

Resistance to Reperfusion Injury Following Short Term Postischemic Administration of Natural Honey in Globally Ischemic Isolated Rat Heart

Haleh Vaez¹, Mehrban Samadzadeh¹, Fahimeh Zahednezhad¹, Moslem Najafi^{2,3*}

¹ Student Research Committee, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.

² Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

³ Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.

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ABSTRACT

Purpose: Results of our previous study revealed that preischemic perfusion of honey before zero flow global ischemia had cardioprotective effects in rat. The present study investigated potential resistance to reperfusion injury following short term postischemic administration of natural honey in globally ischemic isolated rat heart. **Methods:** Male Wistar rats were divided into five groups (n=10-13). The rat hearts were isolated, mounted on a Langendorff apparatus, allowed to equilibrate for 30 min then subjected to 30 min global ischemia. In the control group, the hearts were reperfused with drug free normal Krebs-Henseleit (K/H) solution before ischemia and during 120 min reperfusion. In the treatment groups, reperfusion was initiated with K/H solution containing different concentration of honey (0.25, 0.5, 1 and 2%) for 15 min and was resumed until the end of 120 min with normal K/H solution. **Results:** In the control group, VEBs number was 784 ± 199 , while in honey concentration of 0.25, 0.5, 1 and 2%, it decreased to 83 ± 23 ($P < 0.001$), 138 ± 48 ($P < 0.01$), 142 ± 37 ($P < 0.001$) and 157 ± 40 ($P < 0.01$), respectively. Number and duration of VT and time spent in reversible VF were also reduced by honey. In the control group, the infarct size was $54.1 \pm 7.8\%$, however; honey (0.25, 0.5, 1 and 2%) markedly lowered the value to 12.4 ± 2.4 , 12.7 ± 3.3 , 11.3 ± 2.6 and 7.9 ± 1.7 ($P < 0.001$), respectively. **Conclusion:** Postischemic administration of natural honey in global ischemia showed protective effects against ischemia/reperfusion (I/R) injuries in isolated rat heart. Antioxidant and radical scavenging activity, lipoperoxidation inhibition, reduction of necrotized tissue, presence of rich energy sources, various type of vitamins, minerals and enzymes and formation of NO-contain metabolites may probably involve in those cardioprotective effects.

Introduction

Since ancient times, honey has been used both as a food and medicine.¹ It has been reported to contain about 200 substances,² mainly sugars (95-99% of honey dry matter) and water. Minerals comprise small quantities (0.17%) of honey; contain calcium, copper, iron, manganese, phosphorus and potassium as the most abundant. Enzymes in honey composition are mainly invertase (saccharase), diastase (amylase) and glucose oxidase. Main vitamins that exist in honey are Vit C and B group.³ More than 60 species of bacteria including Gram-positives and Gram-negatives, aerobes and anaerobes are inhibited by natural honey.⁴ It has been shown that honey can inhibit the activation of HIV-1 in latent models of infection.⁵ Also, honey at various concentrations could inhibit the growth of Herpes Simplex Virus-1.⁶ Several studies describe anti-ulcerous capacity of honey.^{7,8} As a wound barrier, it has been used to treat infections in various types of wounds such as burns, abscesses, diabetic and pressure-

caused ulcers, untreated graft donor sites, and infected wounds.⁹ Positive effect of honey as an anticarcinogenic agent was reported in some previous studies.¹⁰⁻¹² The anti-inflammatory action of honey was seen in the previous reports.¹³⁻¹⁵ In addition, antioxidant capacity of honey was previously reported by some research groups.¹⁶⁻¹⁹ Clinically, it has been shown that natural honey consumption in a defined dose improved cardiovascular risk factors and decreased total cholesterol, LDL-C, triacylglycerole, fasting blood glucose, C-reactive protein, and increased HDL-C in overweight or obese people.^{20,21} In animal models, protective effects of chronic oral honey administration against ischemia/reperfusion (I/R)-induced arrhythmias were demonstrated.²² In another study, short term perfusion of enriched Krebs solution with natural honey in a state of preconditioning had antiarrhythmic activity in isolated rat heart.²³ More recently, we showed that preischemic administration of honey

*Corresponding author: Dr. Moslem Najafi (PhD), Department of Pharmacology and Toxicology, Faculty of Pharmacy, Tabriz University of Medical Sciences, 5166414766, Tabriz, Iran. Tel: +98 (411) 3372250, fax: +98 (411) 3344798, E-mail: najafimoslem@yahoo.com

before zero flow global ischemia had cardioprotective effects in rat.²⁴ In the present study, the effects of postischemic perfusion of natural honey on global myocardial ischemia/reperfusion injury were investigated in male rats.

Materials and Methods

Animals

Adult male Wistar rats weighing 270-300 g were supplied by the Laboratory Animal Center, Medical Sciences University of Tabriz, Iran. The rats were divided randomly into five groups (n=10-13) and were acclimated for at least 1 week at a temperature of 21 ± 3 °C and humidity $55\pm 5\%$. The animals were maintained with free access to standard rat food and tap water. The experiments reported were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No 85-23, revised 1985).

Chemicals

Honey (Oskou, East Azerbaijan, Iran), Triphenyltetrazolium chloride (TTC, Sigma), Formalin, NaCl, NaHCO₃, KCl, KH₂PO₄, MgSO₄, CaCl₂, D-glucose (Merck Company), Sodium pentobarbital (Kela Company, Belgium) and Heparin (Daru-pakhsh Company, Iran).

Experimental protocols

To prepare buffer solution for treatment groups, honey was dissolved in Krebs-Henseleit (K/H) solution for providing its different concentrations (0.25, 0.5, 1 and 2%). The rats were anesthetized by pentobarbital sodium (50 mg/kg) and heparinized (300 IU) via intraperitoneal injection.²² The rat hearts were immediately excised and kept in ice cold oxygenated K/H solution (pH=7.4), before the aorta was cannulated on Langendorff apparatus. After mounting the hearts on a non-recirculating langendorff apparatus, they were perfused under constant pressure of 100 mmHg at 37 °C and perfused with K/H solution that previously equilibrated with 95% O₂-5% CO₂.^{22,25} A water-filled polyvinylchloride balloon was attached to a pressure transducer and inserted into the left ventricle (LV) through an incision in the left atrium.^{22,25} The hearts were allowed to equilibrate for 30 min (stabilization) and then subjected to 30 min no flow global ischemia by halting solution perfusion. In the control group, the hearts were reperfused with normal K/H solution for 120 min immediately after global ischemia. However, in the all treated groups, reperfusion was initiated with K/H solution containing different concentration of honey for 15 min and then resumed until the end of 120 min with drug free normal K/H solution.

Evaluation of arrhythmias and infarct size

The ECGs were recorded and analyzed according to the diagnostic criteria advocated in the Lambeth conventions²⁶ for determining cardiac arrhythmias

including number of ventricular tachycardia (VT), number of total ventricular ectopic beats (VEBs=Single+Salvos+VT), duration and incidence of VT and ventricular fibrillation (VF) during first 30 min of reperfusion.

To determine the infarct size, at the end of the 120 min reperfusion period, the hearts were frozen at -20 °C then the ventricle of the hearts were sliced transversely in a plane perpendicular to the apico-basal axis into 2 mm-thick sections.²⁵ The slices then incubated by 1 % (w/v) TTC solution in phosphate buffer (NaHPO₄, 88 mM; NaH₂PO₄ 1.8 mM, pH= 7.4) for 15 min at 37 °C to dye the non-infarcted region.^{25,27} The tissue slices were then fixed in 10% formalin for 24 h and then placed between two glass cover sheets. The sheets cause that the tissue color can clearly be seen also makes a convenient flat surface for directly tracing the dimensions of the infarct and risk zone on a transparent sheet. The slices were drawn onto transparent sheets then by using a computerized planimetry package, the percentage of infarcted tissue within the volume of myocardium at risk was calculated.^{25,27}

Statistics analysis

Data was expressed as Means±SEM except for the incidence of VT and VF which were indicated as percentage. Differences in the incidences of arrhythmias were analyzed by using Fisher's exact test (with Yates correction). The group means were compared by non-parametric Mann-Whitney U-test. The infarct size was analyzed by one way ANOVA. Post-hoc comparisons were done using LSD. Differences were considered as significant for P<0.05.

Results

The effects of different concentrations of natural honey on I/R-induced injuries are summarized in Tables 1-3. As shown in Table 1 and Figure 1, perfusion of K/H solution containing honey for 15 min in the initiation of reperfusion significantly decreased the number of single ectopic beats by all used concentrations. The number of salvos arrhythmias was reduced from 221 ± 100 in the control rats to 8 ± 4 , 10 ± 4 , 14 ± 4 and 4 ± 1 (P<0.01) by 0.25, 0.5, 1 and 2% of honey solution, respectively. In addition, honey markedly lowered the total number of VEBs compared to the control group (Figure 2). In the control group, VEBs number was 784 ± 199 , while in honey concentrations of 0.25, 0.5, 1 and 2%, it was decreased to 83 ± 23 (P<0.001), 138 ± 48 (P<0.01), 142 ± 37 (P<0.001) and 157 ± 40 (P<0.01), respectively. The amount of 153 ± 55 in the control group for VT number was changed to 17 ± 9 (P<0.01), 41 ± 23 (P<0.05), 54 ± 30 (P<0.05), and 72 ± 44 , respectively. At the same time and as shown in Figure 3, duration of VT was also lowered by natural honey from the control value (43 ± 21 sec) to 6 ± 5 , 7 ± 4 , 8 ± 4 (P<0.05 for all) and 9 ± 5 sec in 0.25, 0.5, 1 and 2% of honey, respectively. The time spent in reversible VF showed no-significant reduction by the lower honey

concentrations (0.25, 0.5 and 1%). Moreover, there were no significant differences for incidences of VT, reversible VF, irreversible VF and total VF between the treatment and control groups (Table 2).

In the control group, the percentage of infarct size was 54.1±7.8, while in honey concentration of 0.25, 0.5, 1 and 2%, the value was markedly lowered to 12.4±2.4, 12.7±3.3, 11.3±2.6 and 7.9±1.7 (P<0.001 for all),

respectively (Figure 4). In addition, the same concentrations of honey significantly reduced the infarcted volume from 832±122 mm³ (control) to 125±24, 118±32, 104±25 and 66±16 mm³ (P<0.001), respectively. Risk zone volume (area at risk) in all groups was similar and did not show significant differences between groups (Table 3).

Table 1. Effects of honey (0.25, 0.5, 1 and 2 %) on cardiac arrhythmias during 30 min reperfusion in isolated rat hearts.

Groups	Single number	Salvos number	VT number	VEBs number	VT duration (Sec)	Rev VF duration (Sec)
Control	409±94	221±100	153±55	784±199	43±21	240±76
Honey (0.25 %)	57±14**	8±4**	17±9**	83±23***	6±5*	219±98
Honey (0.5 %)	87±28*	10±4**	41±23*	138±48**	7±4*	218±100
Honey (1 %)	73±15**	14±4**	54±30*	142±37***	8±4*	155±51
Honey (2 %)	79±29**	4±1***	72±44	157±40**	9±5	579±239

Data are represented as Mean±SEM. *P<0.05, **P<0.01, ***P<0.001 versus the control group. VT; ventricular tachycardia, VEBs; ventricular ectopic beats (Single+Salvos+VT), Rev VF; reversible ventricular fibrillation. N=10-13 rats in each group.

Table 2. Effects of honey (0.25, 0.5, 1 and 2 %) on the incidence of cardiac arrhythmias during 30 min reperfusion in isolated rat hearts.

Groups	VT Incidence (%)	Rev VF Incidence (%)	Irr Rev VF Incidence (%)	Total VF Incidence (%)
Control	66	66	27	93
Honey (0.25 %)	50	100	0	100
Honey (0.5 %)	60	100	0	100
Honey (1 %)	40	90	10	100
Honey (2 %)	55	100	0	100

VT; ventricular tachycardia, Irr Rev VF; Irreversible ventricular fibrillation. N=10-13 rats in each group.

Table 3. Effects of natural honey on myocardial infarction size after 30 min zero flow global ischemia followed by 120 min reperfusion in isolated rat hearts.

Groups	Risk zone vol. (mm) ³	Infarcted vol. (mm) ³	Infarct size (%)
Control	1402±99	832±122	54.1±7.8
Honey (0.25 %)	1028±32	125±24***	12.4±2.4***
Honey (0.5 %)	897±22	118±32***	12.7±3.3***
Honey (1 %)	874±19	104±25***	11.3±2.6***
Honey (2 %)	814±23	66±16***	7.9±1.7***

Data are represented as Mean±SEM. ***P<0.001 versus the control group. N=10-13 rats in each group.

Discussion

In the present study, potential protective effects of postischemic administration of natural honey on cardiac arrhythmias and infarct size were investigated in globally ischemic isolated rat heart.

The results of this study clearly showed that treatment with natural honey reduced the total number of different ectopic beats (single, salvos, VT) and also the incidence and duration of ventricular arrhythmias including VT and VF. These effects are ultimately

important, not only because ischemic heart diseases are a leading cause of death, but also using honey as a natural nutritional agent is a novel approach. Present results are substantiated by the results of previous work showing that long time oral honey administration to rat (feeding of rats by different concentrations of honey for 45 days) could reduce arrhythmias and infarct size after I/R.²² In addition, we showed that short term perfusion of natural honey as a preconditioning agent caused antiarrhythmic activity in isolated rat heart.²³

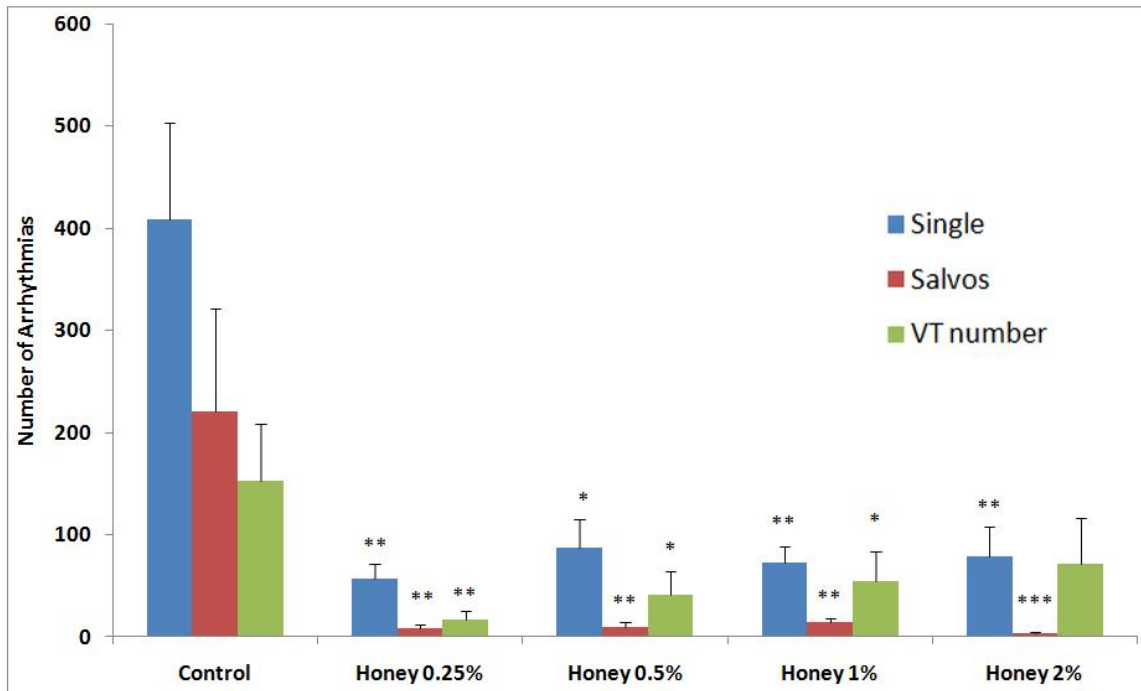


Figure 1. Number of single, salvos and VT in the control and treated groups receiving 0.25, 0.5, 1 and 2 % of honey for 15 min at the beginning of reperfusion. Data are represented as Mean±SEM. *P<0.05, **P<0.01, ***P<0.001 versus the control group. N=10-13 rats in each group.

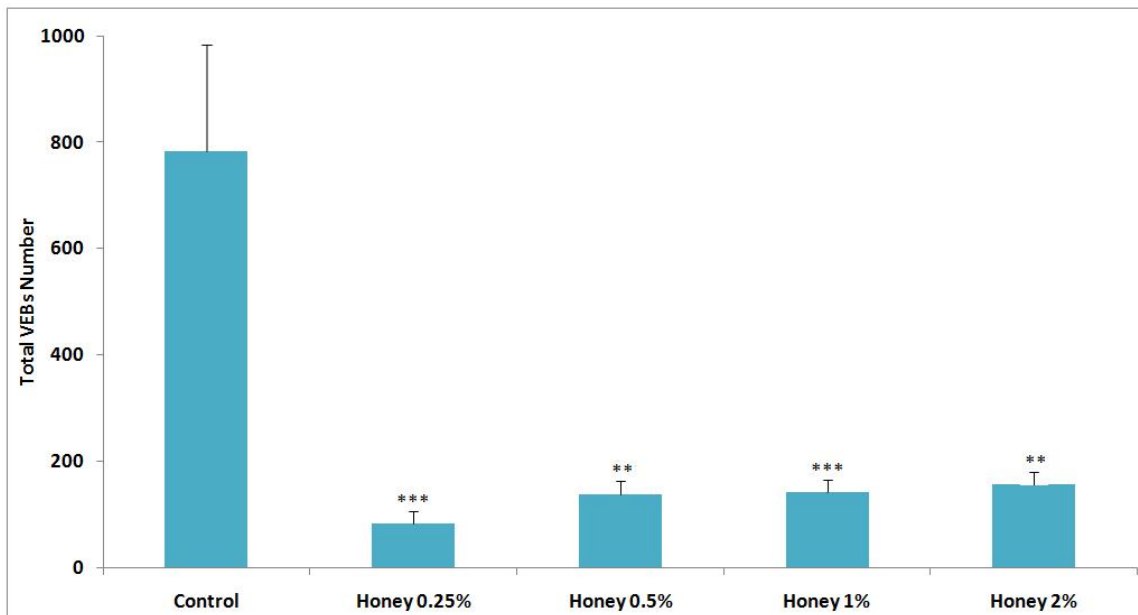


Figure 2. The total number of ventricular ectopic beats (VEBs) in the control and treated groups receiving 0.25, 0.5, 1 and 2 % of honey for 15 min at the beginning of reperfusion. Data are represented as Mean±SEM. **P<0.01, ***P<0.001 versus the control group. N=10-13 rats in each group.

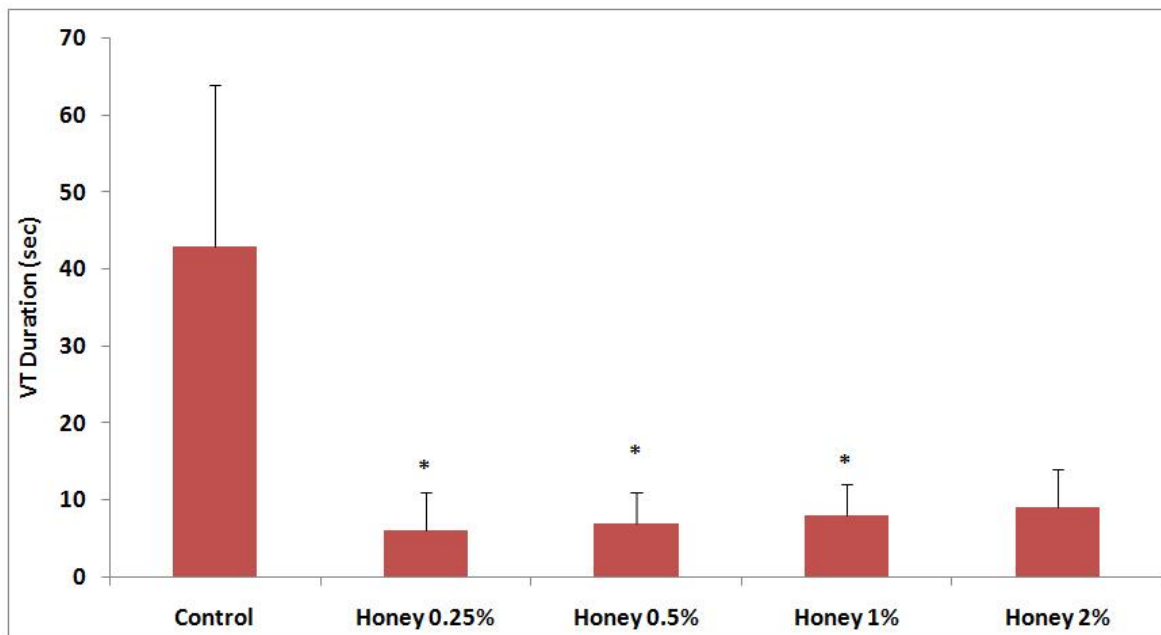


Figure 3. Duration of ventricular tachycardia (VT) in the control and treated groups receiving 0.25, 0.5, 1 and 2 % of honey for 15 min at the beginning of reperfusion. Data are represented as Mean±SEM. *P<0.05 versus the control group. N=10-13 rats in each group.

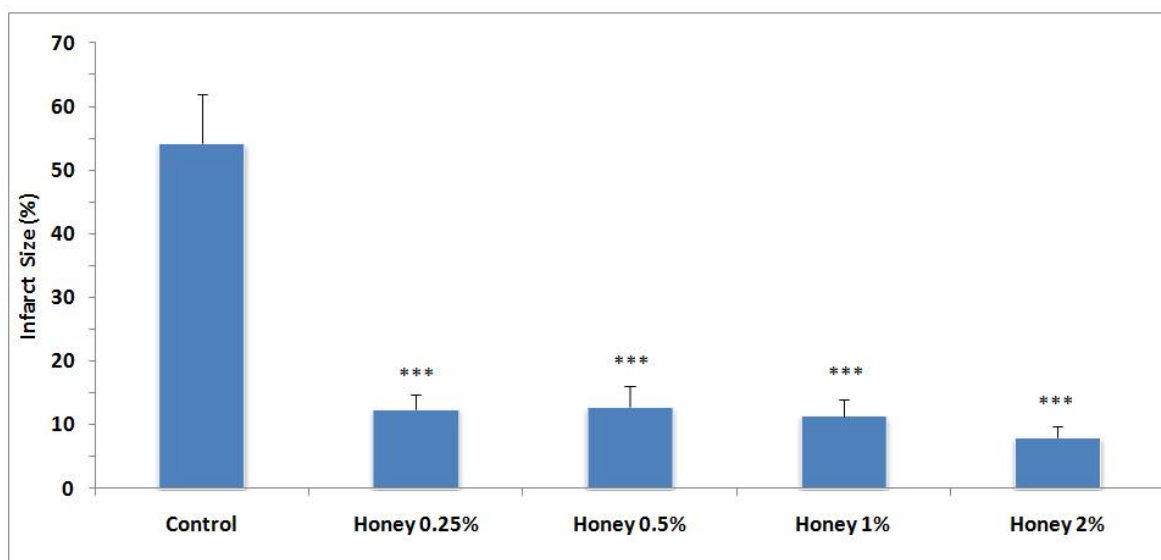


Figure 4. Myocardial infarction size in the control and treated groups receiving 0.25, 0.5, 1 and 2 % of honey for 15 min at the beginning of 120 min reperfusion. Data are represented as Mean±SEM. ***P<0.001 versus the control group. N=10-13 rats in each group.

In another study at the set of regional ischemia, pharmacologic postconditioning by using natural honey produced similar protective effects against I/R-induced arrhythmias.²⁸ More recently, preischemic administration of honey for 15 min before 30 min zero flow global ischemia had marked and significant reduction in the number, duration and incidence of most arrhythmia types after I/R.²⁴ Basically, the mechanism of action of honey is not clear completely; however, honey contains many trace elements, such as copper, zinc, antioxidants, and unidentified materials. Its content of fructose and glucose and ratio of them might have an important role in such useful

functions.^{20,21} In addition, it seems that the beneficial effects of natural honey in heart may in part be relevant to antioxidant capacity. When cells suffer from I/R injury, the sudden generation of reactive oxygen species (ROS) can dramatically disrupt the balance between ROS and antioxidants in injured tissue and cause an increased demand on the antioxidant defense system. In such situation, natural antioxidants including superoxide dismutase (SOD), catalase and glutathione peroxidase are depleted gradually, accompanied by accumulation of ROS. In this dilemma, natural products can play a significant role in neutralizing ROS and increasing the activity of natural antioxidants.

Honey has been found to contain significant antioxidant agents including glucose oxidase, catalase, ascorbic acid, flavonoids, phenolic acids, carotenoid derivatives, organic acids, Maillard reaction products, amino acids and proteins.²⁹ It has demonstrated that honey can increase the antioxidant level of body such as β -carotene by 3%, vitamin C concentration by 47% and glutathione reductase by 7%.²⁰ Rakha et al. (2008) showed that natural wild honey may exert its cardioprotective and therapeutic effects against epinephrine-induced cardiac disorders and vasomotor dysfunction directly, via its significant antioxidant capacity.³⁰ A significant correlation between the antioxidant activity, the phenolic content of honey and the inhibition of the lipoprotein oxidation of human serum was found.¹⁷ Also in a study of comparing fructose-fed rats and honey-fed rats, honey receiving group had a higher plasma α -tocopherol level, and a higher α -tocopherol/triacylglycerol ratio, as well as lower plasma nitrate levels and lower susceptibility of the heart to lipid peroxidation, which can be a leading cause of less free radical production and so decreasing myocardium injuries.³¹ Many epidemiological studies have shown that regular flavonoid intake reduced the risk of cardiovascular diseases³² by protective effects of mainly antithrombotic, antiischemic, antioxidant, and vasorelaxant activity.³³ All natural honeys almost contain flavonoides (such as apigenin, pinocembrin, kaempferol, quercetin, galangin, chrysin and hesperetin).³⁴⁻³⁶ It seems that the mentioned effects could be expected from flavonoids component of honey to reduce I/R injuries. Furthermore, natural honey contains nitric oxide (NO) metabolites which are known indicators for cardiovascular disease risk.²⁰ By increasing NO level, honey might have a protecting role in such conditions.

As mentioned earlier, natural honey contains various kinds of minerals such as calcium, potassium, chlorine, sodium, iron, magnesium and zinc.^{17,34} Maybe, some of these minerals may be relevant to the antiarrhythmic effects of honey. Hypokalemia can result in arrhythmia and cardiac arrest in acute myocardial infarction.³⁷ Zinc is attributed to have an inhibitory action on the free radical formation in the heart and magnesium inhibits the voltage dependent calcium channels and may prevent cardiac arrhythmias. Effects of sodium is through the function of Na^+ / Ca^{2+} exchange and alter Ca^{2+} level within the cardiac cells and reduce the incidence of ventricular arrhythmias.³⁰

Although all used concentrations of honey exerted protective effect, however, 0.25% had partially better antiarrhythmic activity in this model of study. This finding is similar to the result of our previously reported work.²⁴ On the other hand, the existence of high amount of glucose in higher concentrations of honey may change glucose to lactate in ischemic phase and result in electrical and contractility disturbances in the heart.²² In addition to arrhythmias, the results of present study showed that the infarct size (another

important parameter to evaluate the severity of acute myocardial infarction) was significantly reduced by all used honey concentrations. As well as antiarrhythmic activity, it has shown that short term administration of honey as a preconditioning or postconditioning agent during 30 min regional ischemia produced anti-infarction activity in isolated rat heart.^{23,28} In consistent with the above findings, in a recent another study, preischemic administration of honey before zero flow global ischemia caused potent reduction in infarct size in isolated rat heart.²⁴ All probable mechanism which mentioned for antiarrhythmic effects of honey also can attribute in reducing infarct size. Perhaps, antioxidant activity, scavenging of free radicals and the existence of high energy sources in honey composition (such as fructose and glucose) may be the most important potential cardioprotective mechanisms of honey.

Conclusion

By considering all mechanism of action and our findings, it may be concluded that postischemic administration of natural honey after 30 min global ischemia followed by 120 min reperfusion can prevent isolated rat hearts against I/R injuries and consequently protect heart by antiarrhythmic activity and reducing infarction size. Antioxidant and radical scavenging activity, lipoperoxidation inhibition, presence of rich energy sources, various type of vitamins, minerals and enzymes and formation of NO-contain metabolites may probably involve in those cardioprotective effects of natural honey in such conditions. Future studies are required to determine the exact protective mechanism (s) of honey.

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Conflict of interest

The authors report no conflicts of interest.

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