Prolonged preconditioning with natural honey against myocardial infarction injuries

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Abstract: Potential protective effects of prolonged preconditioning with natural honey against myocardial infarction were investigated. Male Wistar rats were pre-treated with honey (1%, 2% and 4%) for 45 days then their hearts were isolated and mounted on a Langendorff apparatus and perfused with a modified Krebs-Henseleit solution during 30 min regional ischemia followed by 120 min reperfusion. Two important indexes of ischemia-induced damage (infarction size and arrhythmias) were determined by computerized planimetry and ECG analysis, respectively. Honey (1% and 2%) reduced infarct size from 23±3.1% (control) to 9.7±2.4 and 9.5±2.3%, respectively (P<0.001). At the ischemia, 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, honey produced significant reduction in the incidences of VT, total and Rev VF, duration and number of VT. The results showed cardioprotective effects of prolonged pre-treatment of rats with honey following myocardial infarction. Maybe, the existence of antioxidants and energy sources (glucose and fructose) in honey composition and improvement of hemodynamic functions may involve in those protective effects.

Keywords: Honey, myocardial infarction, ischemia, reperfusion, rat.

INTRODUCTION

Honey has had a valued place in traditional medicine for centuries (Zumla and Lulat, 1989; Chowdhury, 1999). However, it has a limited use in modern medicine due to lack of scientific support (Ali et al., 1991). For a long time, it has been observed that honey can be used to overcome liver, cardiovascular and gastrointestinal problems (Ezz El-Arab et al., 2006). Honey is a natural product with very complex chemical composition. It is composed primarily of fructose and glucose but also contains fructo-oligosaccharides (Chow, 2002). It mainly contains sugar (95-99% of honey dry matter) and water. Fructose (38.2%) and glucose (31.3%) are the most content of sugars in honey composition (Moundoi et al., 2001). 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 ascorbic acid, thiamine, riboflavin, nicotinic acid, panthenolic acid and pyridoxine are also found (Olaitan et al., 2007). The ancient Egyptians, Assyrians, Chinese, Greeks and Romans employed honey for wounds and diseases of the gut (Zumla and Lulat, 1989). Laboratory studies and clinical trials have shown that honey is an effective broad-spectrum antimicrobial agent (Jeddar et al., 1985; Zaghoul et al., 2001; Al-Waili et al., 2005; Ezz El-Arab et al., 2006). It has been suggested that pure honey is bactericidal for many pathogenic organisms, including various gram-negative and gram-positive bacteria (Zumla and Lulat, 1989; Asadi-Pooya et al., 2003). An antifungal action has also been observed for some yeasts and species of Aspergillus and Penicillium, as well as all the common dermatophytes (Brady et al., 1997). Avicenna, the great Iranian scientist and physician, almost 1000 years ago, had recommended honey as one of best remedies in the treatment of tuberculosis (Asadi-Pooya et al., 2003). In an inflammatory model of colitis, intrahepatic honey administration was as effective as prednisolone treatment (Bilsel et al., 2002). The effect of the topical application of honey on recurrent attacks of herpes lesions was significantly better than acyclovir cream in adult patients (Al-Waili, 2004). Research has also indicated that honey may possess anti-inflammatory activity and stimulate immune responses within a wound (Lusby et al., 2002; Tonks et al., 2003). Honey has also antineoplastic activity in an experimental bladder cancer (Sewllam et al., 2003). Other medicinal properties of honey and other hive products have been described for a variety of medicinal and nutritional purposes by other studies (Molan, 2001; Meda et al., 2004; Snow and Manley-Harris, 2004).

Previously, 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 (Najafi et al., 2008a). However, there is no report regarding effects of prolong pre-treatment of rats with honey (long time pre-
conditioning) against myocardial infarction injuries. Therefore, in the present study, potential protective effects of prolonged preconditioning with natural honey were investigated in male rats.

MATERIALS AND METHODS

Animals
Male Wistar rats weighing 270-300 g were used in this study. They were housed in the Animal House of Tabriz University of Medical Sciences at a controlled ambient temperature of 25±2°C, relative humidity between 40-60% with a 12-h light/12-h dark cycle (lights on at 7:00 a.m.) and were given food and water ad libitum. 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 in 1985).

Surgical procedure
The rats were pretreated by heparin (300 IU, i.p) then anaesthetized with sodium pentobarbital (50 mg/kg, i.p) (Najafi et al., 2010). As soon as deep anesthesia was achieved, thoraxes were opened (Trueblood et al., 2000) and the hearts were rapidly and carefully excised and mounted via the aorta on a standard Langendorff perfusion apparatus with a perfusion pressure of 100 cmH2O. Modified Krebs-Henseleit (K/H) solution containing (in mM): NaCl (118.5), NaHCO3 (25.0), KCl (20.3), NaH2PO4 (1.8), MgSO4 (1.2), KH2PO4 (1.2), D-glucose (12.0) and CaCl2 (1.7) was used as the perfusion medium gassed by 95% O2 5% CO2, pH= 7.4) for 15 min at 37°C to dye the non–infarcted region (Zacharowski et al., 2001; Najafi et al., 2010). This procedure resulted in the normally perfused tissue being stained blue, non- infarcted, non-perfused tissue stained brick red, infarcted tissue remaining unstained and appeared pale (Kim et al., 2001). 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 (Hausenloy et al., 2003; Najafi et al., 2010).

Classification and analysis of ischemia/reperfusion (I/R)-induced ventricular arrhythmias
Based on the Lambeth conventions (Walker et al., 1988), the ECGs were analyzed to determine the total number of ventricular ectopic beats (VEBs), the number of beats occurring as ventricular tachycardia (VT), and the incidence and duration of VT and ventricular fibrillation (VF) during both ischemia and reperfusion phases (Najafi et al., 2008b, Najafi et al., 2008c).

Protocols of animal feeding with prepared honey concentrations
To prepare the required concentrations of honey, it was purchased from Oskou (East Azerbaijan, Iran) and different amounts of honey were completely dissolved in the drinking water of rats. The rats were allocated randomly to four groups (n=10-14 in each group). Except the drug free control group (group A), the others were fed with different amounts of honey. The rats were allocated randomly to four groups (n=10-14 in each group). Except the drug free control group (group A), the others were fed with concentrations of natural honey on myocardial infarction size and volume. The rats were fed with 0%, 1%, 2% and 4% (W/V) concentrations of natural honey.

Measurement of myocardial infarction size
To measure myocardial infarction size and volume, at the end of reperfusion period, the ligature around the LAD coronary artery re-tied and the heart was slowly perfused with 2-3 ml of saline solution containing 0.25% Evans blue dye (w/v) via the side arm of the aortic cannula (Najafi et al., 2008a; Najafi et al., 2010). The hearts were frozen, and then the ventricles of the frozen hearts sliced transversely in a plane perpendicular to the apico–basal axis into 2 mm thick sections. The slices incubated with 1% (w/v) thiphenyl tetrazolium chloride (TTC) solution in phosphate buffer (NaH2PO4, 88 mM; NaH2PO4 1.8 mM, pH= 7.4) for 15 min at 37°C to dye the non–infarcted region (Zacharowski et al., 2001; Najafi et al., 2010). This procedure resulted in the normally perfused tissue being stained blue, non- infarcted, non-perfused tissue stained brick red, infarcted tissue remaining unstained and appeared pale (Kim et al., 2001). 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 (Hausenloy et al., 2003; Najafi et al., 2010).

STATISTICAL ANALYSIS
Except for the incidence of VT and VF that indicated as percentage, all results expressed as mean±SEM. To compare the number of VT, VEBs and duration of VT, VF between groups, Mann-Whitney non-parametric U-test employed. Analyzing the incidence of VT and VF accomplished by Fisher Irwin test (Chi-square with Yates correction). Infarcted volume and percentage of infarct size were analyzed using one-way ANOVA and then considerable differences were examined by LSD post hoc range test. Differences between groups were considered significant at a level of P<0.05.

RESULTS

Effects of honey on myocardial infarction size
The effects of prolonged preconditioning with different concentrations of natural honey on myocardial infarction size are summarized in table 1. As depicted in fig. 1, in
the control group, the infarct size was 23±3.1% while chronic administration of oral honey (1% and 2%) for 45 days significantly reduced infarct size to 9.7±2.4 and 9.5±2.3%, respectively (P<0.001). Although honey (4%) lowered the infarct size to 16.2±1.6%, however the effect was not statistically significant. Risk zone volume (area at risk) in the all groups was similar and did not show significant differences between them (table 1).

**Effects of honey on ischemic arrhythmias**

The effects of different concentrations of prolonged preconditioning with orally administered honey on ischemic arrhythmias are summarized in table 2. As shown in fig. 2, 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 also led to a reduction in the number of VEBs by 1% and 2% (P<0.05 and P<0.01, respectively) (Fig 2). In addition, incidence and duration of reversible VF (Rev VF) were significantly lowered by honey 2% (P<0.05). Moreover, 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).

**Effects of honey on reperfusion arrhythmias**

Table 2 summarizes the effects of different concentrations of orally administered natural honey on reperfusion arrhythmias.

At the reperfusion phase, 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). As shown in fig. 2, administration of natural honey (1% and 2%) also lowered incidence of Rev VF from 82% (in the 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).

**Table 1:** Myocardial infarct size in the control and treated groups with honey (1, 2 and 4%) for 45 days subjected to 30 min regional ischemia followed by 120 min reperfusion.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number</th>
<th>Risk zone Vol (mm³)</th>
<th>Infarct size (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>428±148</td>
<td>23±3.1</td>
</tr>
<tr>
<td>Oral Honey (1%)</td>
<td>10</td>
<td>500±156</td>
<td>9.7±2.4*</td>
</tr>
<tr>
<td>Oral Honey (2%)</td>
<td>10</td>
<td>527±121</td>
<td>9.5±2.3*</td>
</tr>
<tr>
<td>Oral Honey (4%)</td>
<td>10</td>
<td>453±103</td>
<td>16.2±1.6</td>
</tr>
</tbody>
</table>

Data are represented as mean±SEM. *P<0.001 versus the control group by using one-way ANOVA with LSD post test.

**Table 2:** Effects of prolonged preconditioning with natural honey on ischemia-induced arrhythmias

<table>
<thead>
<tr>
<th>Groups</th>
<th>VT number</th>
<th>VEBs number</th>
<th>VT duration (Sec)</th>
<th>Rev VF duration (Sec)</th>
<th>Rev VF Incidence (%)</th>
<th>VT incidence (%)</th>
<th>Total VF incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>473±166</td>
<td>941±224</td>
<td>74±30</td>
<td>65±42</td>
<td>50</td>
<td>90</td>
<td>71</td>
</tr>
<tr>
<td>Oral Honey (1%)</td>
<td>26±14**</td>
<td>421±143*</td>
<td>4±2**</td>
<td>2±2</td>
<td>11</td>
<td>33*</td>
<td>11*</td>
</tr>
<tr>
<td>Oral Honey (2%)</td>
<td>72±54*</td>
<td>241±116*</td>
<td>12±9*</td>
<td>0*</td>
<td>0*</td>
<td>33*</td>
<td>0*</td>
</tr>
<tr>
<td>Oral Honey (4%)</td>
<td>247±101</td>
<td>53±109</td>
<td>37±11</td>
<td>20±15</td>
<td>43</td>
<td>71</td>
<td>43</td>
</tr>
</tbody>
</table>

*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.
Prolonged preconditioning with natural honey

Fig. 3: 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 the control group. N=10-14 rats in each group.

DISCUSSION

In the present study, potential protective effects of prolonged preconditioning with natural honey were investigated in isolated rat heart. The results clearly showed that long term administration of honey caused marked and potent protective activity against myocardial infarction injuries as reduction of infarct size in this model of study. Compared to the control value, honey (1%, 2% and 4%) lowered infarct size 58, 59 and 30%, respectively. However, the highest used concentration of honey (4%) did not produce significant reduction in infarct size. In addition, our results indicated that prolonged preconditioning with natural honey produces antiarrhythmic effects following myocardial infarction. 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 concentration used of natural honey (1%) caused marked antiarrhythmic effects where incidence of total VF, duration, incidence and number of VT showed significant reduction. As well as 1% honey, administration of 2% 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 (Ahmed et al., 2003), however, 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 (Schramm et al., 2003; Noori et al., 2004; Chepulis, 2007; Bahrami et al., 2008; Yaghoobi et al., 2008). The results of our previous work revealed that short time acute preischemic administration (acute preconditioning) of natural honey (0.25, 0.5 and 1%) had antiarrhythmic and cardioprotective activities in isolated rat heart (Najafi et al., 2008a). Despite some methodological differences between the above studies (different administration period and concentration of honey), the results of current study are in consistent with our 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 (Najafi et al., 2008a). 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. (White, 1979; Chow, 2002) have potential role to diminish infarct size and arrhythmias. 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 (Ghelfo et al., 2002; Ghelfo et al., 2003; Table 3: Effects of prolonged preconditioning with natural honey on reperfusion-induced arrhythmias.

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

*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.
et al., 2007; Zalibera et al., 2008). In a study, pretreatment of anesthetized normal or stressed rats with natural wild honey (5 g/kg) for 1 hour 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 (Rakha et al., 2008). Little is known about the individual components of honey that 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 (Schramm et al., 2003). Strong scavenging free radicals also was shown by some bee products containing higher level of phenolic compounds (such as propolis) (Shimazawa et al., 2005). It also inhibited oxidative stress which may be partly responsible for its neuroprotective activity during in vitro cell death and in vivo focal cerebral ischemia (Shimazawa et al., 2005). 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 (Ghelfod et al., 2002). 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 (Zalibera et al., 2008). In addition, some findings indicated that honey has anti-inflammatory effect and cause a reduction in necrosis tissues (Asadi-Pooya et al., 2003; Johnson et al., 2005).

CONCLUSION

By considering the results, it may be concluded that prolonged administration of honey as a preconditioning agent can recover ischemic-reperfused heart and consequently has anti-infarct and anti-arrhythmic activities. Probably, antioxidant and radical scavenging activity, presence of rich energy sources, many vitamins, minerals and enzymes may involve in the cardioprotective effects of natural honey. Future studies are required to determine the exact protective mechanism(s) of honey.

REFERENCES

Hausenloy JD, Duchen RM and Yellon MD (2003). Inhibiting mitochondrial permeability transition pore


