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Original article

Effects of Chronic Oral Administration of Natural Honey on Ischemia/Reperfusion-induced Arrhythmias in Isolated Rat Heart

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Abstract

Objective(s)
In this study, effects of chronic administration of oral natural honey against ischemia/reperfusion (I/R)-induced cardiac arrhythmias were investigated in isolated rat heart.

Materials and Methods
Male Wistar rats were divided into four groups (n= 10-14 rats in each group) and fed with natural honey (1%, 2% and 4% dissolved in the drinking water) for 45 days except for the control group. After anesthesia, the rats' hearts were isolated quickly, mounted on a Langendorff apparatus and perfused with a modified Krebs-Henseleit solution during stabilization, 30 min regional ischemia followed by 30 min reperfusion. The ECGs were recorded throughout the experiments to analyze cardiac arrhythmias based on the Lambeth conventions.

Results
In the ischemic phase, honey (1%) significantly reduced (P<0.05) the number and duration of ventricular tachycardia (VT). Honey (1% and 2%) also significantly decreased number of ventricular ectopic beats (VEBs). In addition, incidence and duration of reversible ventricular fibrillation (Rev VF) were lowered by honey 2% (P<0.05). During reperfusion time, VT incidence was 73% in the control group, however natural honey (1%) decreased it to 22% (P<0.05). Honey also produced significant reduction in the incidences of total VF, Rev VF, duration and number of VT.

Conclusion
For the first time, the results of present study demonstrated protective effects of chronic oral honey administration against I/R-induced arrhythmias in isolated rat heart. Antioxidant activity, the existence of energy sources such as glucose and fructose and improvement of some hemodynamic functions might be responsible for these effects.

Keywords: Arrhythmia, Honey, Ischemia, Rat, Reperfusion

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Introduction

Cardiac arrhythmias remain a major source of morbidity and mortality in developed countries (1). In cardiac surgery and myocardial infarction, ventricular arrhythmias such as ventricular tachycardia (VT) and ventricular fibrillation (VF) are the most important causes of mortality (2). Myocardial ischemia/reperfusion (I/R) results in significant damage to the heart. Ischemic condition results in the inhibition of fatty acid metabolism then accumulation of their toxic metabolites in the heart. These molecules have been shown to be deleterious to the recovery of myocardial function of the reperfused heart (3, 4) and have been shown as a cause of ventricular arrhythmias (5).

Honey is a natural substance produced by honey bees from nectar or blossoms or from the secretion of living parts of plants or excretions of plants (6). It primarily contains sugar (95–99 % of honey dry matter) and water. Fructose (38.2 %) and glucose (31.3 %) are the most abundant content of sugars in honey composition (7). Small quantities of minerals such as potassium (the most abundant), calcium, copper, iron, manganese and phosphorus are present in honey. The main enzymes in natural honey are invertase, diastase and glucose oxidase. In addition, several vitamins such as vitamins C, B_1 (thiamine), riboflavin, nicotinic acid, panthothenic acid and B_6 are also found (8). Although honey has been applied for medicinal purposes since ancient times (9), it has a limited use in modern medicine due to lack of scientific support (10). Inhibitory effects of natural honey have been reported on around 60 species of bacteria including aerobes and anaerobes, Gram-positives and Gram-negatives (11). Antifungal action has been observed for some yeasts and species of *Aspergillus* and *Penicillium*, as well as all the common *dermatophytes* (8). Honey has been used as wound barrier against tumor implantation in laparoscopic oncological surgery (12). It also has a potential therapeutic role in the treatment of gingivitis and periodontal disease (12). In addition, some findings indicated that honey has anti-inflammatory effect and causes a reduction in necrosis tissues (13, 14). Antioxidant activity of natural honey was previously reported by many researchers (15-18). In a cultured endothelial cell line subjected to oxidative stress, a multifloral origin honey showed potent activity against free radicals, suppression/prevention of cell damage, inhibition of cell membrane oxidation, reduction of intracellular reactive oxygen spices (ROS), and production and recovery of intracellular reduced glutathione (GSH). Flavonoids and phenolic acids were the main compounds of honey mediating the above-mentioned protective effects (19). In natural wild honey, the total antioxidant capacity was found to be very pronounced as relatively high levels of reduced glutathione, vitamin C and superoxide dismutase activity (20). In the study of Gheldof *et al*, acute consumption of buckwheat honey significantly increased oxygen radical absorbance capacity in human serum (17). Schramm *et al* described the effects of oral buckwheat honey (1.5 g/kg) on the antioxidant and reducing capacities of plasma in healthy adults. Following consumption of the agent, plasma total-phenolic content and antioxidant capacities increased. It seems that phenolic antioxidants may augment defenses against oxidative stress (21). In another study, 10% honey feeding for 6 weeks in rats produced significant reduction in HbA1c, triglyceride levels and weight gain compared to sucrose and mixed sugars (22). Bahrami *et al* demonstrated that administration of oral natural honey for 8 weeks in 48 diabetic type 2 patients had beneficial effects on body weight, total cholesterol, low-density lipoprotein-cholesterol (LDL-C) and triglyceride reduction and increasing high-density lipoprotein-cholesterol (HDL-C) (23). In addition, consumption of natural honey (70 g daily for 30 days) ameliorated cardiovascular risk factors and reduced total cholesterol, LDL-C, triacylglycerole, C-reactive protein (CRP), fasting blood glucose (FBG), and increased HDL-C in overweight or obese individuals (24). In the study of Noori *et al*, single dose oral honey and consumption of honey (15 days) in human subjects reduced

cholesterol, LDL-C, and TG and increased HDL-C. Furthermore, TG and LDL-C levels were lowered by natural honey in patients with hypertriglyceridemia and hyperlipidemia. Compared with dextrose and sucrose, honey caused lower elevation of PGL in diabetic patients (25).

Almost all the previous reported effects of honey against cardiovascular risk factors are focused on lipid profiles and free radicals. Recently, we have demonstrated that short time perfusion of enriched Krebs solution with natural honey for 10 min before to 10 min after ischemia (pharmacologic preconditioning) had antiarrhythmic activity in isolated rat heart (26). However, there is no report regarding cardioprotective effects of chronic oral administration of natural honey against I/R injuries. Therefore, in the present study, effects of chronic administration of oral honey on I/R-induced cardiac arrhythmias were investigated in isolated rat heart.

Materials and Methods

Feeding animals with prepared honey concentrations

Male Wistar rats weighing 270-300 g were used in this study. The rats were divided into four groups (n=10-14 in each group) and were fed by different concentrations of honey (1%, 2% and 4%) for 45 days, except for the control group. The natural honey was purchased from Oskou (East Azerbaijan, Iran). To prepare the required concentrations of honey, different amounts of honey was completely dissolved in the drinking water of rats then the rats were fed with the solutions during 45 days.

Animals and surgical procedure

The rats were pretreated by heparin (300 IU, i.p.) then anaesthetized with sodium pentobarbital (50 mg/kg, i.p.) (27). The hearts were excised rapidly and mounted on a non-recirculating Langendroff apparatus under 100 mmHg pressures at 37 °C and perfused throughout the experiments with modified Krebs-Henseleit (K/H) solution containing (in mM): NaCl (118.5), $NaHCO_3$ (25.0), KCl (4.8), $MgSO_4$ (1.2), KH_2PO_4 (1.2), D-glucose (12.0) and $CaCl_2$ (1.7) which was gassed with 95% O_2-5% CO_2. A fluid filled balloon introduced into the left ventricle and inflated to give a preload of 8–10 mmHg (27, 28). After 20 min stabilization, the hearts were subjected to 30 min regional ischemia by temporary occlusion of left anterior descending (LAD) coronary artery followed by 30 min reperfusion. The recorded ECGs by Powerlab data recorder and analyzer (ADInstruments, Australia), were analyzed based on the Lambeth conventions to determine the total number of ventricular ectopic beats (VEBs), the number of beats occurring as ventricular tachycardia (VT), the incidence and duration of VT and ventricular fibrillation (VF) during 30 min ischemia and 30 min reperfusion (29, 30).

Statistical analysis

Except for the incidence of VT and VF which were indicated as percentage, all results were expressed as mean±SEM. To compare the number of VT, VEBs and duration of VT, VF between groups, Mann-Whitney U test (non-parametric) was employed. Analyzing the incidence of VT and VF was accomplished by Fisher Irwin test (Chi-square with Yates correction) (29, 31). Differences between groups were considered significant at a level of $P<0.05$.

Results

The effects of different concentrations of orally administered natural honey on I/R-induced arrhythmias are summarized in Table 1 and 2.

As shown in Table 1 and Figure 1, at the ischemic condition (30 min), natural honey (1% and 2%), reduced the number of VT from 473±166 in the control group to 26±14 ($P<0.01$) and 72±54 ($P<0.05$), respectively. Duration of VT was decreased by the same concentrations ($P<0.01$ and $P<0.05$, respectively). Honey (1% and 2%) also led to a reduction in the number of VEBs ($P<0.05$ and $P<0.01$, respectively). In addition, incidence and duration of reversible VF (Rev VF) were significantly lowered by honey 2%

Table 1. Effects of oral honey on cardiac arrhythmias during 30 min ischemia in isolated rat hearts.

Groups	VT number	VEBs number	VT duration (Sec)	Rev VF duration (Sec)	Rev VF incidence (%)	VT incidence (%)	Total VF incidence (%)
Control	473±166	941±224	74±30	65±42	50	90	71
Oral Honey (1%)	26±14**	421±143*	4±2**	2±2	11	33*	11*
Oral Honey (2%)	72±54*	241±116*	12±9*	0*	0*	33*	0*
Oral Honey (4%)	247±101	533±109	37±11	20±15	43	71	43

*P<0.05, **P<0.01 versus control group. VT; ventricular tachycardia, VEBs; ventricular ectopic beats (Single+Salvos+VT), Rev VF; reversible ventricular fibrillation. N= 10-14 rats in each group.

Table 2. Effects of oral honey on cardiac arrhythmias during 30 min reperfusion in isolated rat hearts.

Groups	VT number	VEBs number	VT duration (Sec)	Rev VF duration (Sec)	VT incidence (%)	Rev VF incidence (%)	Total VF incidence (%)
Control	140±42	355±101	25±7	71±31	73	82	91
Oral Honey (1%)	7±5**	257±139	1±1**	52±46	22*	33*	33**
Oral Honey (2%)	49±32	214± 87	8±5	82±81	67	33*	50
Oral Honey (4%)	39±16	101±23*	6.5±3	272±223	71	57	57

*P<0.05, **P<0.01 versus control group. VT; ventricular tachycardia, VEBs; ventricular ectopic beats (Single+Salvos+VT), Rev VF; reversible ventricular fibrillation. N= 10-14 rats in each group.

(P<0.05). Moreover, the honey (1% and 2%) reduced the incidence of VT and total VF significantly compared to the control group (P<0.05). However, natural honey (4%) did not produce considerable changes in the incidence, duration and number of ischemic arrhythmias (Table 1).

At the reperfusion phase as shown in Figure 2, VT incidence was 73% in the control group, however natural honey (1%) decreased it to 22% (P<0.05). In addition, total VF incidence, duration and number of VT showed significant reduction by this concentration (P<0.01). Administration of natural honey (1% and 2%) also lowered incidence of Rev VF from 82% (in control group) to 33% (P<0.05). Also, natural honey (4%) significantly reduced number of VEBs (P<0.05) in comparison with the control group (Table 2).

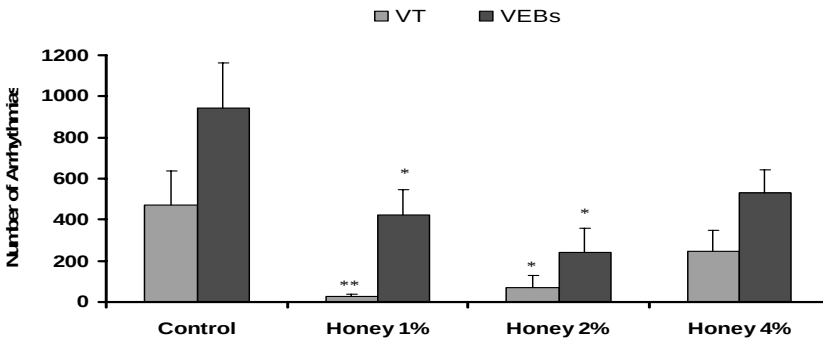

Figure 1. The total number of ventricular ectopic beats (VEBs) and ventricular tachycardia (VT) in the control and honey (1%, 2% and 4%) groups during 30 min ischemia. Data are represented as Mean±SEM.*P<0.05, **P<0.01 versus control group. N=10-14 rats in each group.

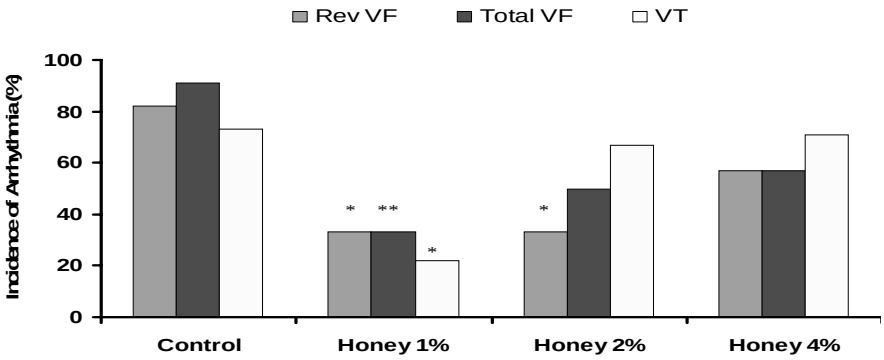

Figure 2. The incidence (%) of ventricular tachycardia (VT), reversible ventricular fibrillation (Rev VF) and total ventricular fibrillation (Total VF) during 30 min reperfusion in the control group and isolated rat hearts feeding by different concentrations of honey (1%, 2% and 4%).*P<0.05, **P<0.01 versus control group. N= 10-14 rats in each group.

Discussion

In the present study, potential antiarrhythmic effects of chronic administration of natural honey were investigated in isolated rat heart. The results clearly showed that chronic oral administration of natural honey produced antiarrhythmic effects against I/R-induced arrhythmias. During ischemia, using natural honey (especially 1%) showed significant reduction in the number of VT, VEBs, VT duration and incidences of VT and total VF. Despite decreasing ischemic arrhythmias by honey (4%), the effect was not statistically significant compared to the control group. At the reperfusion phase, the lowest used concentration of natural honey (1%) caused marked antiarrhythmic effects where incidence of total VF, duration, incidence and number of VT showed significant reduction. In addition to 1% honey concentration administration of 2% of honey lowered Rev VF incidence by 49% in comparison with the control group. Although natural honey has been applied for medicinal purposes since ancient times (9), 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 (21-25). The results of our previous work revealed that short time acute pre-ischemic administration of natural honey (0.25, 0.5 and 1%) as a pharmacologic preconditioning agent had antiarrhythmic and cardioprotective activities in isolated rat heart (26). Despite some methodological differences between the above-mentioned studies (different administration period and concentration of honey), the results of current study are consistent with the previous work. That is, both acute and chronic administration of natural honey protected isolated rat heart against I/R-induced arrhythmias in our model of study.

It seems that the low concentrations of oral honey are more effective than higher used concentrations. 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 (26). Although protective mechanism of honey is not clear, we suggested that similar to chronic administration of medications to prevent angina or other cardiovascular diseases, long time feeding of the rats with proper concentrations of honey for 45 days produced enough adaptation and prophylaxis against I/R injuries. The effect of honey in such condition is similar to ischemic preconditioning as well. Also we suggested that chronic effects of various chemical compounds in honey composition especially rich energy sources (such as glucose and fructose), vitamins, minerals, etc. (32, 33) have potential role to diminish I/R injuries. Antioxidant activity of honey and scavenging of free radicals demonstrated in some previous studies may play important role in the above-mentioned protective effects of honey as well (15-18, 34, 35). In a study, pretreatment of anesthetized normal or stressed rats with natural wild honey (5 g/kg) for 1 hr 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 and therapeutic 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 such as magnesium, sodium, and chlorine), and/or indirectly by stimulating release of nitric oxide from endothelium through the influence of vitamin C (20).

Little is known about the individual components of honey which are responsible for its antioxidant activity. In the study of Schramm *et al*, oral consumption of buckwheat honey (1.5 g/kg), plasma total-phenolic content and antioxidant capacities increased (21). Gheldof *et al* (2003) carried out a study to characterize the phenolics and other antioxidants present in honeys from seven floral sources. Chromatograms of the phenolic nonpolar fraction of the honeys indicated that most honeys have similar but quantitatively different phenolic profiles. Many of the identified flavonoids and phenolic acids have been previously described as antioxidants and may augment defenses against oxidative stress (15, 21). Strong scavenging free radicals were also shown by some bee products containing higher level of phenolic compounds (such as propolis) (36). It also inhibited oxidative stress which may be partly responsible for its neuroprotective activity against *in vitro* cell death and *in vivo* focal cerebral ischemia (36).

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, Maillard reaction products, and possibly other minor components (15). Regarding the existence of many organic compounds with antioxidant and radical-scavenging activity in honey composition, it seems that honey has the potential capability to serve as an important source of natural antioxidants in human nutrition (35).

Conclusion

By considering the data, it may be concluded that chronic oral administration of honey can recover ischemic-reperfused isolated rat hearts and consequently has anti-arrhythmic activity. Antioxidant and radical scavenging activity, presence of rich energy sources, many vitamins, minerals and enzymes may involve in the cardioprotective effects of natural honey in isolated rat heart. Future studies are required to determine the exact protective mechanism (s) of honey.

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