

# Zero Flow Global Ischemia-Induced Injuries in Rat Heart Are Attenuated by Natural Honey

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## ARTICLE INFO

### Article Type:

Research Article

### Article History:

Received: 5 June 2012

Accepted: 20 June 2012

ePublished: 25 June 2012

### Keywords:

Natural honey  
Global ischemia  
Arrhythmia  
Infarction  
Preischemic administration  
Isolated rat heart

## ABSTRACT

**Purpose:** In the present study, effects of preischemic administration of natural honey on cardiac arrhythmias and myocardial infarction size during zero flow global ischemia were investigated in isolated rat heart. **Methods:** The isolated hearts were subjected to 30 min zero flow global ischemia followed by 120 min reperfusion then perfused by a modified drug free Krebs-Henseleit solution throughout the experiment (control) or the solution containing 0.25, 0.5, 1 and 2% of natural honey for 15 min before induction of global ischemia (treated groups), respectively. Cardiac arrhythmias were determined based on the Lambeth conventions and the infarct size was measured by computerized planimetry. **Results:** Myocardial infarction size was  $55.8 \pm 7.8\%$  in the control group, while preischemic perfusion of honey (0.25, 0.5, 1 and 2%) reduced it to  $39.3 \pm 11$ ,  $30.6 \pm 5.5$  ( $P < 0.01$ ),  $17.9 \pm 5.6$  ( $P < 0.001$ ) and  $8.7 \pm 1.1\%$  ( $P < 0.001$ ), respectively. A direct linear correlation between honey concentrations and infarction size reduction was observed ( $R^2 = 0.9948$ ). In addition, total number of ventricular ectopic beats were significantly decreased by all used concentrations of honey ( $P < 0.05$ ) during reperfusion time. Honey (0.25, 0.5 and 1 %) also lowered incidence of irreversible ventricular fibrillation ( $P < 0.05$ ). Moreover, number and duration of ventricular tachycardia were reduced in all honey treated groups. **Conclusion:** Preischemic administration of natural honey before zero flow global ischemia can protect isolated rat heart against ischemia/reperfusion injuries as reduction of infarction size and arrhythmias. Maybe, antioxidant and free radical scavenging activities of honey, reduction of necrotized tissue and providing energy sources may involve in these cardioprotective effects of honey.

## Introduction

Honey is a natural product with very complex chemical composition containing at least 181 different substances.<sup>1</sup> It is composed primarily of fructose and glucose but also contains fructo-oligosaccharides,<sup>1</sup> many amino acids, vitamins, minerals and enzymes.<sup>2</sup> The composition of honey varies according to the plant origin on which the bee feeds. However, all honeys almost contain flavonoids (such as apigenin, pinocembrin, kaempferol, quercetin, galangin, chrysin and hesperetin), phenolic acids (such as ellagic, caffeic, p-coumaric and ferulic acids), ascorbic acid, tocopherols, catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), Millard reaction products and peptides, most of them work together to provide a synergistic antioxidant effect.<sup>3-7</sup>

Honey has had a valued place in traditional medicine for centuries.<sup>8,9</sup> However, it has a limited use in modern medicine due to lack of scientific support.<sup>10</sup> For a long time, it has been observed that honey can be used to

overcome liver, cardiovascular and gastrointestinal problems.<sup>11</sup> Ancient Egyptians, Assyrians, Chinese, Greeks and Romans employed honey for wounds and diseases of the intestine.<sup>12</sup> From the past few decades, honey was subjected to laboratory and clinical investigations by several research groups. The most remarkable discovery was antibacterial activity of honey that has been mentioned in numerous studies.<sup>13,14</sup> Natural honey exhibits bactericidal activity against many organisms including *Salmonella*, *Shigella*, *E. coli*,<sup>3,15</sup> some methicillin-resistant *Staphylococcus aureus* strains<sup>14</sup> and *Helicobacter pylori*.<sup>9</sup> Avicenna, the great Iranian scientist and physician, almost 1000 years ago, had recommended honey as one of best remedies in the treatment of tuberculosis.<sup>16</sup> An antifungal action has also been observed for some yeasts and species of *Aspergillus* and *Penicillium*, as well as all the common dermatophytes.<sup>17</sup> Effectiveness of honey to treat severely infected cutaneous wounds also was

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confirmed in recent clinical case studies.<sup>18</sup> In an inflammatory model of colitis, honey was as effective as prednisolone treatment.<sup>19</sup> Research has also indicated that honey may possess anti-inflammatory activity and stimulate immune responses within a wound.<sup>20,21</sup> Al-Waili and Boni (2003) demonstrated anti-inflammatory effects of honey in humans after ingestion of 70 g of honey. They showed that consumption of honey lowered mean plasma concentrations of thromboxane B<sub>2</sub>, and prostaglandin E<sub>2</sub> and F<sub>2α</sub>.<sup>22</sup> Honey, interestingly, has shown to prevent reactive oxygen species (ROS)-induced low density lipoprotein (LDL) oxidation in some *in vitro* studies, thus exhibiting beneficial cardiovascular protection.<sup>23,24</sup> Honey has also antineoplastic activity in an experimental bladder cancer.<sup>25</sup> Other medicinal properties of honey and other hive products have been described for a variety of medicinal and nutritional purposes by other studies.<sup>26-28</sup>

To date, majority of studies have focused on the potential health benefits of honey for human, but its efficacy on cardiovascular disease such as arrhythmia and myocardial infarction is not completely understood. Previously, we showed cardioprotective effects of chronic oral administration of natural honey (for 45 days) against ischemia/reperfusion (I/R) injuries in rat.<sup>29</sup> In the present study, effects of preischemic administration of natural honey on cardiac arrhythmias and myocardial infarction size during zero flow global ischemia were investigated in isolated rat heart.

### Materials and Methods

The following chemicals were purchased: Honey (Oskou, East Azerbaijan, Iran), Triphenyltetrazolium chloride (TTC) (Sigma), Formalin, NaCl, NaHCO<sub>3</sub>, KCl, KH<sub>2</sub>PO<sub>4</sub>, MgSO<sub>4</sub>, CaCl<sub>2</sub>, D-glucose (Merck Company), Sodium pentobarbital (Kela Company, Belgium) and Heparin (Daru-pakhsh Company, Iran).

### Animals and surgical procedure

Male Wistar rats weighing 270-320 g were divided into five groups randomly as a control and four treated groups (n=10-14 in each group). The rats were pretreated with intraperitoneal (i.p.) injection of 300 IU heparin then anaesthetized by sodium pentobarbital (50-60 mg/kg, i.p.). The hearts were excised rapidly and mounted on a non-recirculating Langendorff apparatus under constant pressure of 100 mmHg at 37°C and perfused with modified Krebs-Henseleit (K/H) solution that previously equilibrated with 95% O<sub>2</sub>-5% CO<sub>2</sub>. A latex fluid filled balloon was inserted into the left ventricle and inflated to give a preload of 8-10 mmHg.<sup>30</sup> All the hearts were subjected to 20 min stabilization, 15 min preischemic time, 30 min zero flow global ischemia followed by 120 min reperfusion. In the control group, the hearts were perfused by normal drug free K/H solution throughout the experiments, while in the treated groups, they were perfused with K/H solution containing 0.25, 0.5, 1 and

2% of natural honey during 15 min preischemic period before induction of global ischemia. Similar to the control group, drug free K/H solution were perfused to treated isolated hearts during stabilization and reperfusion. At stabilization time, any animal with experienced persistent arrhythmias was discarded.<sup>31-33</sup> Anepicardial ECG was recorded continuously by a physiograph during the experiment. Based on the Lambeth conventions,<sup>34</sup> the ECGs were analyzed to determine the number of single ectopic beats, salvos (couplets and triplets), ventricular tachycardia (VT), the total number of ventricular ectopic beats (VEBs), incidence and duration of VT and reversible ventricular fibrillation (Rev VF) and incidence of irreversible ventricular fibrillation (Irrev VF) during the first 30 min of reperfusion time.<sup>35</sup> 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).

### Measurement of myocardial infarction size

To determine the infarction size, at the end of 120 min reperfusion, the hearts were frozen, and then sliced transversely in a plane perpendicular to the apico-basal axis into 2 mm thick sections. The slices were incubated by 1% (w/v) TTC solution in phosphate buffer (pH=7.4) for 15 min at 37°C to dye the non-infarcted region.<sup>36,37</sup> This procedure resulted in the normally perfused tissue being stained blue, non-infarcted, non-perfused tissue stained brick red and infarcted tissue remaining unstained and appeared pale. After fixing the slices in formalin 10% (v/v) for 24 h, the infarct size was determined by using a computerized planimetry package.<sup>38-40</sup>

### Statistical analysis

Except for the incidence of VT and VF which are expressed as percentage, all the other results are expressed as Mean±SEM. To compare the number of VEBs, single ectopic beats, salvos, VT and duration of VT and VF between groups, the Mann-Whitney non-parametric U-test was employed. For analyzing the incidence of VT and VF, Fisher exact test (Chi-square with Yates correction) was used. The mean percentage of infarct size was analyzed using one-way ANOVA and then significant differences were examined by LSD post hoc range test. Differences between groups were considered significant at a level of P<0.05.

### Results and Discussion

The effects of administration of natural honey on reperfusion-induced cardiac arrhythmias after 30 min zero flow global ischemia are summarized in Table 1. As shown in the table 1 and Figure 1, perfusion of K/H solution containing natural honey for 15 min before induction of global ischemia significantly decreased the total number of VEBs. In the control group, VEBs

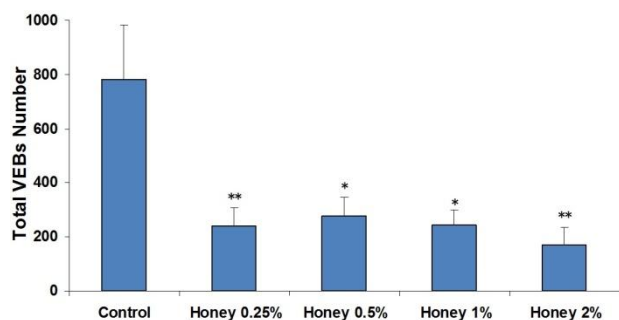
number was  $784 \pm 200$ , while the value was decreased by natural honey (0.25, 0.5, 1 and 2%) to  $240 \pm 68$  ( $P < 0.01$ ),  $277 \pm 68$  ( $P < 0.05$ ),  $242 \pm 57$  ( $P < 0.05$ ) and  $173 \pm 62$  ( $P < 0.01$ ), respectively). The number of single ectopic beats decreased by all used honey concentrations of honey, however as illustrated in

Figure 2, the effect was statistically significant only by 1 and 2 % honey ( $P < 0.01$ ). Natural honey (0.25, 0.5, 1 and 2%) also markedly lowered the number of salvos arrhythmias compared to the control group ( $P < 0.01$  for all). Number and duration of VT were also reduced by natural honey (Table 1 and Figure 2, Figure 3).

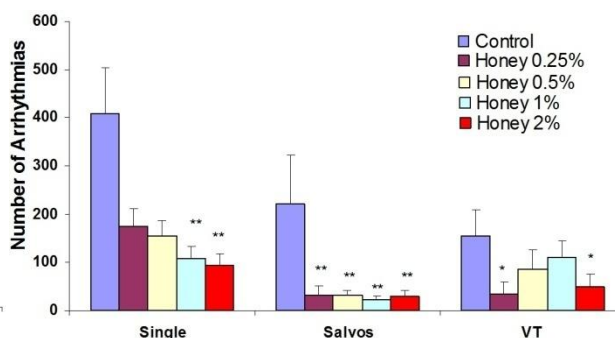
**Table 1.** Effects of administration of natural honey (0.25, 0.5, 1 and 2 %) on reperfusion- induced cardiac arrhythmias after 30 min zero flow global ischemia in isolated rat heart.

Groups	Reperfusion arrhythmias						
	VEBs number	VT number	VT Duration(sec)	Rev VF Duration (sec)	Rev VF Incidence (%)	Irrev VF Incidence (%)	VT Incidence (%)
Control	$784 \pm 200$	$154 \pm 55$	$22 \pm 7$	$248 \pm 76$	60	40	67
Honey (0.25%)	$240 \pm 68^{**}$	$34 \pm 24^*$	$5 \pm 3^*$	$234 \pm 99$	60	0*	40
Honey (0.5%)	$277 \pm 68^{**}$	$86 \pm 40$	$12 \pm 5$	$180 \pm 63$	100	0*	80
Honey (1%)	$242 \pm 57^*$	$111 \pm 34$	$14 \pm 4$	$219 \pm 53$	85	0*	61
Honey (2%)	$173 \pm 62^{**}$	$50 \pm 27^*$	$7 \pm 3^*$	$205 \pm 54$	67	17	42

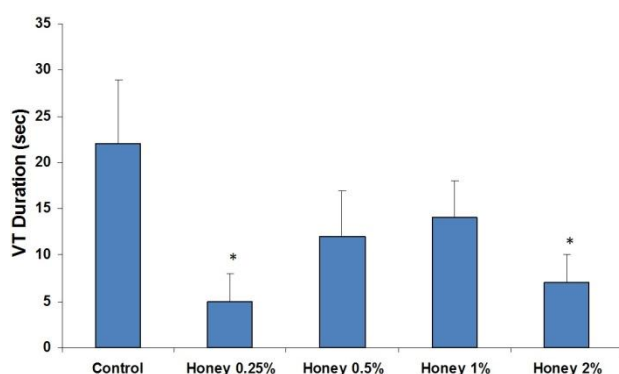
\*\*  $P < 0.01$ , \*  $P < 0.05$  versus the control group. N=10-14 in each group. VT: Ventricular Tachycardia, VEBs: Ventricular Ectopic Beats (Single+Salvos+VT), Rev VF: Reversible Ventricular Fibrillation, Irrev VF: Irreversible Ventricular Fibrillation



**Figure 1.** The total number of ventricular ectopic beats (VEBs: Single+Salvos+VT) in the control and treated groups receiving 0.25, 0.5, 1 and 2 % of honey for 15 minutes before induction of 30 min zero flow global ischemia. Data are represented as Mean±SEM. \* $P < 0.05$ , \*\* $P < 0.01$  versus the control group. N=10-14 in each group.



**Figure 2.** Number of single, salvos and VT (Ventricular Tachycardia) arrhythmias in the control and treated groups receiving 0.25, 0.5, 1 and 2 % of honey for 15 minutes before induction of 30 min zero flow global ischemia. Data are represented as Mean±SEM. \* $P < 0.05$ , \*\* $P < 0.01$  versus the control group. N=10-14 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 minutes before induction of 30 min zero flow global ischemia. Data are represented as Mean±SEM. \* $P < 0.05$  versus the control group. N=10-14 in each group.

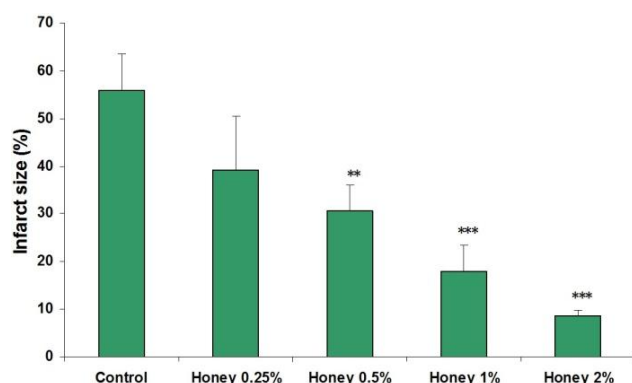
The time spent in reversible VF was  $248 \pm 76$  sec in the control group and different concentrations of honey reduced the time not statistically significant. In

addition, honey significantly lowered the incidence of Irrev VF by 0.25, 0.5 and 1 % concentrations ( $P < 0.05$ ), however, the incidences of VT and Rev VF did not show significant reduction. Effects of honey on the myocardial infarction size are summarized in table 2. Perfusion of the isolated rat hearts by K/H solution containing natural honey for 15 min before global ischemia markedly reduced infarct size by all used concentration. As shown in Figure 4, the infarct size was  $55.8 \pm 7.8\%$  in the control group, while short-term pretreatment of the hearts by 0.5, 1 and 2% of honey solution produced significant reduction in the size of myocardial infarction from the control value to  $30.6 \pm 5.5\%$  ( $P < 0.01$ ),  $17.9 \pm 5.6\%$  ( $P < 0.001$ ) and  $8.7 \pm 1.1\%$  ( $P < 0.001$ ), respectively. The effect was not significant by 0.25%. In addition, similar to infarct size results, natural honey significantly decreased the infarcted volume of ischemic hearts in comparison with the control group value (Table 2). A direct linear correlation ( $R^2 = 0.9948$ ) between honey concentrations and reduction of infarct size was observed (Figure 5).

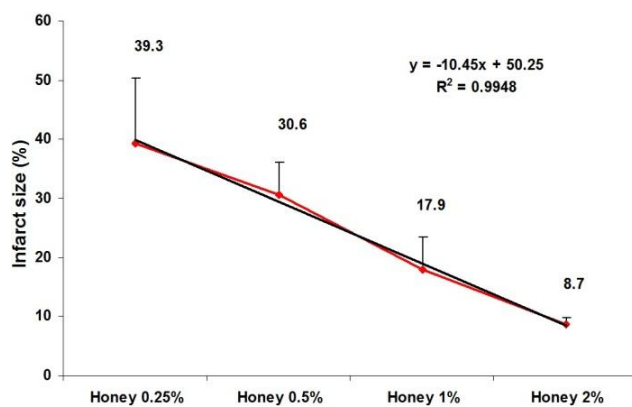
**Table 2.** Effects of administration of natural honey (0.25, 0.5, 1 and 2 %) on myocardial infarction size after 30 min zero flow global ischemia followed by 120 min reperfusion in isolated rat heart.

Groups	Risk zone vol.(mm) <sup>3</sup>	Infarcted vol.(mm) <sup>3</sup>	Infarct size (%)
Control	1278±58	723±113	55.8±7.8
Honey (0.25%)	1325±115	592±190	39.3±11.0
Honey (0.5%)	1249±39	390±75 **	30.6±5.5**
Honey (1%)	1152±50	232±91 **	17.9±5.6***
Honey (2%)	1148±27	101±13 ***	8.7±1.1***

Data are represented as Mean±SEM. \*\*P<0.01, \*\*\*P<0.001 versus the control group. N=10-14 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 minutes before induction of 30 min zero flow global ischemia followed by 120 min reperfusion. Data are represented as Mean±SEM. \*\*P<0.01, \*\*\*P<0.001 versus the control group. N=10-14 in each group.



**Figure 5.** Relationship between myocardial infarct size reduction and honey concentrations (0.25-2%) in isolated rat hearts. Data are represented as Mean±SEM. N=10-14 in each group.

## Discussion

Cardiac arrhythmias remain a major source of morbidity and mortality in developed countries.<sup>41</sup> In the course of cardiac surgery and myocardial infarction, ventricular arrhythmias such as VT and VF are the most important cause of mortality.<sup>42</sup> The results of this work showed that honey can protect isolated rat hearts against reperfusion-induced cardiac arrhythmias after 30 min zero flow global ischemia. In this condition,

perfusion of K/H solution containing natural honey reduced the total number of VEBs, the number of single ectopic beats and salvos arrhythmias compared to the control group. In addition, number and duration of VT and incidence of Irrev VF were reduced by some concentrations of natural honey. Additionally, honey reduced duration and incidence of reversible VF. Although honey has been applied for medicinal purposes since ancient times,<sup>43</sup> however, as mentioned before, there is not enough report on its effects against I/R injuries in the literature. In the case of cardiovascular diseases, most of the previous studies are focused on honey's effects against cardiovascular risk factors such as hyperlipidemia and production of free radicals.<sup>44-48</sup> The results of a previous study in 2011 revealed that chronic administration of honey (feeding of rats by different concentrations of honey for 45 days) had antiarrhythmic effects.<sup>29</sup> In spite of some methodological differences between the present and the above study (such as type of ischemia, experimental protocols and the administration period of honey), findings of both studies are in consistent with them. That is, acute short-term or chronic administration of honey protects isolated rat heart against I/R-induced arrhythmias<sup>29</sup> regardless of ischemia type. Although all used concentration of honey showed this protective effect, it seems that 0.25% had partially better antiarrhythmic effects in this model of study. Probably, the existence of high amount of glucose in higher concentrations of honey may change glucose to lactate in ischemic condition then causes electrical and contractility disturbances in the heart.<sup>29</sup> In other study, pretreatment of anesthetized normal or stressed rats with natural wild honey (5 g/kg) for 1 h prior to adrenaline injection (100 mcg/kg) could protect them from epinephrine-induced vasomotor dysfunction and cardiac disorders and preserved the positive inotropic effect of adrenaline. The authors concluded that natural wild honey might cause its cardioprotective effects against adrenaline-induced cardiac and vasomotor dysfunction directly (*via* its high total antioxidant capacity and enzymatic and nonenzymatic antioxidants, besides its substantial quantities of mineral elements and/or indirectly by stimulating release of nitric oxide from endothelium through the influence of vitamin C.<sup>6</sup> Under conditions of I/R that weaken the cardiac antioxidant system and make the tissue overexposed to ROS production, the myocardium may be subjected to oxidative damage.<sup>49</sup> Probably, the antioxidant activity of honey and scavenging of free radicals that demonstrated in some previous studies may play important role in the above protective effects of honey as well.<sup>19,24,50-53</sup> In general, the antioxidant capacity of honey appeared to be a result of the combined activity of a wide range of compounds including phenolics, peptides, organic acids, enzymes, Millard reaction products, and possibly other minor components.<sup>53</sup> As a result, it may be suggested that protective effects of natural honey against I/R-induced arrhythmias may

also be related to its both nonenzymatic (such as GSH and ascorbic acid) and enzymatic antioxidants (such as SOD and CAT).<sup>4,6</sup> Moreover, honey is extraordinarily rich in minerals, mainly calcium, potassium, chlorine, sodium, iron, magnesium and zinc.<sup>3,6,51</sup> It seems that some of these minerals may be responsible, in part, for the anti-arrhythmic effects of honey. The data linking hypokalemia with arrhythmia and cardiac arrest in acute myocardial infarction are fairly strong.<sup>54</sup> Hypokalemic ventricular ectopy is suppressed by potassium replacement.<sup>54</sup> It has been reported that zinc has an inhibitory action on the free radical formation in the heart, since it interferes with the processes that initiate arrhythmias. Magnesium, by inhibiting the voltage-dependent calcium channels, may prevent cardiac arrhythmias. Also, sodium, through the process of  $\text{Na}^+/\text{Ca}^{2+}$  exchange, can prevent the increase of  $\text{Ca}^{2+}$  ions within the cardiac cells and reduce the incidence of ventricular arrhythmias.<sup>6</sup> Our findings also demonstrated that pretreatment of isolated rat hearts by natural honey caused significant and potent cardioprotection against myocardial infarction as one of the most important predominant determinants of I/R induced injuries (figure 2). Administration of honey (0.25, 0.5, 1 and 2%) 15 min before zero flow global ischemia followed by 120 min reperfusion lowered the infarction size 29.5, 45.1 ( $P<0.01$ ), 67.9 ( $P<0.001$ ) and 84.4% ( $P<0.001$ ), respectively versus the control group value (100%). Regarding the used concentration range of honey in our model, reduction of infarct size is concentration dependent and there is a direct linear relationship (with an equation of  $y=-10.45x+50.25$  and  $r^2=0.9948$ ) between honey concentration and its protective effect (figure 5). Therefore, the higher concentrations of honey (particularly 1 and 2%) are more effective than lower concentrations for decreasing myocardial infarction. Similar to antiarrhythmic effects, reduction of infarct size in this work is in consistence with the findings of previous study.<sup>29</sup> However, the exact cardioprotective mechanisms of natural honey in reducing infarct size are not clear yet. Regarding the antioxidant and free radical scavenging activity of natural honey,<sup>7,19,53</sup> we proposed that the above properties may be responsible, in part, for infarct size reduction in ischemic reperfused heart. Other potential mechanisms to decrease myocardial infarct size by honey may include anti-inflammatory effects,<sup>12,20,22</sup> decrease in the measurement of necrotized tissue<sup>21,22</sup> and antiarrhythmic effects.<sup>29</sup> 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 the data, it may be concluded that preischemic administration of natural honey before zero flow global ischemia followed by 120 min reperfusion can protect isolated rat hearts and

consequently has antiarrhythmic activity and reducing infarction size. Antioxidant and radical scavenging activity, presence of rich energy sources, many vitamins, minerals and enzymes may probably involve in the cardioprotective effects of natural honey in such conditions. Future studies are required to determine the exact protective mechanism (s) of honey.

### Acknowledgment

The present investigation was supported by the Research Affairs of Tabriz University of Medical Sciences, Tabriz, Iran. This article is based on a thesis submitted for Pharm Dr degree (No.3523) in Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran.

### Conflict of interest

The authors report no conflicts of interest.

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