebs salts were obtained from Merck Co. (Germany). Gallic acid was obtained from Sigma (St. Louis, MO USA). GSK650394 (GSK) was obtained from MedChemExpress (USA). Creatine kinase-MB isoenzyme and lactate dehydrogenase (LDH) were received from the Pars Azmoon Co. (Iran). Cardiac troponin-I is a selected biomarker of cardiac damage as measured with a specific kit obtained from Monobind Inc. (Lake Forest, California, USA).

Animal grouping

Sixty male adult Wistar rats (250-300 g) were received from the animal house of Ahvaz Jundishapur University of Medical Sciences one week before initiation of the experiments and had free access to water and food during the 12:12 hour light-dark cycles under controlled temperature conditions (22 ± 2 °C). The protocols of the experiments were approved by the Research Ethics Committee of the Center of Research & Laboratory Animal at Ahvaz Jundishapur University of Medical Sciences (ID: IR.AJUMS.ABHC.REC.1400.032, dated: 2021-05-25). The rats were randomized into six groups with 10 in each group (Table 1). Gallic acid was dissolved in normal saline and administered (30 mg/kg by gavage) once daily for 10 days (18). In the groups that received GSK, five min before the onset of an ischemic attack, the isolated hearts were perfused with Krebs-Henseleit solution containing 1 μ mol of GSK (19) prepared in DMSO (18). For the groups that did not receive gallic acid or GSK, the medication vehicles were given instead. Ischemia induction was performed by complete closure of the infusion flow for 30 min followed by reperfusion for 60 min (18).

Table 1. Animal treatments used in the present study.

Groups*	Gallic acid(mg/kg)	GSK [#] (μmol)
Sham	0	0
Gallic acid	30	0
Isc**	0	0
Isc + G	30	0
Isc + GSK	0	1
Isc + G + GSK	30	1

*, 10 animals per group; **, ischemic/reperfusion groups; #, GSK650394 (SGK1 inhibitor).

Cardiac isolation and ischemia induction

The animals were anesthetized by injecting HCl ketamine (50 mg/kg) and xylazine (5 mg/kg) intraperitoneally. To prevent blood clots, heparin was injected intraperitoneally (1000 U/kg) (18). The trachea was then cannulated and attached to the rodent's ventilator (UGO BASILE, model 7025), to allow artificial breathing to ambient air after opening the chest and removing the ribs. The aorta was cannulated by inserting a stainless-steel cannula through an incision in its wall and secured by a suture. The heart was immediately infused with Krebs-Henseleit solution which consisted of glucose, MgSO₄, KH₂PO₄, NaCl, KCl, NaHCO₃, and CaCl₂ (11.1 mM, 1.2 mM, 1.18 mM, 118 mM, 4.75 mM, 25 mM, and 1.75 mM, respectively) equilibrated by 5% CO₂ and 95% O₂ at pH of 7.4. Subsequently, hearts were excised and transferred into the Langendorff device containing Krebs-Henseleit solution. The isolated heart was continuously perfused with the solution by a peristaltic pump at a constant flow rate of 7 ml.min⁻¹ at 37 °C (20). During the experiment, the buffer was bubbled with carbogen (95% O₂-5% CO₂) to maintain a pH of 7.4. The ischemia was induced by the no flow method for 30 min as described in the previous section and then re-perfused for 60 min (18).

Cardiac function parameters

The hearts were placed in a 37 °C coated glass chamber and left for 25–30 min to reach equilibrium before all experiments. A rubber balloon filled with water, connected to a pressure transducer with a stainless-steel needle, was inserted into the left ventricle through the left atrium to measure left ventricular pressure (LVP). The balloon

volume was adjusted so the LV end-diastolic pressure (EDP) was 5-10 mm Hg. A Powerlab system (Powerlab, AD instrument, Australia) was used to record and analyze the signal from the pressure transducer. End-diastolic pressure, left ventricular developed pressure (LVDP), the maximum rates of LVP rising (+dp.dt⁻¹ max) and falling (-dp.dt⁻¹ max) as indices of myocardial contractility, the rate pressure product (RPP; the product of LVDP and heart rate), and lead II of ECG were recorded. All hearts were perfused for 25–30 min before ischemia to stabilize the LVP and coronary perfusion pressure (CPP) and then exposed to 30 min of no-flow global ischemia followed by 60 min of reperfusion (18).

Electrocardiogram parameters and heart rate

To record the electrical activity, hook electrodes were attached to the isolated heart. A lead II electrocardiogram was recorded to calculate the RR and QT intervals and QRS complex voltage. ECG recordings were performed using Bio Amp tools and a Powerlab system. Because the QT interval varies with heart rate changes, corrected QT (QTc) was reported according to Bazett's formula (21).

Bazett's formula: QTc (QT corrected for heart rate) = $QT/\text{square root of } RR$

In all groups, heart rate was also measured using the RR interval during the ECG measurement (22).

Inflammatory factors evaluation

Tissue TNF-α and IL-6 levels were used as inflammatory biomarkers. Cardiac tissues were homogenized and centrifuged. Inflammatory biomarker concentrations in the supernatants were measured according to the kits'

instructions using an ELISA reader (Biotek, USA) (23).

Evaluation of myocardial injury

To evaluate ischemia-induced myocardial damage, perfusion solution samples were collected from hearts 20 min after reperfusion to measure cTnI, LDH, and CK-MB activity. The measurements were performed using an ELISA reader according to the kits' instructions (24).

Statistical analysis

Data were analyzed with SPSS version 22 and are expressed as means \pm standard deviations. The normal distribution of the data was checked using the Kolmogorov-Smirnov goodness test. Groups were compared using one-way analysis

of variance, followed by Tukey's multiple comparison test. P values less than 0.05 were considered statistically significant.

Results

Cardiac function parameters

The cardiac perfusion pressures did not differ significantly between the gallic acid-treated and sham groups but were significantly less in the Isc, Isc + G, and Isc + GSK groups than in the sham group (###). Perfusion pressures in the Isc + G, Isc + GSK, and Isc + G + GSK were significantly greater than that in the untreated ischemic group, and the differences between the treated groups were also significant (* vs. ** vs. ***) Perfusion pressures in the Isc + G and Isc + GSK groups were significantly less than that in the Isc + G + GSK group (\$\$) (Fig. 1).

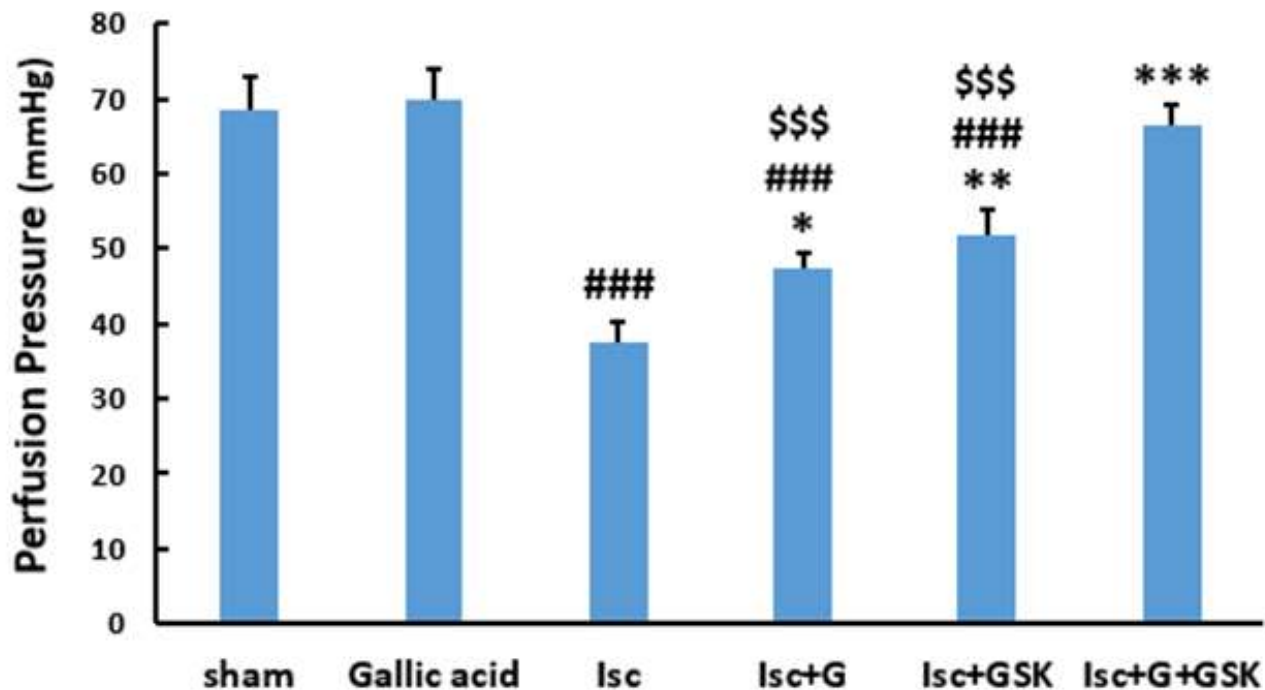


Fig. 1. The effects of gallic acid (G, 30 mg/kg/daily by gavage) and GSK (1 μ mol) on perfusion pressure (mean \pm SD, n = 10). ### P < 0.001 vs. sham. *** P < 0.001, ** P < 0.01, and * P < 0.05, vs. Isc. \$\$\$ P < 0.001 vs. Isc + G + GSK (One way ANOVA followed by Tukey's post hoc test).

Cardiac contractility did not differ significantly between the gallic acid-treated and sham groups. Cardiac contractility maximum rising and falling rates (+dp. d t^{-1} and -dp. d t^{-1} , respectively) in LVP were significantly less in all the Isc groups than in the sham group (### and ##). Contractility was

significantly greater in the Isc + G, Isc + GSK, and Isc + G + GSK groups than in the Isc group and the differences between the groups were also significant (* vs. ** vs. ***). Contractility was significantly less in the Isc + G group than in the Isc + G + GSK group (\$) (Figs. 2A and 2B).

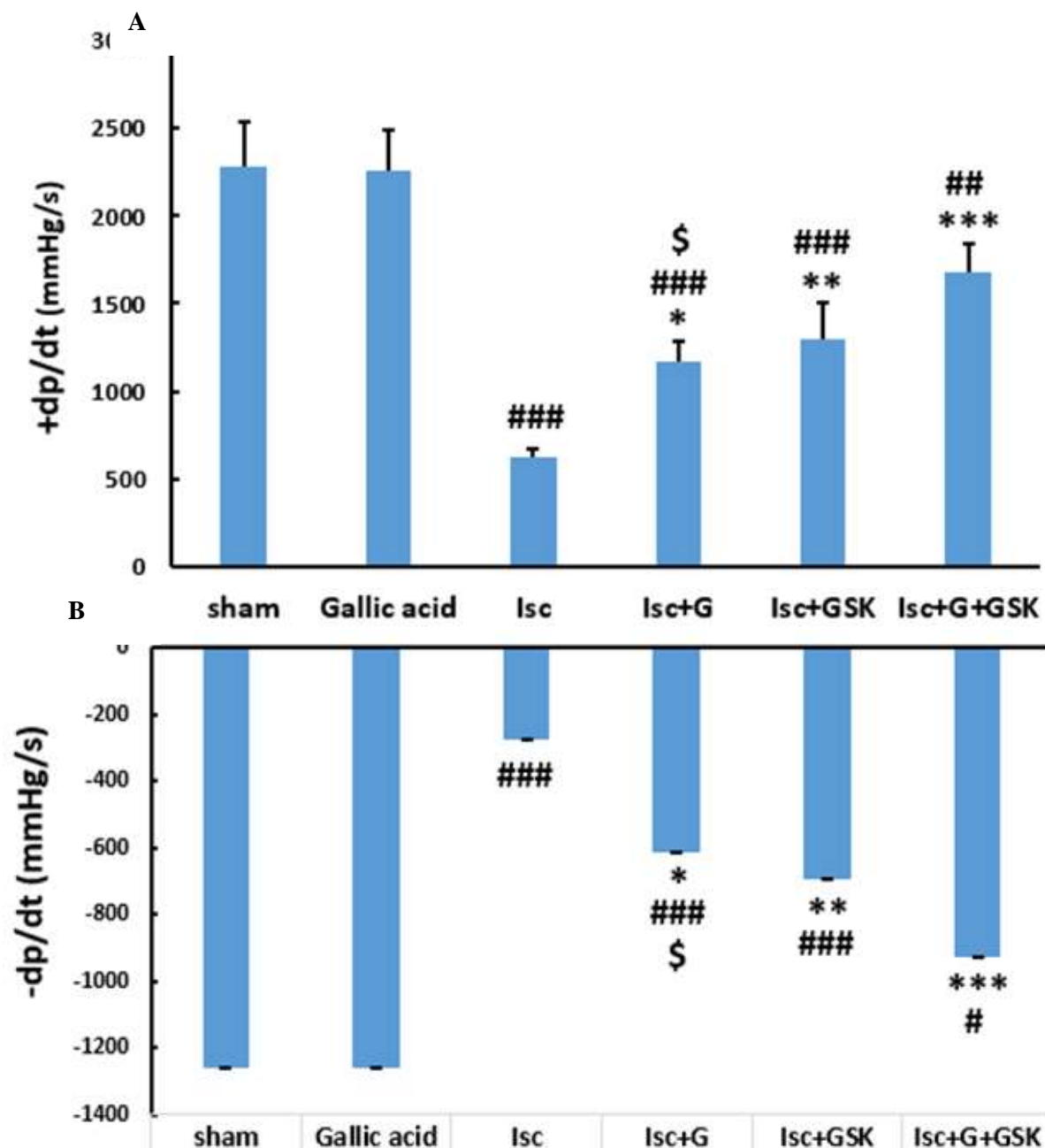


Fig. 2. The effects of gallic acid (G, 30 mg/kg/daily by gavage) and GSK (1 μ mol) on myocardial contractility (mean \pm SD, n = 10). ### P < 0.001, ## P < 0.01, and # P < 0.05, vs. sham. *** P < 0.001, ** P < 0.01, and * P < 0.05, vs. Isc. \$ P < 0.05 vs. Isc + G + GSK (One way ANOVA followed by Tukey's post hoc test).

Heart rate did not differ significantly between the gallic acid-treated and sham groups, nor was it significantly affected by ischemia or ischemia plus treatment with gallic acid or GSK alone; however, it was significantly greater in the Isc + G + GSK than in the Isc group (*) (Fig. 3A).

LVDP and RPP did not differ significantly between the gallic acid-treated and sham groups but were significantly less in all the Isc

groups than in the sham group (###). Treatment with gallic acid or GSK had no significant effect on LVDP or RPP relative to the Isc group, but both parameters were significantly greater in the groups treated with both drugs combined than in the Isc group (***), while those parameters in the Isc groups treated with either drug were significantly less than in the groups treated with both drugs combined (\$\$ and \$) (Figs. 3B and 3C).

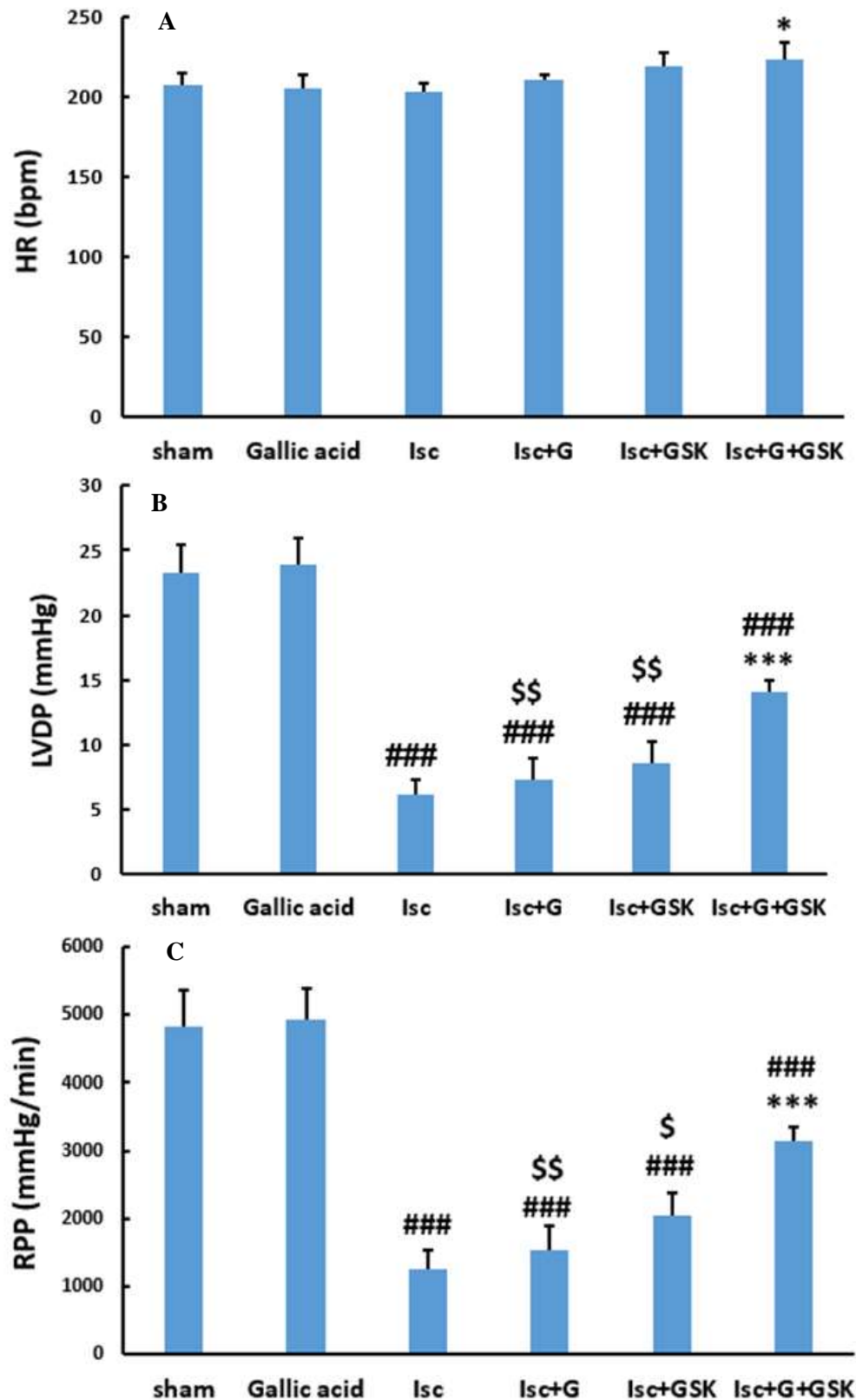


Fig. 3. The effects of gallic acid (G, 30 mg/kg/daily by gavage) and GSK (1 μ mol) on cardiac function parameters: heart rate (HR, A), left ventricular developed pressure (LVDP, B), and rate pressure product (RPP, C) (mean \pm SD, n = 10). ### P < 0.001 vs. sham. *** P < 0.001, and * P < 0.05, vs. Isc. \$\$ P < 0.01, and \$ P < 0.05, vs. Isc + G + GSK (One way ANOVA followed by Tukey's post hoc test).

QRS voltage did not differ significantly between the gallic acid-treated and sham groups but was significantly less in all the Isc groups than in the sham group (### and ##). Treatment with gallic acid or GSK alone had no significant effect; however, QRS voltage was significantly greater in the group treated with both drugs than in the Isc group (*). QRS voltage in the Isc + G group was significantly

less than in the Isc + G + GSK group (\$) (Fig. 4A).

QTc did not differ significantly between the gallic acid-treated and sham groups but was significantly greater in the Isc and Isc + G groups than in the sham group (### and ##). QTc was significantly less in the Isc + GSK and Isc + GSK + G groups than in the Isc group (* and **) (Fig. 4B).

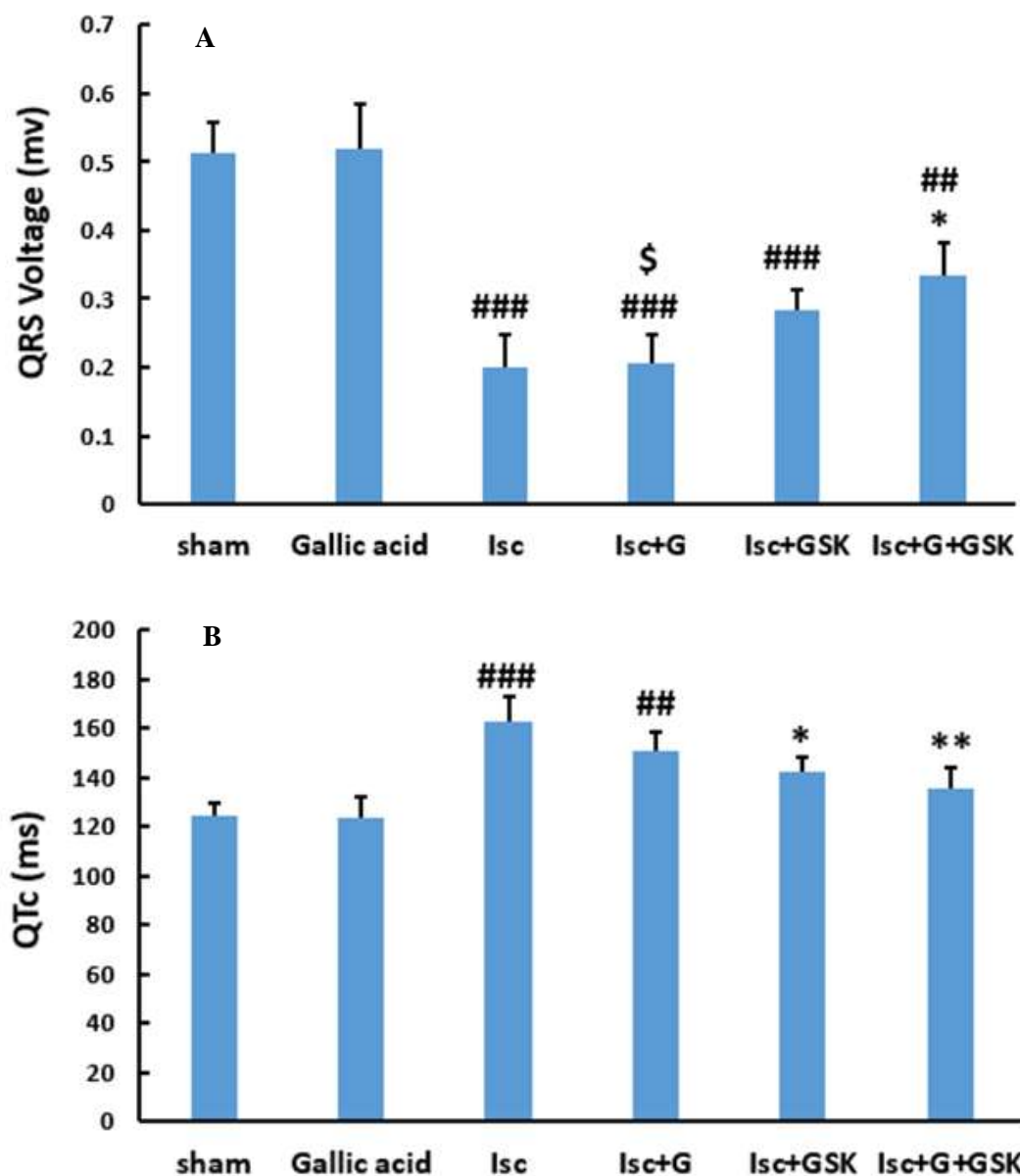


Fig. 4. The effects of gallic acid (G, 30 mg/kg/daily by gavage) and GSK (1 μ mol) on the electrical parameters QRS voltage (A) and QTc (B) (mean \pm SD, n = 10). ### P < 0.001, and ## P < 0.01, vs. sham. ** P < 0.01, and * P < 0.05, vs. Isc. \$ P < 0.05 vs. Isc + G + GSK (One way ANOVA followed by Tukey's post hoc test).

Enzyme activity

Creatine kinase-MB activity did not differ significantly between the gallic acid-treated and

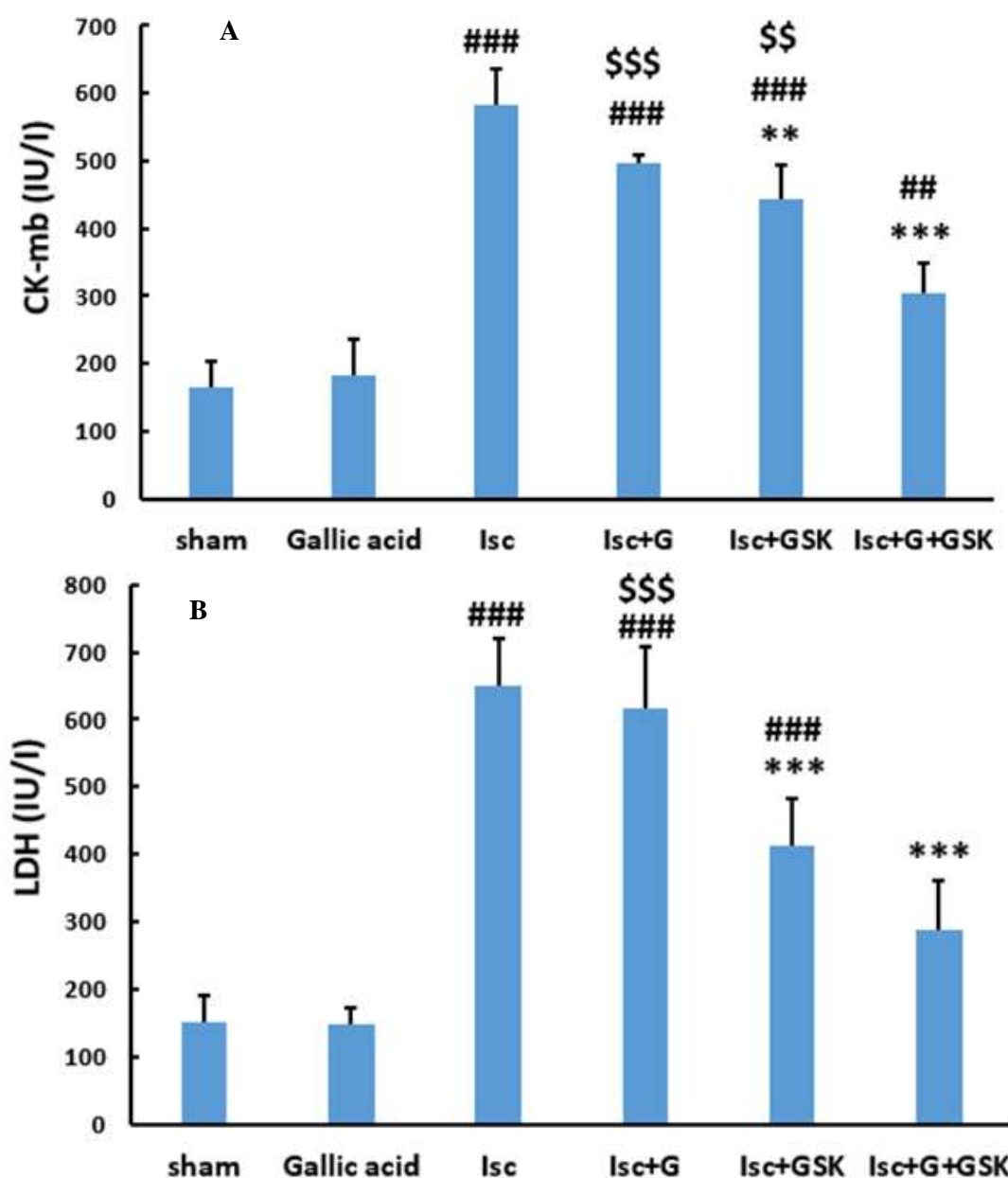
sham groups but was significantly greater in all the Isc groups than in the sham group (### and ##). Treatment of the Isc group with gallic acid

alone had no significant effect on CK-MB activity relative to Isc; however, CK-MB activity was significantly less in the Isc + GSK and Isc + G + GSK groups than in the Isc group (** and ***). Creatine kinase-MB activity was significantly greater in the Isc + G and Isc + GSK groups than in the Isc + G + GSK group (\$\$\$ and \$\$) (Fig. 5A).

LDH activity in the gallic acid group did not differ significantly from that in the sham group but was significantly greater in the Isc, Isc + G, and Isc + GSK groups than in the sham group (###). LDH activity was significantly less in the

Isc + GSK and Isc + G + GSK groups than in the Isc group (*), and was significantly greater in the Isc + G group than in the Isc + G + GSK group (\$\$\$) (Fig 5B).

Cardiac troponin-I activity in the gallic acid group did not differ significantly from that in the sham group but was significantly greater in the Isc and Isc + G groups than in the sham group (###). Cardiac troponin-I activity was significantly less in the Isc + GSK and Isc + G + GSK groups than in the Isc group (***), and significantly greater in the Isc + G group than in the Isc + G + GSK group (\$\$\$) (Fig. 5C).



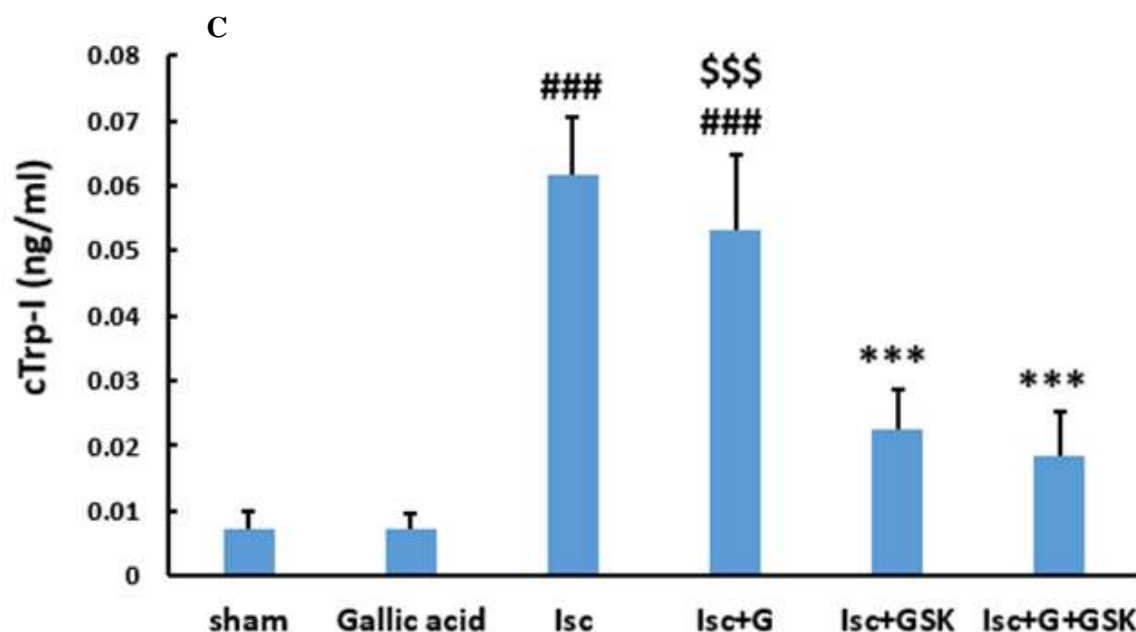
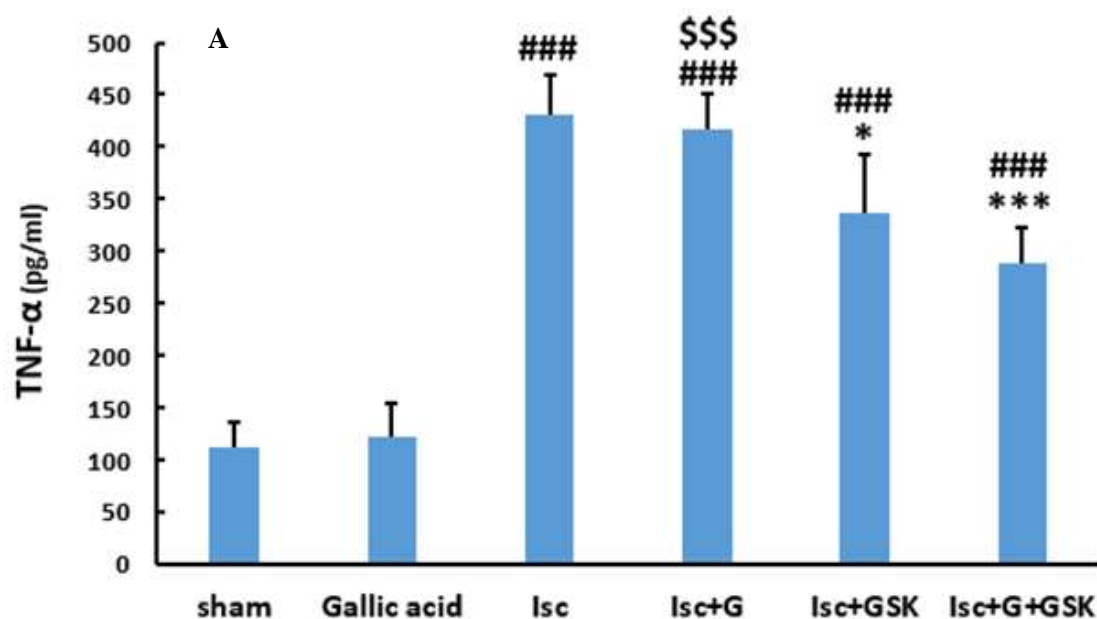


Fig. 5. The effects of gallic acid (G, 30 mg/kg/daily by gavage) and GSK (1 μ mol) on creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), and cardiac troponin-I (cTnI), markers of myocardial injury (mean \pm SD, n=10). ### P < 0.001, and ## P < 0.01, vs. sham. *** P < 0.001, and ** P < 0.01, vs. Isc. \$\$\$ P < 0.001, and \$\$ P < 0.01, vs. Isc + G + GSK (One way ANOVA followed by Tukey's post hoc test).

Inflammation factors in the heart

TNF- α concentration did not differ significantly between the gallic acid-treated and sham groups but was significantly greater in all the Isc groups than in the sham group (###), and significantly less in the Isc + GSK and Isc + G + GSK groups than in the Isc group (* and ***). TNF- α was greater in the Isc + G group than in the Isc + G + GSK group (\$\$\$) (Fig. 6A).

The IL-6 concentration did not differ significantly between the gallic acid-treated and sham groups but was significantly greater in all the Isc groups than in the sham group, although less so in the Isc + G + GSK group than in the other groups (# vs. ###). IL-6 was also significantly less in the Isc + G + GSK group than in the Isc group (*) (Fig. 6B).



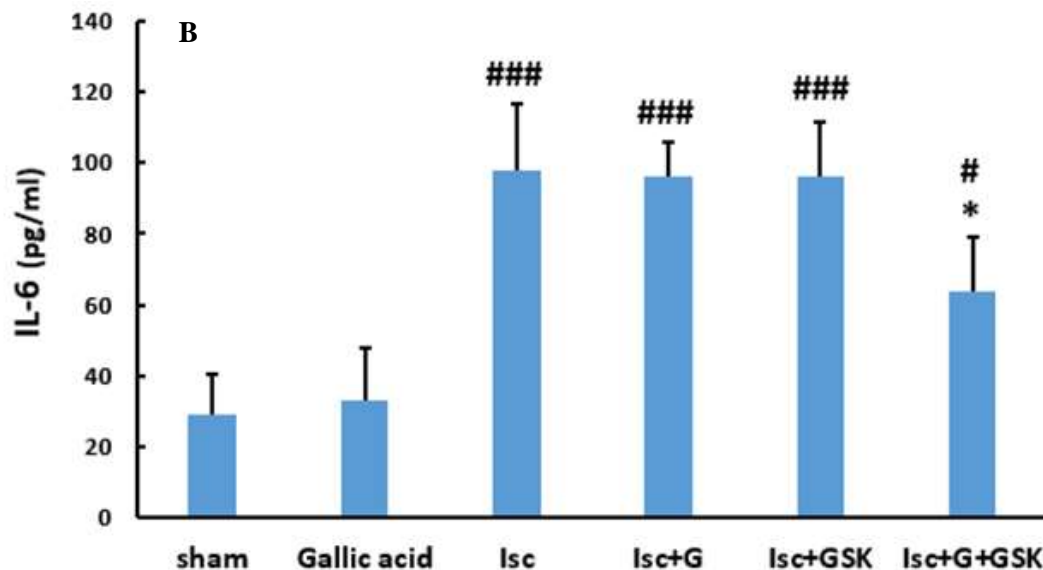


Fig. 6. The effects of gallic acid (G, 30 mg/kg/daily by gavage) and GSK (1 μ mol) on the pro-inflammatory cytokines tumor necrosis factor- α (TNF- α) and interleukin 6 (IL-6) (mean \pm SD, n=10). ### $P < 0.001$, and # $P < 0.05$, vs. sham. *** $P < 0.001$, and * $P < 0.05$, vs. Isc. \$\$\$ $P < 0.001$ vs. Isc + G + GSK (One way ANOVA followed by Tukey's post hoc test).

Discussion

In this study, we examined gallic acid and the SGK1 inhibitor GSK alone or combined as a strategy to better manage I/R-induced myocardial injury. The results revealed the protective effects of gallic acid and GSK on myocardial injuries. Gallic acid and GSK significantly protected ventricular functions. This was indicated by the increase in myocardial contractility, inflammatory cytokines TNF- α and IL-6, cardiac enzyme markers CK-MB, LDH, and cTnI, and other cardiac parameters. In addition, the most important result was that the two drugs combined improved heart function more than either medication alone.

Consistent with these findings, other studies have shown that gallic acid, as a potent natural antioxidant, has useful and strong anti-inflammatory, immune, antioxidant, and antiatherogenic properties (25, 26). In vitro studies have shown that gallic acid interacts with multinucleated leukocytes to produce its effects. Gallic acid may inhibit inflammation through the removal of superoxide anions, inhibition of myeloperoxidase release and activity, and participation in the accumulation of active NADPH-oxidase (27, 28).

The present work found also that SGK1 inhibition, mediated by GSK, not only improved almost all cardiac functions, but also enhanced the protective effect of gallic acid, and that the coadministration of gallic acid and GSK was more protective than the administration of either compound alone. These protective effects are indicated by restoring reduced ventricular functions such as perfusion pressure, LVDP, RPP, and myocardial contractility. Consistent with our results, gallic acid has been reported to have a protective effect on left ventricular (LV) dysfunction in a rat diabetes model (17).

It has been shown that SGK1, by activating the STAT3 pathway, may play an essential role in the recruitment and activation of macrophages, leading to angiotensin II-induced cardiac fibrosis (29). Furthermore, SGK1 has been reported to have a key pressure-related role in regulating inflammatory response and cell fate in re-perfused ischemic myocardium (19).

In this study gallic acid and GSK administration, either alone or combined, restored the QT interval. It has also been shown that SGK1 affects the cardiac electrical cycle QT interval. Because the QT interval

indicates left and right ventricular electrical depolarization and repolarization, perhaps SGK1 is not necessary to maintain normal QT, but could potentially shorten the QT interval (30). Additionally, an increase in body mass index (31) and a shortening of the QT interval, as well as an increase in blood pressure, were observed (32, 33). The increase in body mass index could be partly attributed to increased activation of the intestinal glucose transporter SGLT1 (31) and increased cardiac repolarization rate due to increased cardiac K⁺ channel activation KCNE1 (34). Therefore, the regulation of transporters and channels by SGK1 may explain the coincidence of hypertension, obesity, and heart action potential depletion (35).

Our results also showed that I/R increased IL-6 and TNF- α concentrations in heart tissue. GSK and gallic acid + GSK reduced TNF- α , while gallic acid + GSK reduced IL-6 (Figs. 6A and 6B). These results are consistent with other data that showed inhibition of SGK1 by EMD638683 may improve angiotensin II-induced myocardial inflammation and fibrosis by inhibiting the activation of inflammasome NLRP3 (15). Activation of the proinflammatory cytokine pathway in myocardial injury has been established in several studies (36-38). The expression of proinflammatory factors including IL-1 β , TNF- α , and IL-6 is greatly enhanced in experimental models of myocardial infarction. It is widely accepted that proinflammatory factors such as TNF- α initiate irreversible loss of large numbers of cardiomyocytes after myocardial I/R injury (39).

In this study, 30 min of ischemia followed by 60 min of reperfusion induced myocardial damage, as demonstrated by elevated CK-MB, LDH, and cTnI concentrations in coronary perfusion samples 20 min after reperfusion (Figs. 5A, 5B, and 5C, respectively), consistent with previous reports (40, 41). The data also demonstrate that gallic acid and GSK are effective at reducing enzyme levels after I/R and their combined effect is greater than either alone,

which means they provide increased protection against heart muscle damage when combined. Previous studies have shown that the CK-MB increase is strongly correlated with infarct size, and the LDH increase in myocardial infarction may be due to prolonged ischemic injury (41, 42). Our results agree with other studies demonstrating that cardiac enzymes are increased in I/R injury (40, 41). In our hands, administration of GSK and GSK + gallic acid reduced this increase.

In summary, this study demonstrated that gallic acid and GSK can act as potent anti-inflammatory and protective agents in a rat model of cardiac I/R injury. In addition to improving cardiac function, they reduced the myocardial injury markers CK-MB, LDH, and cTnI, and the proinflammatory cytokines TNF- α and IL-6. Generally, our results showed that gallic acid and GSK as an SGK1 inhibitor could have anti-inflammatory effects and improve cardiac function in I/R injury. Furthermore, the simultaneous use of both compounds may significantly increase and potentiate these curative effects.

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Conflicts of interest

The authors have no relevant financial or non-financial interests to disclose.

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Ethics Approval

The protocols of the experiments were approved by the research ethics committee of the Research Center & Experimental Animal House at Ahvaz Jundishapur University of

Medical Sciences according to the ethical principles and national norms and standards for conducting Medical Research in Iran (ID: IR.AJUMS.ABHC.REC.1400.032, dated: 2021-05-25).

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