Cardioprotective Effect of Garlic Juice on the Isolated Rat Heart in Ischemia- Reperfusion

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Abstract

Background: It has been reported that the chronic oral administration of garlic homogenate protected the rat heart from in vitro ischemic reperfusion injury. However, the biological effects of garlic juice on the heart are expected to be different from oral administration of it.

Objective: the present study was designed to investigate the effect of garlic juice on the isolated rat heart in ischemia- reperfusion.

Methods: Rat isolated, perfused hearts were subjected to 30 min baseline measurement followed by 40 min normothermic global ischemia and 45 min reperfusion. Garlic juice (0.01 mg/ml) was added to the perfusion solution 20 min before ischemia in the test 1 and 5 min before and 10 min after ischemia in test 2. Different cardiac variables including left ventricular developed pressure (LVDP), heart rate (HR) and coronary flow (CF) were measured. Rate pressure product (RPP) was calculated, and released lactate dehydrogenase (LDH) enzyme in effluent was measured in reperfusion.

Results: Garlic juice significantly increased CF before ischemia in both test groups. The released LDH enzyme at the first minute and the recovery of RPP and LVDP on the 45th minute of reperfusion were significantly better in the test group 2 in comparison to the control.

Conclusion: The result of the present study shows that garlic juice has a vasodilator activity and protects the isolated ischemic rat heart when it was administrated in reperfusion. It is probably mediated by antioxidant activity of allicin as a principal bioactive compound of garlic juice.

Keywords: Isolated rat heart, Garlic juice, Myocardial function, Ischemia-reperfusion

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Introduction

Garlic (*Allium sativum*) grows in all parts of the world and the bulb has been used extensively in both traditional and popular medicine [1]. It's reported beneficial actions include: antidiabetic, antimicrobial, antithrombotic, antihypertensive, antihyperlipidemic, antitumor, and antioxidant effects [2 - 6]. Oxidative stress plays a major role in the biochemical and pathological changes associated with myocardial ischemic-reperfusion injury [7]. Use of plant extracts has been identified as a promising therapeutic approach to combat oxidative stress associated with ischemic heart disease [8]. The antioxidant properties of garlic are well documented [9] and it has been shown that many of the medicinal properties of garlic are associated with its antioxidant properties [10]. Both epidemiological and experimental studies have claimed that garlic has significant beneficial effects in ischemic heart disease [11, 12]. Raw garlic is the commonest way of garlic consumption. It has been shown that chronic oral administration of garlic homogenate protected the rat heart from in vitro ischemic reperfusion injury, oxidative stress [13] and oxidative damage induced by ischemia and reperfusion [7]. Raw garlic homogenate augmented endogenous antioxidants along with reduction of basal lipid peroxidation in rat heart in a dose dependent manner [13]. Furthermore, it has been found that an aqueous extract of raw garlic scavenges hydroxyl radicals, superoxide anion and inhibits lipid peroxidation [7, 14]. Allicin (allyl 2-propenethiosulfinate or diallyl thiosulfinate) is thought to be the principal bioactive compound present in aqueous garlic extract or raw garlic homogenate. When garlic is chopped or crushed, allinase enzyme, present in garlic, is activated and acts on alliin (present in intact garlic) to produce allicin [13]. In addition, it has been shown that garlic exerts its therapeutic effect by increasing nitric oxide production [15, 16] and it has been demonstrated that garlic and the active metabolite allicin are capable of eliciting a (nitric oxide) NO-dependent relaxation in rat-isolated arteries, which is mediated via activation of NO formation [17]. Meanwhile garlic/allicin has a unique vasoregulatory effects [18], it has been reported that allicin is virtually undetectable in blood circulation after garlic ingestion because it decomposes to form other compounds [19]. Allicin is an unstable [9, 19] and short – lived compound [20] which has not been detected in serum or urine after ingestion of raw garlic [10] and it was found to disintegrate in the blood a few minutes after its administration, both in vitro in human blood and in vivo in rats [20]. Therefore, the biological effects of garlic juice on the heart are expected to be obviously different from oral administration of it. Although there are many reports about the effect of chronic oral administration of raw garlic on the cardiovascular system, there aren't enough data about the effect of garlic juice on the ischemic heart. Regarding to this fact that different garlic preparations have different properties, the aim of this study was to examine the possible beneficial effects of garlic juice on the myocardial function of the isolated rat heart in ischemia and reperfusion.

Materials and Methods

General

All experiments were approved by the Ethics Committee of Kermanshah University of Medical Sciences, and all animals used in the present study received humane care in compliance to institutional animal care

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guidelines.

Preparation of garlic juice

Garlic was purchased from the local market. The peeled garlic cloves were weighed and finely grinded in pestle and mortar. Garlic paste was squeezed out through double cheesecloth to obtain the extract. Next, the extract was passed through Watman filter paper Grade 40 (8 µm) and was stored at –20 degrees of centigrade [3]. The processes yielded 48.5 ml of garlic juice with solid content of 282-mg/ml. Dilutions were prepared in distilled water on the day of the experiment.

Experimental preparation

Male Wistar rats (250 - 350 gr) were anaesthetized by intraperitoneal administration of 60 mg/kg pentobarbital sodium (Sigma). Hearts were excised and immediately arrested in ice-cold Krebs solution. The hearts were rapidly cannulated and retrogradely perfused through the aorta in a noncirculating Langendorff apparatus with Krebs solution (containing in mmol/Lit: NaCl 118, NaHCO3 25, KCl 4.8, KH2PO4 1.2, MgSO4 1.2, Glucose 11 and CaCl2 1.2) at pH 7.4. The buffer was bubbled with 95% O2 5% CO2 at 37°C and perfusion was performed under a constant hydrostatic pressure of 90 cm H2O. Following removal of the left atrial appendage, a deflated water filled latex balloon was inserted through the mitral valve in to the left ventricle. This balloon was connected via a rigid polyethylene tube to a pressure transducer (MLT 844; AD Instruments), which in turn was connected via a power lab (model ML825; AD Instruments) to a computer for continuous monitoring of cardiac performance. At the beginning of the experiment, the balloon volume was adjusted to achieve a stable end diastolic pressure of 5-10 mmHg. This volume was then kept constant for the duration of the study. The index of myocardial function was left ventricular developed pressure (LVDP), which was defined as peak systolic pressure minus end diastolic pressure, heart rate (HR; cardiac spontaneous rhythm was counted per min), and the rate pressure product (RPP=LVDP×HR). Coronary flow was measured by timed collections of the coronary effluent [21].

Experimental protocol

Baseline data were recorded after a 30 min stabilization and equilibration period. Global normothermic ischemia was induced by clamping the aortic cannula. The temperature was maintained by immersing the heart in perfusion medium at 37°C. Hearts in the control group (n=8) were subjected to global ischemia for 40 min, followed by reperfusion for 45 min. In addition to this protocol, hearts in the test group 1 (n=8) were perfused for 20 min before ischemia, with krebs solution, containing10mg/Lit Garlic juice. In the test group 2 (n=7), the hearts were perfused for 5 minutes before and 10 minutes after ischemia with the Krebs solution containing same concentration of the Garlic juice.

The level of ischemia-reperfusion injury was assessed based on the maximum contracture during ischemia, functional recovery and the release of lactate dehydrogenase (LDH) in reperfusion. In order to measure the LDH, coronary effluent was collected at the first minute of reperfusion. The samples were measured using a Cell Cytotoxicity detection kit (LDH) (Roche) using known quantities of LDH (Sigma) as a standard.

Analysis of results

Results are expressed as mean ± S.E.M. Comparisons between datasets were made using paired or unpaired t-test as appropriate

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or ANOVA with a Tukey post test as offered by Graphpad Instat Soft Ware (Version 3.05). Differences were considered to be statistically significant when $p<0.05$.

Results

Cardiac function and coronary flow

The functional parameters including heart rate, left ventricular developed pressure, RPP, and CF at baseline and on the 45th minute of reperfusion are summarized in Table 1. There were no significant differences between groups at baseline. The RPP and CF averages in control group didn't significantly vary throughout the time of experiment before ischemia. Conversely in the test groups, the mean of CF after perfusion of Garlic juice significantly increased in comparison to the baseline in the test groups 1 (12.18 ± 0.68 to 15.85 ± 0.7) and 2 (12.5 ± 1.07 to 14.28 ± 1.13 ml/min) (Figure 1). Also, in the test group 2, which received garlic juice in reperfusion, coronary flow significantly increased at 10^{th} minute of reperfusion (9.58 ± 0.8) in comparison to the control (7.52 ± 0.49) (Figure 2). During ischemia, there weren't any significant differences among the control, test 1 and test 2 groups in maximum contracture (55± 9.24, 61.4± 4.75 and 47± 5.49 mmHg), respectively. However, in the reperfusion period, the recoveries of RPP and LVDP on the 45th minute of reperfusion were significantly better in the test group 2, in comparison to the control (Table 1).

Lactate dehydrogenase (LDH) release

The extent of reperfusion injury in the three groups of hearts was determined from the release of a marker intracellular enzyme into the effluent. The released LDH during the first minute of reperfusion from the hearts in the control, test 1 and test 2 were 2.26 ± 0.16, 2.07 ± 0.35 and 1.28 ± 0.14 miliU/gr of hearts, respectively. The amount of released enzyme was significantly ($p<0.05$) lower in the test group2 in comparison to the control.

Functional recovery percentage

Meanwhile, there aren't significant differences between the test group 1 and the control; in the test group 2 which were treated with garlic juice in reperfusion the recovery percentages of RPP (76.66 ± 7.39%) and LVDP (87.83 ± 6.95) were significantly greater than the control, (48.59 ± 4.38) and (60.06 ± 6.13) respectively, (Fig. 3A & B).

Table 1- The effect of Garlic juice on myocardial function and coronary flow, before and after exposure to 40-minute global normothermic ischemia

Periods & Parameters / Groups	Baseline Values				45 th min of Reperfusion			
	LVDP	HR	CF	RPP	LVDP	HR	CF	RPP
Control	87 ± 4.1	242 ± 3.83	13.7 ± 0.9	21299 ± 1060	52 ± 4.9	202 ± 17	7.6 ± 0.5	10210 ± 860
Test 1	90 ± 5.91	253 ± 10.27	12.1 ± 0.6	22519 ± 1586	63 ± 2.9	217 ± 18	7.6 ± 0.8	14078 ± 1668
Test 2	79 ± 6.2	265 ± 13	12.5 ± 10	20675 ± 1246	67 ± 3.7*	227 ± 7.6	8.7 ± 0.7	15470 ± 1130*

Note: Left ventricular function of Langendorff perfused hearts With/without garlic juice treatment. LVDP: left ventricular developed pressure (mmHg), Heart rate (beat/minute), CF: (Coronary Flow) (ml/min), RPP: (Rate pressure product) (LVDP×HR). Data are means ± SEM of control (n=8), Test 1 (n=8) and test 2 (n=7). *p< 0.05, versus control.

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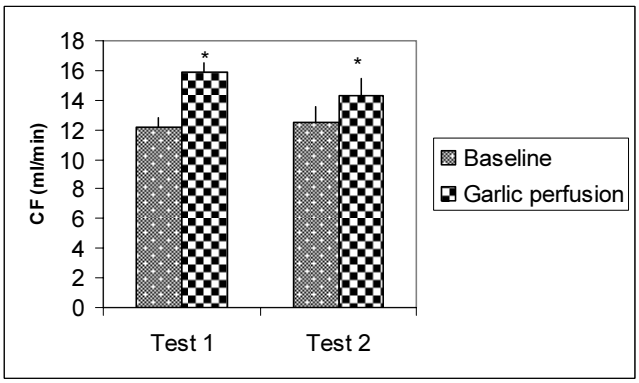


Fig. 1- Coronary flow of the hearts in test groups, before and after garlic juice treatment. Data shown are mean ± SEM of test groups 1 (n=8) and 2 (n=7). * p<0.01 versus baseline

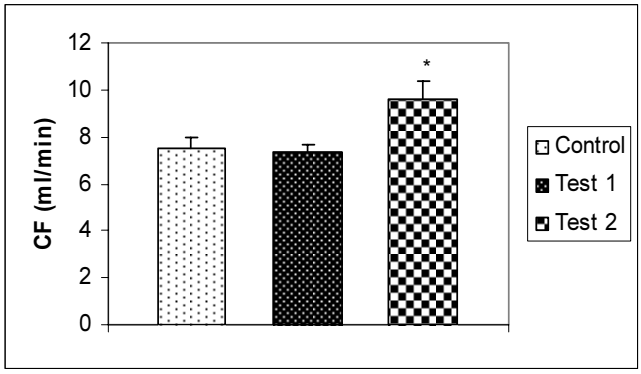


Fig. 2- Coronary flow of the hearts on the 10th minute of reperfusion in the control, test group 1 and test group 2 (in the presence of garlic juice). Data shown are mean±SEM of control (n=8), test groups 1 (n=8) and 2 (n=7). * p<0.05 versus control

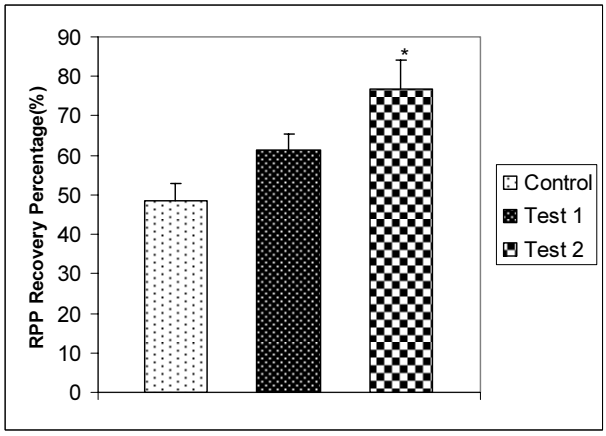


Fig 3A

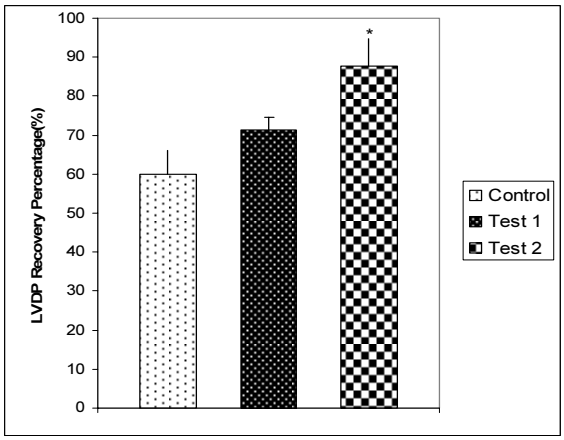


Fig 3B

Fig. 3 - Recovery percentage of the (RPP) rate pressure product (Fig. 3A), and (LVDP) left ventricular developed pressure (Fig. 3B) at the 45th minute reperfusion following the 40-minute global normothermic ischemia in the control and test groups. Data are expressed as the mean ± SEM of control (n=8), Test 1 (n=8) and test 2 (n=7). *p<0.01 versus control

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Discussion

In the present study, the effects of garlic juice on the myocardial function of the ischemia-reperfused isolated rat heart were studied. On the basis of the functional recovery and released LDH from the heart in reprfusion, data showed that the treatment with Garlic juice, before ischemia and during reperfusion, significantly protected the heart and myocardial function in the ischemia-reperfusion. This is a new finding about the direct effect of garlic juice on the heart. Numerous reports have been demonstrated that the allicin is responsible for most biological activities of garlic [18, 22-24]. Since alliinase and alliin are enclosed in different compartment within the garlic clove cells, intact garlic cloves do not contain allicin. When the garlic clove is crushed, alliin and alliinase intract, to form allicin which is thought to be the principal bioactive compound present in aqueous garlic extract or raw garlic homogenate [13]. Hence, on the basis of this fact, allicin is probably responsible for cardioprotective effect of garlic juice on the ischemia-reperfused isolated rat hearts in the present study. In the other study, it has been demonstrated that treatment with allicin-containing garlic significantly protected the rat heart against monocrotaline-induced coronary endothelial dysfunction and prevented the development of right ventricular hypertrophy. It has been concluded that the active garlic ingredient allicin, is most likely responsible for this protective effect [18]. Also, it has been shown that allicin improves postischemic pulmonary artery flow when it was intravenously injected in the beginning of reperfusion [22]. It has been suggested that this protective effect of garlic is mediated via the allicin action on coronary endothelial function and vasoreactivity [18]. Vasodilator action of allicin has been reported in different tissues including feline mesenteric vascular bed [25] and in the pulmonary vascular bed of the rat [26]. Also, it has been shown that garlic improved endothelial function in humans with coronary artery diseases [27]. Consistent with these reports, garlic juice in the present study has significant vasodilator effect on the coronary vessels of the isolated rat heart and significantly increased the coronary flow in both test groups before ischemia and in the test group 2 during reperfusion. It has been reported that allicin has significant vasodilator activity in the pulmonary vascular bed of rat; meanwhile its different metabolites do not posses this vasodilator action. On the basis of this finding, it has been suggested that the allicin analogs should be developed for clinical use [26]. Consistent with these results the finding of the present study confirms the beneficial effects of allicin and according to that suggestion; stable allicin analogs might be useful for clinical purposes. However, garlic juice didn't significantly change the maximum contracture in ischemia and it didn't significantly improve the cardiac performance and its LDH release in the test group 1, which received garlic juice only before ischemia. On the other hand, garlic juice has a cardioprotective effect when it was administrated in reperfusion (Test group 2). It has been shown that myocardial ischemia can lead to cellular damage, not only during ischemia itself, but also during subsequent reperfusion. The reperfusion injury can be reduced by modifying conditions of reperfusion and it has been reported that some drugs have greater protective effect against reperfusion injury than against ischemic injury in isolated hearts [28]. Therefore, this is another new finding of the present study,

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which shows the protective effect of garlic juice in reperfusion, and it could probably be explained by the antioxidant activity of the garlic juice. There are many studies and documents regarding the antioxidant activities of garlic extract and juice [10, 22, 24, 29, 35]. Allicin, as a sulfur-containing compound extracted from garlic, has an antioxidant activity [22] and prevents the lipid peroxidation of liver homogenate in a concentration-dependent manner [24]. In addition, allicin can prevent the formation of free radicals [20, 30-32] and can scavenge the chain-carrying peroxyl radicals of the substrates by transferring its allylic hydrogen to the oxidized substrate [33]. Garlic also has a high phenolic content [34] and it has been shown that the antioxidant activities of garlic extract are directly related to contents of phenolic compounds [35]. Therefore, consistent with the other previous studies, the cardioprotective effect of the garlic juice in the present investigation is probably mediated via the vasoregulatory effects of garlic juice and its antioxidant activity in the ischemia-reperfusion. However, the exact role of these two different mechanisms in cardio protection remains to be elucidated in the future study.

Conclusion

The result of the present study shows that garlic juice protected the isolated ischemic rat heart when it was administrated in reperfusion. It is probably mediated by vasoregulatory effects and antioxidant activity of allicin as a principal bioactive compound of garlic juice.

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