Dietary polyphenol quercetin protects rat hearts during reperfusion: enhanced antioxidant capacity with chronic treatment

İzole sıçan kalplerine uygulanan iskemi/reperfüzyon hasarında quercetinin koruyucu etkisi: Kronik kullanımda artmış antioksidan etkinliği

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ABSTRACT

Objective: Quercetin is an important member of dietary flavonoid family and widely present in red wine and Mediterranean diet. The major objective of the this study is to evaluate the beneficial effects of quercetin in protecting the myocardium from the deleterious effects of ischemia reperfusion (I/R) injury in chronic quercetin treatment with or without an acute quercetin infusion protocols.

Methods: Forty male Sprague-Dawley rats were included in this experimental randomized study/ Langendorff perfused isolated rat hearts were subjected to 60-min of global ischemia period following 60-min of reperfusion. All animals were randomly divided into 4 groups. Group 1 animals were kept as controls. Group 3 and 4 animals received 50 mg/kg quercetin via an intragastric tube for 7 days for chronic treatment. Group 2 and 4 animals received an acute 15 μmol/L infusion for 30 minutes before the onset of ischemia. The myocardial postischemic recovery was compared using hemodynamic data (peak systolic pressure, end-diastolic pressure and $+dP/dt_{max}$), coronary flow, biochemical parameters (lactate dehydrogenase, creatine kinase –MB fraction, cardiac troponin I) from coronary effluent, and oxidative stress markers (malondialdehyde, glutathione, glutathione reductase and nitrite) from heart tissue homogenates in each group.

Results: Quercetin has provided increased preservation in myocardial recovery in both chronic and acute treatment protocols compared to non-treated group. According to all estimated hemodynamic parameters, while the statistical difference between acute treated hearts and control hearts was significant ($p<0.05$); this significance was more clear in chronic treated groups (group III and IV) when compared to control ($p<0.01$). Likewise, biochemical and oxidative stress markers displayed significant differences in acute treated and chronic treated hearts when compared to control ($p<0.05$ and $p<0.01$, respectively).

Conclusion: As a major dietary flavonoid, due to its antioxidant and cytoprotective actions, quercetin has the capacity to protect the myocardial tissue against global ischemia and reperfusion injury. In instances where the molecule is administered for the purpose of acute therapy, this cardioprotective effect of a significant degree can be observed to; however, this potency is further accentuated upon administration as a chronic treatment protocol for seven days. *(Anadolu Kardiyol Derg 2007; 7: 404-10)*

Key words: Quercetin, heart, ischemia/reperfusion, protection, oxidative stress, antioxidant

ÖZET

Amaç: Quercetin Akdeniz diyetinde bol miktarda bulunan polifenol yapıda bir maddedir ve güçlü bir antioksidandır. Bu çalışmanın amacı sıçan kalplerine kronik tedavi ve akut infüzyon olarak verilen quercetinin iskemi-reperfüzyon hasarına karşı koruyucu etkinliğini saptamaktır.

Yöntemler: Bu deneysel randomize çalışmaya kırk adet erkek Sprague-Dawley sıçan dahil edildi. İzole kalpler Langendorff sistemine asılarak Krebbs-Henseleit solüsyonu ile perfüze edildi. Kalpler 60 dakika global iskemiyi takiben 60 dakika reperfüzyon uygulandı. Sıçanlar rasgele 4 gruba ayrıldı. Grup 1 kalpler kontrol grubu olarak kabul edildi. Grup 3 ve grup 4'deki sıçanlara deney öncesi bir hafta boyunca 50 mgr/kg oral quercetin verildi. Grup 2 ve grup 4 kalplere deney esnasında 30 dakika boyunca 15 μmol/lt quercetin infüzyonu uygulandı. Hemodinamik veriler (sistolik basınç, diyastol sonu basınç, dP/dt ve koroner akım miktarları), koroner akım örneklerinden laktat dehidrogenaz, kreatin kinaz-MB, kardiyak troponin I ve miyokardiyal doku örneklerinde oksidatif stres belirleyicileri (malondialdehid, glütatyon, glütatyon reduktase ve nitrit) çalışıldı.

Bulgular: Quercetin uygulanan gruplarda reperfüzyon periyodunun her fazında hemodinamik veriler ve biyokimyasal parametreler kontrol grubuna göre belirgin olarak daha iyi miyokardiyal iyileşmeyi işaret etmiştir. Hemodinamik veriler incelendiğinde, akut tedavi edilen kalpler ile kontrol grubu arasındaki fark istatistiksel olarak anlamlı iken ($p<0.05$), kronik tedavi edilen grup 3 ve 4 kalplerde kontrol grubuna kıyasla izlenen hemodinamik iyileşme belirgin olarak daha iyi idi ($p<0.01$). Benzer şekilde ölçülen biyokimyasal parametreler ve oksidatif stres belirleyicileri hem akut, hem de kronik tedavi edilen kalplerde kontrol grubuna göre anlamlı olarak daha iyi idi ($p<0.05$ ve $p<0.01$, sırasıyla).

Sonuç: Quercetin, antioksidan etkinliği ile oksidatif strese bağlı doku hasarını azaltarak global iskemi sonrası reperfüzyonda miyokardiyal iyileşmeyi artırmaktadır. Bu koruyucu etki özellikle kronik tedavi uygulanan kalplerde belirgin olarak izlenmektedir. *(Anadolu Kardiyol Derg 2007; 7: 404-10)*

Anahtar kelimeler: Quercetin, kalp, iskemi/reperfüzyon, koruma, oksidatif stres, antioksidan

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Introduction

Flavonoids are a big group naturally occurring polyphenolic compounds, which are widely presented in fruits and vegetables, and in particular in grapes. In addition to their broad scale of biological effects, they have been reported to possess antioxidant effects as free radical scavengers, hydrogen-donating compounds, singlet oxygen quenchers, and metal ion chelators (1). Quercetin, (3,5,7,3',4'-pentahydroxy flavone) is an important member of flavonoid family, and it daily intake with dietary that is estimated to be up to 16 mg, in particular in Mediterranean diet (2, 3). It has been reported that quercetin inhibited thrombocyte aggregation (4) and had an anti-hypertensive effect through vasodilator action on vascular smooth muscles (5). The studies that were focused on antioxidant efficiency of flavonoids against ischemia/reperfusion (I/R) injury have demonstrated that, quercetin possesses robust protective effects in renal, cerebral and hepatic I/R models (6- 8). Quercetin was also demonstrated to improve contractile function of the left ventricle in experimental myocardial infarction with subsequent 24-hour reperfusion (9). However, studies reporting the effects of this compound against global myocardial I/R injury are scanty.

This study was conducted with the aim of investigating the cardioprotective effects of quercetin against global ischemia and reperfusion injury in rat hearts, and comparison of this efficiency in the chronic treatment with and without an acute infusion protocols.

Methods

Animals

This study was approved by The Osmangazi University Institutional local animal care and use committee. Male Sprague-Dawley rats, 250-300 g body weight (BW) were used for this study. Animals were fed with a standard rat feed and allowed to drink water ad libitum. Animals were deprived of food for 12 h before the experiments. They were housed in temperature controlled (20–25^0 C) cages with a 12-h dark and 12-h light cycles. All procedures were performed in sterilized conditions.

Drug

Quercetin was purchased from Sigma, St. Louis, MO, USA, and dissolved in ethanol and then both diluted with tap water to a concentration of 15 mg/ml for oral administration, and diluted with physiological saline to achieve an infusion concentration of 15 μmol/L. Final ethanol concentration in both oral and infusion quercetin cocktails was 0.2 % (vol/vol) (10).

Isolated Heart Preparation

All rats were heparinized (300 IU/kg BW) via femoral vein and anaesthetized with 50 mg/kg BW of sodium pentobarbital administered by intraperitoneal injection. Rat hearts were rapidly excised through a median sternotomy, and immersed into ice-cold heparinized saline solution during preparation, and then mounted on the stainless steel cannula of the modified Langendorff perfusion apparatus via aorta. Aortic perfusion was initiated at a constant perfusion pressure of 80 mm Hg with gassed (95% O_2, 5% CO_2) Krebs-Henseleit bicarbonate buffer (K-H) (in mmol/L: NaCl 118; KCl 4.7; $CaCl_2$ 2.0; $MgSO_4$ 1.2; $NaHCO_3$ 25; $KH2PO_4$ 1.2; Glucose 11.1) while the pH was at maintained 7.4 to 7.5. A pulmonary arteriotomy was performed to allow free drainage of coronary sinus effluent. The heart temperature was monitored using a thermistor probe placed in the right ventricle wall, and kept constant at 37 ^{0}C during the stabilization, ischemia and reperfusion periods. The right atrium was removed, and the hearts were paced to 300 beats/minute at 4 volts using an external pacemaker (HSE Stimulator P, Type 201, Hugo Sachs Electronic, Germany). A water filled latex balloon was inserted in the left ventricle cavity through a small incision in the left atrium, and connected to a pressure transducer (Transpac II, Abbott, Bedford, MA) by rigid polyethylene tubing. The balloon volume was adjusted to produce a 10 mm Hg of diastolic pressure. Hemodynamic data from the latex balloon were analyzed using a real time data acquisition and analysis system (BIOPACK MP 30 system, Santa Barbara, CA) and displayed on a monitor during the experiment. The hearts were excluded from the experiment if they displayed lower contractile function ($+dP/dt_{max}$ values below 3000) or severe arrhythmia.

Experimental Design

Forty rats were randomly divided into four groups (Fig. 1):

Group 1 (n=10): These rats were reserved as control group and received 0.2 % (vol/vol) ethanol in tap water via an intra-gastric tube once daily for seven days and saline infusion containing 0.2% (vol/vol) ethanol for 30 minutes after stabilization period during the experiment. Animals in this group were not subjected to quercetin treatment.

Group 2 (n=10): Acute treatment group. These rats were received tap water containing 0.2 % (vol/vol) ethanol via an intra-gastric tube for seven days. Hearts were then subjected to 15 μmol/L quercetin infusion for 30 minutes after stabilization period during the experiment.

Group 3 (n=10): Chronic treatment group. Rats were subjected to 50 mg/kg BW quercetin treatment via an intra-gastric tube once daily for seven days. Hearts in this group were received saline infusion containing 0.2 % (vol/vol) ethanol for 30-minute after stabilization period during the experiment.

Group 4 (n=10): Chronic and acute treatment group. These rats were pretreated with 50 mg/kg BW quercetin via an intra-gastric tube once daily for seven days and received 15 μmol/L quercetin infusion for 30-minute after stabilization period during the experiment.

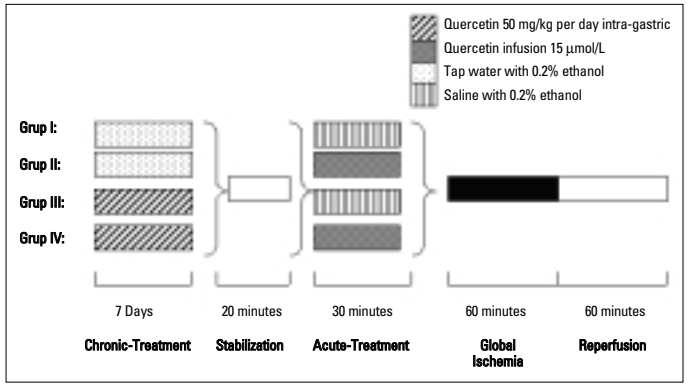

Figure 1. Diagrammatic presentation of the experimental protocol. Group 1 - control, Group 2 - acute treatment, Group 3 - chronic treatment, Group 4 - chronic and acute treatments

Since the chronic quercetin and ethanol exposure did not lead to any deleterious effects in the rats, 24 hours after this treatment, the hearts were perfused on Langendorff apparatus. All hearts underwent a 20-min of stabilization period. Then, following the 30-min of infusion period, all hearts in each group were then arrested with crystalloid cardioplegia solution (Plegisol, Abbott, USA) and subjected to 60-minute of global ischemia. Pacing was stopped during ischemia. Following the ischemic period, the hearts were then reperfused for a 60-minute period with K-H solution at 37 °C and paced at 300 beats/minute.

Hemodynamic Measurements

Left ventricular pressure waveforms (obtained from left ventricular latex balloons) were analyzed, and peak systolic pressure (PSP), end-diastolic pressure (EDP), and the maximum rate of increase and decrease of left ventricular pressure ($+dP/dt_{max}$) were recorded at the end of the stabilization period and at 20-minute intervals throughout the reperfusion period. Coronary effluent was collected in a reservoir for one minute and measured as coronary flow (CF) after the stabilization period and at 20-min intervals during reperfusion.

Biochemical Assay

The coronary effluent was collected throughout the stabilization and reperfusion periods separately, and samples were stored at -80 °C. Ischemic damage was assessed using lactate dehydrogenase (LDH) level (11), creatine kinase (CK-MB) activity (12), troponin I (cTnI) level (13). The LDH and CK-MB activities were determined in Hitachi 917 automated analyzer by using commercial kits supplied from Roche Diagnostic (Mannheim, Germany). Troponin I levels were measured in ACS: 180 automated chemiluminescence system using commercial kits supplied from Bayer Diagnostics (Cedex, France).

Evidence of oxidative stress was determined from heart tissue homogenates using glutathione (GSH), malondialdehyde (MDA), and glutathione reductase (GR) content analysis. Following reperfusion period, at the end of the experiment, the hearts were rapidly arrested by immersing in ice-cold K-H solution, and stored at –80 °C. The tissues were homogenized in 0.1 M phosphate-buffer (pH 7.4) with Ultra Turrax homogenizer (IKA T18 basic, Wilmington NC, USA). The homogenates were centrifuged 5000 rpm at +4 °C for 10 min, and the supernatants were removed, and assayed for MDA, GSH and GSSGR levels. Levels of GSH (14), MDA (15) and GR content (16) were measured by the methods referenced.

Tissue nitric oxide levels were correlated with nitrite determination from myocardial homogenates. Nitrite level was determined by using diazotization method. This method depends on diazotization of nitrite with an aromatic amine (sulphonamide) and formation of colorful azo derivative with N-(1-Naftil) etilendiamine (NEDD) (Griess reaction). Then it was measured on spectrophotometrically at 545-555 nm wave length (17).

Statistical Analysis

Results were expressed as means±S.E.M. Statistical analysis was performed using ANOVA (variance analysis for multiple comparisons). The normality of data for each group was analyzed using Shapiro-Wilk test. The post hoc Tukey multiple range test was used after ANOVA analysis and $p<0.05$ was accepted as considering statistical significance.

Results

Hemodynamic recovery (Table 1)

In the present study, quercetin infusion did not modify contractile function in each group. No significant difference was observed in hemodynamic parameters during stabilization and quercetin infusion periods ($p>0.05$).

Table 1. Pre-ischemic and post-ischemic hemodynamic data and coronary flow values

Periods of ischemia and reperfusion	Group*	Systolic pressure		Diastolic pressure		+dP/dtmax		Coronary flow	
		mm Hg control	p** value vs control	mmHg	p** value vs control		p** value vs control	mL/min	p** value vs control
Stabilization Period	1	119.3±5.7		12.6±0.8		4250±22		18.1±1.1	
	2	120.2±6.1	1.000	13.5±0.4	0.860	3961±25	0.826	18.4±0.7	0.976
	3	118.3±5.1	0.999	13.2±0.8	0.953	4247±26	1.000	20.3±1.1	0.882
	4	111.7±6.2	0.795	13.1±0.9	0.972	4242±20	1.000	20.3±1.1	0.939
Reperfusion 20. min	1	70.1±1.9		38.4±1.4		1030±47		9.9±0.4	
	2	80.4±2.2	0.011	33.2±1.3	0.013	1413±67	0.018	11.8±0.4	0.033
	3	90.0±2.4	0.001	27.9±0.7	0.001	1784±92	0.001	13.9±0.5	0.001
	4	90.3±2.1	0.001	28.6±0.8	0.001	1767±12	0.001	13.8±0.3	0.001
Reperfusion 40. min	1	70.4±1.6		38.0±1.3		1065±36		10.2±0.3	
	2	78.4±1.8	0.029	33.4±1.2	0.026	1470±67	0.011	11.9±0.3	0.011
	3	87.0±2.1	0.001	29.0±0.7	0.001	1845±10	0.001	13.5±0.3	0.001
	4	86.9±1.9	0.001	29.0±0.9	0.001	1833±10	0.001	13.6±0.4	0.001
Reperfusion 60. min	1	9.6±1.9		37.2±0.9		1050±37		10.9±0.3	
	2	79.0±1.2	0.013	33.2±0.9	0.030	1448±91	0.011	13.1±0.5	0.024
	3	88.5±2.0	0.001	29.3±0.9	0.001	1824±10	0.001	15.3±0.6	0.001
	4	88.4±2.6	0.001	29.2±1.0	0.001	1787±94	0.001	15.5±0.5	0.001

The values are expressed as the mean±SEM
*- one way ANOVA analysis
** - post hoc Tukey multiple range test
Group 1 - control, Group 2 - acute treatment, Group 3 - chronic treatment, Group 4 - chronic and acute treatments

Peak systolic pressure: The PSP values obtained from chronic pretreated groups (group 3 and 4) did not differ significantlu at any time during reperfusion (p>0.05). However, group 1 (control) and group 2 (acute treatment group) PSP values were significantly lower than that of groups 3 (chronic treatment group) and 4 (acute and chronic treatment group) (p<0.01 and p<0.05, respectively). Additionally, acute treated hearts in group 2 exhibited statistically higher PSP values than non-treated hearts in group 1 (p<0.05) (Fig. 2).

End-diastolic pressure: According to ANOVA test, no significant difference was observed between group 3 and group 4 hearts during reperfusion (p>0.05). The EDP values obtained from group 2 hearts were significantly higher than in chronic pretreated (groups 3 and 4) hearts throughout reperfusion (p<0.05). However, statistical significance was more evident in group 1 control hearts compared with groups 3 and 4 (p<0.01) throughout reperfusion period. Likewise, EDP values from group 2 were also statistically lower compared to group 1 during all phases of reperfusion period (p<0.05) (Fig. 2).

+dP/dtmax: The chronic pretreated groups (groups 3 and 4) have demonstrated significantly higher +dP/dtmax values at every time points of reperfusion period compared to group 2 (p<0.05) and group 1 (p<0.01) hearts. Acute treated hearts (group 2) exhibited significantly higher +dP/dtmax values than that non-treated hearts (group 1) during all phases of reperfusion (p<0.05) No significant difference was observed between group 3 and 4 throughout reperfusion period (p>0.05) (Fig. 3).

Coronary flow: No significant difference was observed between groups 3 and 4 during reperfusion (p>0.05). Coronary flow values of groups 3 and 4 were significantly higher than of both acute treated group 2 (p<0.05) and non-treated group 1 (p<0.01) at 20th , 40th and 60th minutes of of reperfusion. It was also significantly higher in group 2 compared to group 1 throughout reperfusion (p<0.05) (Fig. 3).

Biochemical results (Table 2)

There were no significant differences in any measured biochemical parameters among the all groups following stabilization period (p>0.05). No significant enzymatic difference was observed between chronic pretreated groups 3 and 4 during reperfusion (p>0.05). However, LDH, CK-MB and cTnI levels from chronic pretreated groups were significantly lower than in both acute treated (group 2) and non-treated (group 1) groups during reperfusion period (p<0.05 and p<0.01, respectively). Significantly less biochemical damage was also determined in group 2 compared to group I throughout reperfusion (p<0.05) (Fig. 4).

Oxidative stress indicators

The MDA levels obtained from groups 3 and 4 were significantly lower than in groups 2 (p<0.05) and 1 (p<0.01) during reperfusion. Likewise, GSH and GR levels were significantly higher in pretreated groups 3 and 4 than in groups 2 and 1

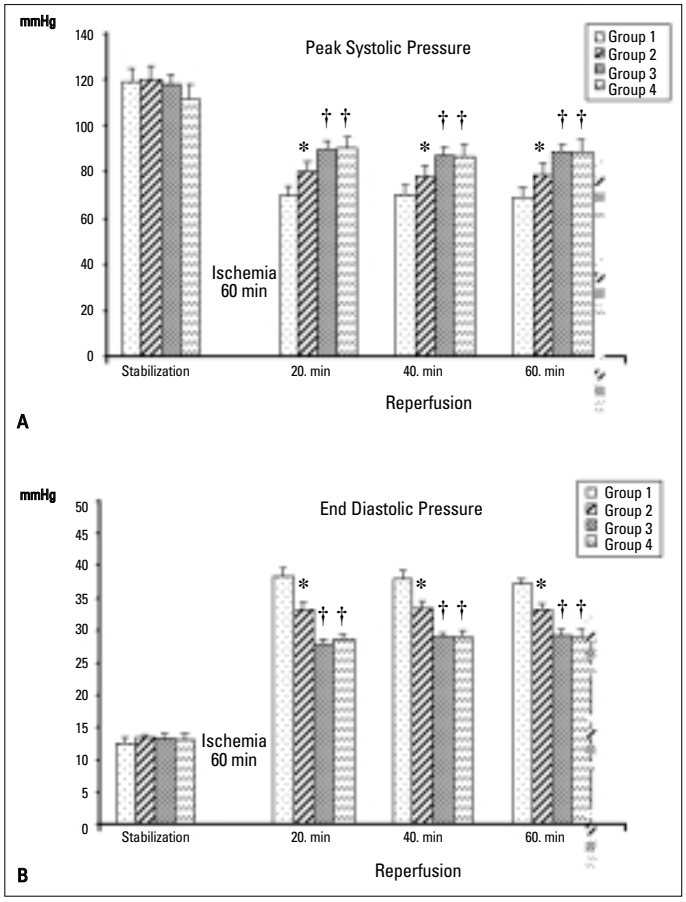

Figure 2. Effects of Quercetin on peak systolic pressure (A) and end-diastolic pressure (B) The values are expressed as the mean±SEM One way ANOVA analysis and post hoc Tukey multiple range test - *p<0.05 and † p<0.01 as compared with control group

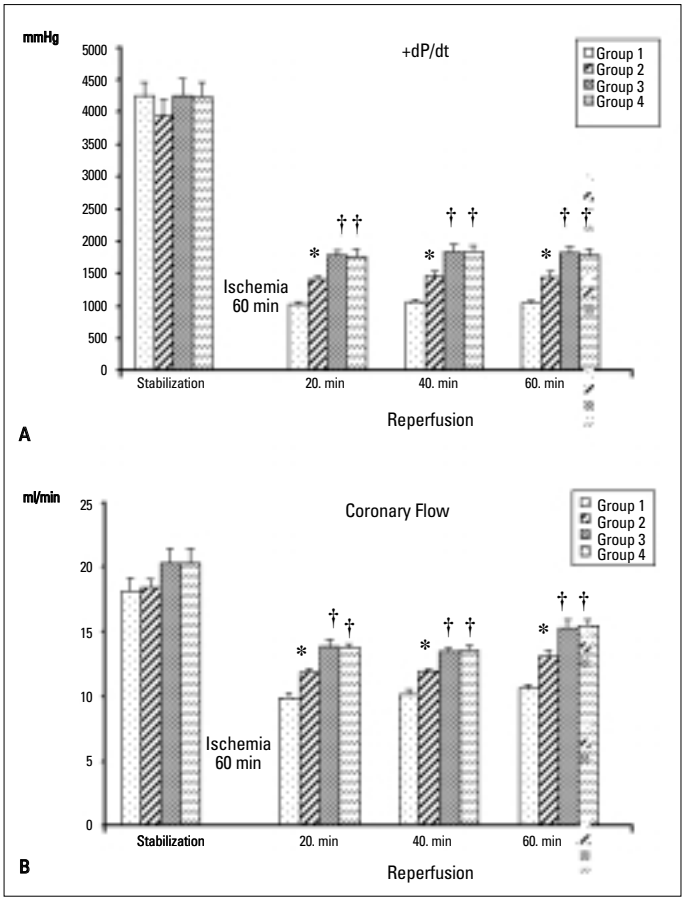

Figure 3. Effects of Quercetin on +dP/dtmax values (A) and coronary flow (B) measurements. The values are expressed as the mean ± SEM One way ANOVA analysis and post hoc Tukey multiple range test - * p<0.05 and † p<0.01 as compared with control group

(p<0.05 and p<0.01, respectively). No significant differences were observed in determined markers between groups 3 and 4 (p>0.05). Markers obtained from group 2 hearts were signaled a significantly more potent antioxidant effect than in group 1 hearts (p<0.05) (Fig. 5).

Nitrite levels in group 1 were significantly lower than those in group 2, 3 and 4 (p<0.01). Likewise, determined nitrite levels from myocardial homogenates of group 2 were significantly lower than in groups 3 and 4 levels (p<0.01). Nitrite levels from groups 3 and 4 were similar (p>0.05) (Fig. 5).

Discussion

In this study, we demonstrated that natural flavonoid quercetin that is found in abundance in the daily diet could protect the heart against global I/R injury and that this effect was more significant in the chronic treatment protocols. The I/R complex is a multidimensional process leading the generation of reactive oxygen species (ROS) and oxidative stress, which results in severe tissue damage (18). Under normal conditions, ROS, which are generated during cellular functions, are eliminated by intrinsic antioxidant enzyme systems like superoxide dismutase (SOD), glutathione peroxidase, and catalase (18). Especially during the early stages of reperfusion, tissue concentrations of ROS increase partly due to increased production and partly due to insufficient levels of antioxidant systems (19). In the myocardial tissue, with the initiation of such a process, increasing

Table 2. Biochemical results

Variables	Group*	Stabilization Period		Reperfusion Period	
			p** vs control		p** vs control
LDH, IU/L	1	35.3±6.9		278±12	
	2	44.7±6.4	0.999	238±7	0.022
	3	45.9±8.0	0.978	201±6	0.001
	4	44.8±8.2	0.938	199±5	0.001
CK-MB, IU/L	1	25.3±1.6		128±5.1	
	2	25.9±2.1	0.983	103±2.8	0.027
	3	24.3±2.7	1.000	80.3±5.6	0.004
	4	21.1±2.1	0.973	80.6±8.5	0.001
cTn I, mg/L	1	0.85±0.1		3.72±0.1	
	2	0.97±0.1	0.895	2.95±0.1	0.018
	3	0.89±0.1	0.994	2.10±0.2	0.001
	4	0.92±0.1	0.958	2.17±0.1	0.001
MDA, nmol/g pro	1			120.1±7.6	
	2			99.66±3.1	0.036
	3			79.24±3.2	0.001
	4			80.11±4.1	0.001
GSH, nmol/g pro	1			7.35±0.2	
	2			8.98±0.7	0.011
	3			10.52±0.9	0.001
	4			10.53±0.8	0.001
GR, nmol/min/ mg pro	1			16.0±0.9	
	2			21.3±1.3	0.017
	3			25.8±0.7	0.001
	4			26.1±1.1	0.001
Nitrite, mmol/g tissue	1			122.69±21	
	2			278.27±31	0.001
	3			426.50±38	0.001
	4			433.63±40	0.001

The values are expressed as the mean±SEM
*- one way ANOVA analysis
**- post hoc Tukey multiple range test
Group 1- control, Group 2- acute treatment, Group 3- chronic treatment, Group 4- chronic and acute treatments
CK-MB- creatine kinase activity, cTn I- cardiac troponin I, GSH- glutathione, GR- glutathione reductase, LDH- lactate dehydrogenase, MDA- malondialdehyde

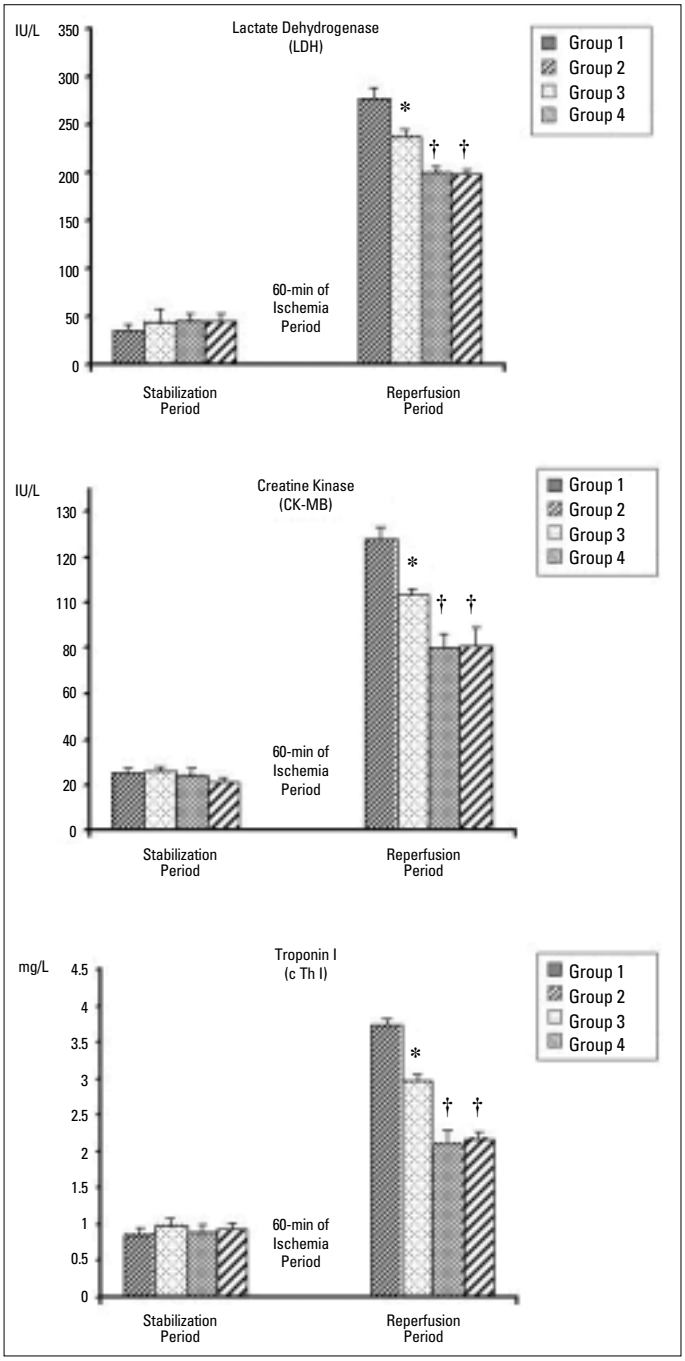

Figure 4. Effects of Quercetin on lactate dehydrogenase (LDH) level (top), creatine kinase (CK-MB) activity (middle) and troponin I (cTnI) (bottom) levels. The values are expressed as the mean±SEM
One way ANOVA analysis and post hoc Tukey multiple range test - *p<0.05 and † p<0.01 as compared with control group

amounts of ROS result in I/R damage (20) and finally lead to myocardial injury (21). Reactive oxygen species scavenging agents and antioxidant molecules have the capacity to partially reduce or eliminate deleterious effects induced by I/R injury (22).

Being an important member of the flavonoid family, quercetin is an antioxidant molecule found in fruits and vegetables, mainly in grapes and red wine. The example is the so-called French paradox; despite the consumption of fat rich diet and heavy smoking habits, people living in southern parts of France are identified to have low levels of coronary heart disease and this was related to frequent consumption of wine and Mediterranean diet containing quercetin and similar antioxidant flavonoids (5). Appreciation of ischemia reducing effects of quercetin brought together several studies demonstrating its protective capacity against I/R injury in several different tissues (6-8). Quercetin that was subsequently administered as chronic treatment decreased the myocardial damage by preserving mitochondrial functions in a global ischemia and reperfusion model (23). In our study, quercetin was used as chronic treatment with and without an acute infusion; in addition to investigating the cardioprotective effects of the compound, we aimed at delineating the differences in the efficacies of chronic and acute treatment protocols. Previous study (6), have also reported the efficacy of quercetin in prevention of I/R injury.

Quercetin exerts its antioxidant effects by scavenging free O2 and OH radicals on one hand and by inhibiting xanthine oxidase activity and lipid peroxidation on the other (24). In the study we present, the levels of MDA, which is an indicator of the damage induced by free radicals on lipid tissue were found to be lower in the

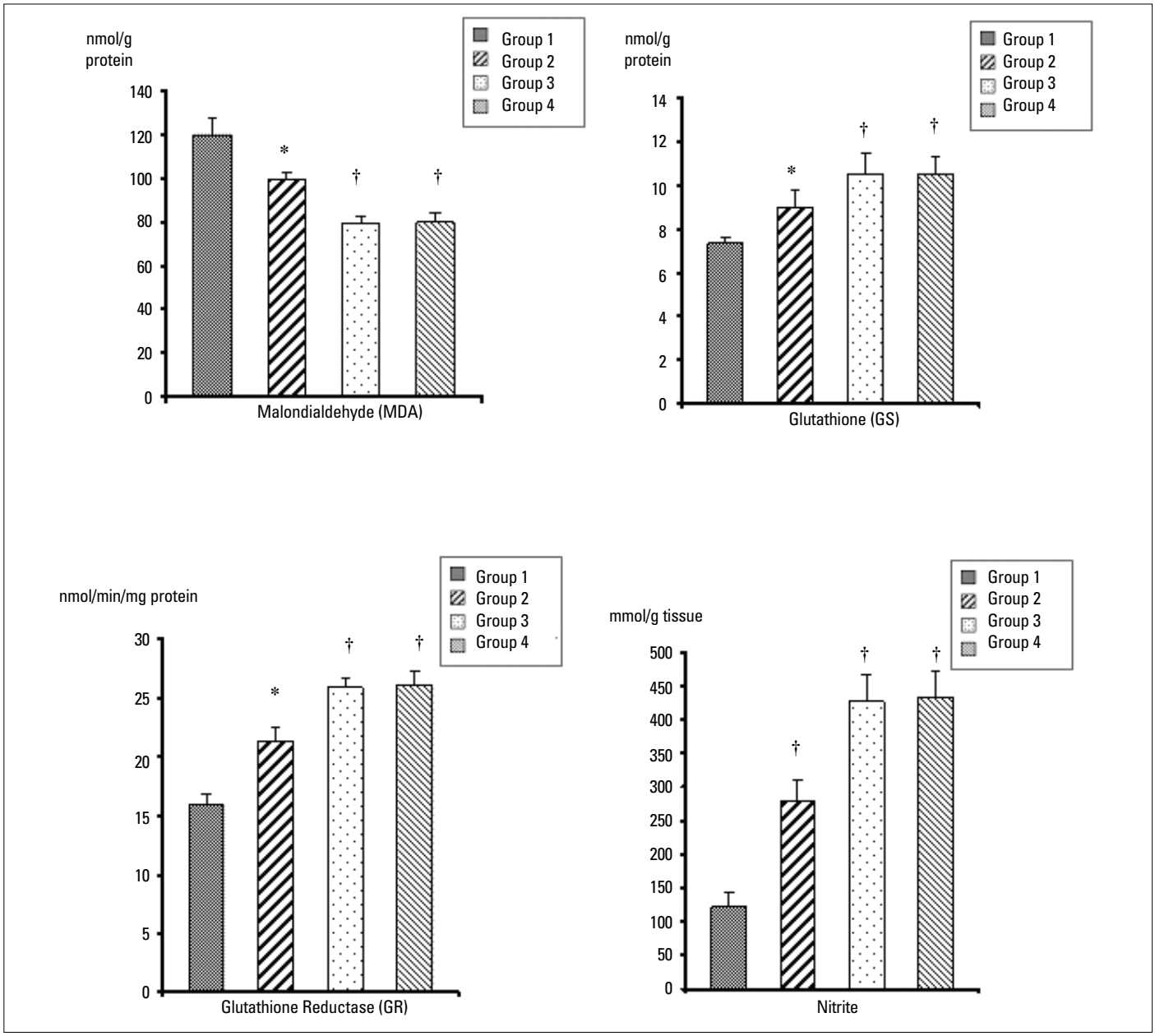

Figure 5. Effects of Quercetin on malondialdehyde (top left), glutathione (top right), glutathione reductase (bottom left) and nitrite (bottom right) measurements
The values are expressed as the mean±SEM
One way ANOVA analysis and post hoc Tukey multiple range test - *p<0.05 and † p<0.01 as compared with control group

quercetin groups compared to control group. In a similar manner, levels of GSH and glutathione reductase that were defense mechanisms against ROS damage were found to be higher in quercetin groups, which indicates that quercetin treatment decreases oxidative stress through its antioxidant properties. Thus, lower levels of ischemic enzymes as well as better hemodynamic performance in quercetin administered groups indicate that I/R injury is of lesser level in these hearts. Furthermore, strong cardioprotective effects obtained by quercetin might be due to preconditioning like effects in addition to antioxidant properties. Earlier, it was demonstrated that quercetin increases the secretion of adenosine, which is an important mediator of preconditioning mechanism (25). Besides, together with quercetin treatment, production of cytoprotective NO increases thereby protecting the tissues from I/R damage. (26). High concentrations of NO in the cardiac tissue may trigger preconditioning mechanism and decrease myocardial ischemia damage (27). Nitrite levels in our study indicated high levels of NO in the myocardial tissue treated with quercetin. This brings forward the pharmacological preconditioning inducing property that contributes to the cardioprotective effects of quercetin. Quercetin and other grape flavonoids increase endothelial NO levels and smooth muscle relaxation through the up-regulation of cyclic GMP and induce vasodilatation (28). This condition explains the high levels of coronary blood flow observed in groups receiving quercetin.

Both hemodynamic data and biochemical parameters pointed out to a better improvement with the use of chronic treatment protocol when compared to acute therapy protocol. This result might be due to effective concentrations of quercetin in the heart following seven days of administration. However, the improvement in the acute dose group was still better than those in control group.

Limitations of the study

The limitation of this study is that we have no opportunity to measure tissue levels of quercetin in the heart.

Conclusion

As a flavonoid that can be consumed in significant amounts in the daily diet; quercetin due to its antioxidant and cytoprotective effects, has the capacity to protect the myocardial tissue against global ischemia and reperfusion injury. In instances where the molecule is administered for the purpose of acute therapy, this significant cardioprotective effect can be observed; however, this potency is further accentuated upon administration as a chronic treatment protocol for seven days. Although we might speculate that the superiority of this chronic treatment protocol in our study was due to having effective tissue concentrations in the heart, we certainly need studies of larger scale to validate this argument.

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