Original Article

Protection of Ischemic and Reperfused Rat Heart by Aqueous Extract of Urtica Dioica

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Background: Urtica dioica (U.D) has widely been used in traditional medicine for its hypotensive and vasodilatory effects. The objective of this study was to clarify the effects of aqueous extract of Urtica dioica on isolated ischemia- reperfused heart.

Methods: The heart of male wistar rats were isolated and perfused according to langendorff method. In the control group (n = 13) the hearts were subjected to three steps of stabilization (30 min), normothermic global ischemia (40 min) and reperfusion (45 min). In addition, before and after ischemia, the aqueous extract of U.D (200 mg/ml) was added to perfusion solution in the test group (n=14). Different cardiac variables including left ventricular pressure, heart rate and coronary flow were measured and rate pressure product was calculated.

Results: Results showed that left ventricular pressure (59.11±4.7) and rate pressure product (13680±1136) in 45th minute of reperfusion in the test group were significantly (P=0.0187 and 0.0321 respectively) greater than the control group (39.1±6.0, 9480±1480) respectively. These findings indicated decreased cardiac damage following ischemia in the test group, compared with that of control group.

Conclusion: Results of the present study showed that the aqueous extract of U.D, increased the tolerance of isolated rat hearts against ischemic damage. This effect can be explained by potent antioxidant activity of the U.D extract, suggesting its clinical use in ischemic heart disease.

Keywords: Ischemia and Reperfusion, Isolated Heart, Urtica Dioica

Introduction

Urtica Dioica (U.D) has widely been used in traditional medicine as hypotensive and anticancer agent in Iran[1] and Turkey.[2] This plant has known biological effects such as, natriuretic and hypotensive in the rat,[3] as well as cardiovascular effects,[4] stimulation of lymphocyte proliferation,[5] activation of neutrophils,[2] antiproliferative effect on human prostate cancer cells[6] and antirheumatic activity.[7] Its antimicrobial, analgesic and antiulcer,[8] and hepatoprotective[9] properties of U.D have also been reported.

The negative inotropic, hypotensive and vasodilatory effects of U.D extract has been reported in another study.[3,4] It has been concluded that vasorelaxing effect of U.D has been mediated by nitric oxide.[4] Despite numerous beneficial effects of U.D in medicine, there are not enough data on the effect of this plant on the ischemic and reperfused heart. In regard to the potent antioxidant activity of U.D reported in other studies[8-10] this plant is a good candidate for reducing myocardial injury in the ischemia and reperfusion.

It has been well known that free radicals produced in ischemia reperfusion are one of the main factors of cellular injury including vascular endothelial damage.[11] Antioxidant substances neutralize or remove free radicals and reduce cardiovascular damage during ischemia.[12] Considering the antioxidant activity of U.D, the aim of this study was to investigate the effect of its extract on ischemia – reperfused isolated rat heart.

Patients and Methods

This investigation was approved by the ethics

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committee of Kermanshah University of Medical Sciences and performed on male wistar rats (300–350 gr). All animals used in present study received human care, in compliance with the institutional animal care guidelines. Animals were anesthetized by intra peritoneal administration of 60 mg/kg Sodium pentobarbital (Sigma, Stein heim, Germany).

Hearts were excised and immediately arrested in ice-cold Krebs Solution (0-4°c). They were then rapidly cannulated and retrogradely perfused through the aorta with krebs Solution (containing in mmol/L: Sodium chloride 118, Sodium hydrogen carbonate 25, Potassium chloride 4.8, Potassium dihydrogen phosphate 1.2, Magnesium sulphate 1.2, Glucose 11 and Calcium chloride 1.2) at PH 7.4.[13] The solution thus prepared was filtered through the whatman paper cat. No 1002 125. The buffer was bubbled with 95% O_2 and 5% Co_2 at 37 Cº and perfusion was performed under a constant pressure of 90 cm H_2O. Following removal of the left arterial appendage, a deflated water filled poly ethylene balloon was inserted through the mitral valve into the left ventricle. This balloon was connected via a rigid polyethylene tube to a pressure transducer (MLT844; AD Instruments), which in turn was linked via a powerlab (model ML825; AD Instruments) to a computer. The balloon volume was adjusted to achieve a stable end diastolic pressure of 5-10 mmHg. The index of myocardial function was left ventricular developed pressure (LVP in mm Hg), which was defined as peak systolic minus end diastolic pressure and heart rate as beat per minute (BPM). Rate pressure product (RPP) was calculated as: RPP = LVP × HR.[13] Coronary flow (CF) was measured by timed collections of the coronary effluent. In control group (n=13), baseline data were recorded after 20-30 minutes stabilization and equilibration period. In the second step, global normothermic ischemia was induced by clamping the aortic cannula. The temperature was maintained at 37Cº by immersing the heart in Krebs medium.[13] Hearts in control group were subjected to global ischemia for 40 minutes followed by the third stage including reperfusion for 45 minutes. The rate of damage resulting from ischemia was then determined by comparing the first stage with different cardiac parameters after passing ischemia.

For the second group (n=14), test stages was performed similar to the first group, differing in that the extract of U.D (200 µg/ml) was added to krebs solution, 10 minutes before ischemia and 10 minutes from the beginning of reperfusion.

Extraction procedure

The maceration method was used for preparation of U.D aqueous extract. The dried and ground preparation of the aerial part (50 gr.) of U.D was infused in 250 ml of water and incubated for 48 hours. Finally, the aqueous extract was filtered and dried by distillation in rotary evaporator.[14]

Statistical analysis

The changes of cardiac parameters were compared by paired (in each group) and unpaired (between groups) t test. Differences were considered to be statistically significant when $P<0.05$.

Results

The values of cardiac parameters in different groups and stages are shown in Table 1.

Cardiac performance was appropriate in two control and test groups at the baseline period as

Table 1. Cardiac parameters before and after exposure to a 40-minutes global normothermic ischemia in the control and test group

Experimental period / Groups	Baseline values				Forty-fifth minute of reperfusion			
	LVP	HR	CF	RPP	LVP	HR	CF	RPP
Control Group (n=13)	81.7±2.4	277.5±7.0	13.8±0.5	22558±213	40.0±6.0	265±29.5	8.4±0.7	9480±1480
Test Group (n=14)	88.2±2.3	275.3±12.1	14.2±0.5	23758±807	59.1±4.7*	233.7±11.1	9.5±1.9	13680±1136**

Left ventricular function of the hearts in different experimental groups. LVP- left ventricular developed pressure (mm Hg), HR- heart rate (beat/min), CF- coronary flow (ml/min) and RPP- rate pressure product (LVP×HR). Data are mean ± SEM of control (n=13) and test groups (n=14). *P=0.0187 and **P= 0.0321 versus control.

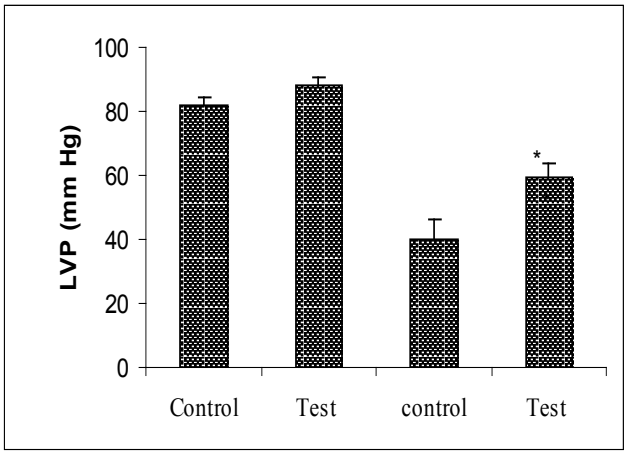

Figure 1: Left ventricular pressure (LVP mm Hg), before and after exposure to a 40 minute global normothermic ischemia in control (n=13) and test groups (n=14). *P=0.0187 versus control.

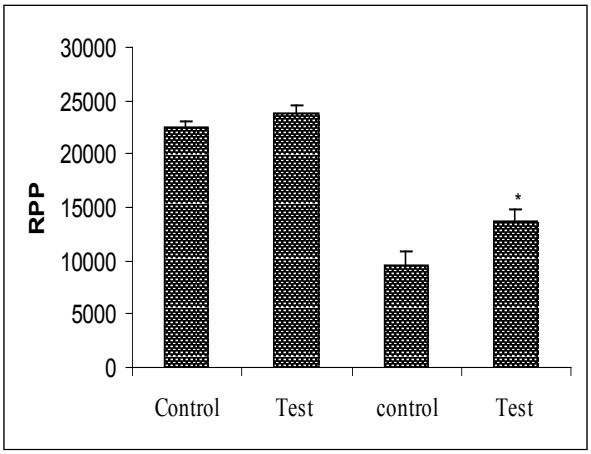

Figure 2. Rate pressure product (RPP= BMP × LVP) at baseline and forty fifth minute of reperfusion following 40 minutes global normothermic ischemia in control (n=13) and test groups (n=14). *P=0.0321 versus control.

represented in the first column of table 1. For example baseline RPP was greater than 20,000 in two groups. Also there were not any significant differences in cardiac parameters of two groups at this period. In the test group, application of the U.D extract (200 µg/ml) before ischemia didn't significantly change cardiac performance (HR, LVP and RPP) in comparison to its baseline. As represented in the second column of table 1, 45 minute reperfusion after 40 minute global normothermic ischemia resulted in ischemia-reperfusion injury and decrease the cardiac performance in two groups in comparison to their related baseline.

However, the decline of cardiac performance in reperfusion period was significantly lower in the test group which received U.D. extract, compared with control group. In this connection, the values of LVP and RPP in test group were significantly greater than those of the control group (Figs. 1, 2). Therefore the ischemia reperfusion injury was decreased in test group.

Our findings showed that there was lesser damage due to ischemia of heart and a more pronounced recovery of the cardiac function in test group. Consistent with these results, the value of left ventricular diastolic pressure after 45 minutes of reperfusion in the control (51.35±2.3 mm Hg) was significantly greater than the test group (38.93±4.06 mm Hg). In this respect, cardiac damage following ischemia led to an increase in diastolic pressure, a further evidence of the lower cardiac damage in the test group (Fig. 3).

Discussion

The findings of the present study demonstrated a reduction in ischemic damage of isolated rat hearts following administration of aqueous extract of U.D. Our results showed a better preservation of the functional cardiac parameters in the reperfusion period, including LVP, RPP and decreased left ventricular diastolic pressure in the test group.

Myocardial ischemia and reperfusion leads to cardiac dysfunction.[11] Decreased cardiac dysfunction in the test group showed the cardioprotective effect of U.D. aqueous extract. It has been shown that ischemia reperfusion results in intracellular calcium overload and leads to cellular contracture, which in turn, increases diastolic pressure.[15] Therefore, the cardio protective effect of U.D. aqueous extract has been confirmed by the fact that the rising of LVP in the present study, was significantly reduced in test group following ischemia- reperfusion. Despite numerous reported therapeutic effects of different parts of this plant, to our knowledge, such cardio-protective effect of U.D has not previously been reported. Negative inotropic and vasodilatory effects of this plant are reported in an in vivo study.[4] Considering the reported toxic effects of high doses of this plant extract,[3] a low dose of aqueous extract of U.D was used in the present study. Thus, the different functional cardiac parameters did not change significantly before ischemia. However, using this extract before and after ischemia resulted in improved heart function in the reperfusion period. Consistent with these findings, the same protective effect on the ischemic striped muscle has been

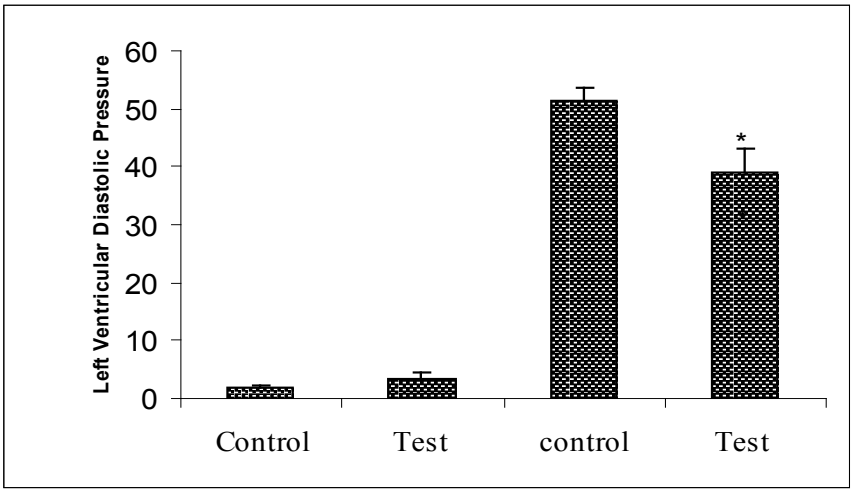

Figure 3. Rate of left ventricular diastolic pressure (mm Hg) at baseline and forty fifth minute of reperfusion following 40 minutes global normothermic ischemia in control (n=13) and test (n=14) groups.*P=0.0153 versus control.

reported in the other study.[16] It has been shown that, U.D. extract pretreatment diminished the antioxidative stress in the ischemic muscle and it has been suggested that U.D. has a cell protection effect against oxidative stress in rat and it is plausible that oxidant activity of this extract leads to a reduction of ischemic damage in stripped muscular tissue.[16] Antioxidant activity of the U.D. extract has been reported in several studies.[17,8] The U.D. extract contained the phenolic compounds. There is a positive relationship between total phenols and antioxidant activity in many plant species.[18] Phenols have scavenging ability because of their hydroxyl groups.[19] It has been reported that the essential phenolic ingredient in the U.D is caffeic malic acid and due to its phenolic compounds, U.D has scavenging ability and stabilizes the lipid peroxidation, which plays an important role in ischemia-reperfusion injury.[16]

In conclusion, the aqueous extract of U.D significantly increased the tolerance of isolated rat heart against damage caused by ischemia-reperfusion. Moreover, in regard to the cardioprotective effect of U.D extract, the consideration of its clinical usage is suggested, and complementary study of its cellular effect is warranted.

Acknowledgments

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