Prolonged preconditioning with natural honey against myocardial infarction injuries

Tahereh Eteraf-Oskouei[1], Elnaz Shaseb[1], Saba Ghaffary[1] and Moslem Najafi[1,2*]
[1]Department of Pharmacology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
[2]Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

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 fallowed 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, panthothenic 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; Zaghloul *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, intrarectal 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-

*Corresponding author: e-mail: Hnajafim@tbzmed.ac.irH H

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 cmH_2O. 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) was used as the perfusion medium gassed with 95% O_2 5% CO_2, pH 7.4 at 37°C throughout the experiment (Najafi *et al.,* 2010). An epicardial ECG was recorded by a polygraph during the experiment using two silver electrodes attached directly to the heart. Epicardial ECG was recorded throughout the experiment. A fluid filled latex balloon was introduced into the left ventricle through incision in the left atrium and inflated to give a pre-load of 8–10 mmHg. A 4/0 braided silk suture was placed around the left anterior descending coronary artery. Following 20 min stabilization, the hearts were subjected to 30 min regional ischemia by temporary occlusion of left anterior descending (LAD) coronary artery followed by 120 min reperfusion (Hausenloy *et al.,* 2003; Najafi *et al.,* 2008b, Najafi *et al.,* 2008c).

Measurement of myocardial infarction size

To measure myocardial infarction size and volume, at the end of reperfusion period, the ligature around the LAD 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) thriphenyl tetrazolium chloride (TTC) solution in phosphate buffer ($NaHPO_4$, 88 mM; NaH_2PO_4 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 infracted 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 by 1%, 2% and 4% (W/V) concentrations of natural honey for 45 days (as groups B, C and D, respectively).

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).

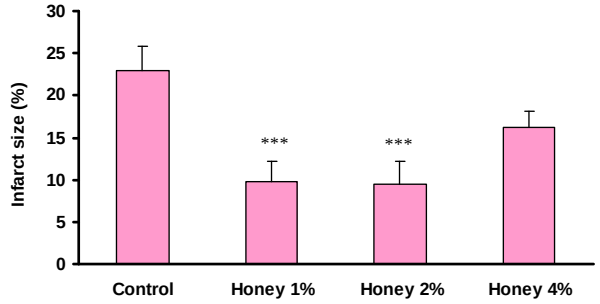

Fig. 1: Myocardial infarct size in the control and isolated rat hearts feeding by honey (1%, 2% and 4%) during 30 min ischemia followed by 120 min reperfusion.
Data are represented as mean±SEM. ***P<0.001 versus the control group. N=10 rats in each group.

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).

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.

Groups	Number	Risk zone Vol (mm)^3	Infarct size (%)
Control	10	428±148	23±3.1
Oral Honey (1%)	10	500±156	9.7±2.4*
Oral Honey (2%)	10	527±121	9.5±2.3*
Oral Honey (4%)	10	453±103	16.2±1.6

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

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).

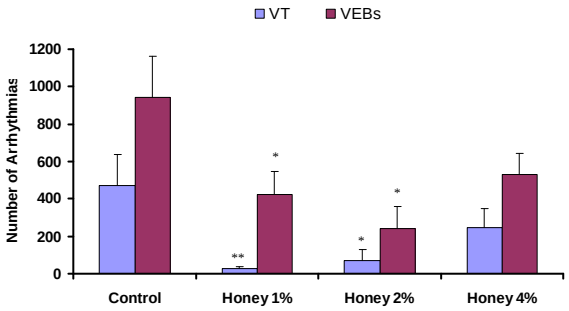

Fig. 2: The total number of ventricular ectopic beats (VEBs) and ventricular tachycardia (VT) in the control and honey treated groups (1%, 2% and 4%) during 30 min ischemia.
Data are represented as mean±SEM.*P<0.05, **P<0.01 versus the control group. N=10-14 rats in each group.

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

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.

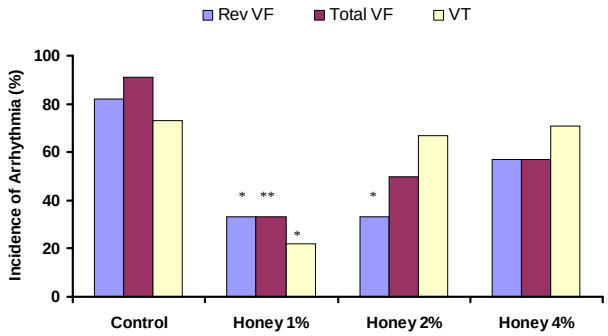

Fig. 3: The incidence (%) of ventricular tachycardia (VT), Reversible Ventricular Fibrillation (Rev VF) and total Ventricular Fibrillation (Total VF) during 30 min reperfusion in the control group and isolated rat hearts feeding by different concentrations of honey (1%, 2% and 4%). *P<0.05, **P<0.01 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 (Gheldof *et al.,* 2002; Gheldof *et al.,* 2003;

Table 3: Effects of prolonged preconditioning with natural honey on reperfusion-induced arrhythmias.

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.

Shimazawa *et al.,* 2005; Baltrusˇaityt *et al.,* 2007; Hegazi *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 against *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 (Gheldof *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

Ahmed AK, Hoekstra MJ, Hage JJ and Karim RB (2003). Honey-medicated dressing: transformation of an ancient remedy into modern therapy. *Ann. Plast. Surg.,* **50**: 143-147.

Ali AT, Chowdhury MN and al Humayyd MS (1991). Inhibitory effect of natural honey on *Helicobacter pylori*. *Trop. Gastroenterol.,* **12**: 139-143.

Al-Waili NS (2004). Topical honey application vs. acyclovir for the treatment of recurrent herpes simplex lesions. *Med. Sci. Monit.,* **10**: 94-98.

Al-Waili NS (2004). Natural Honey Lowers Plasma Glucose, C-Reactive Protein, Homocysteine, and Blood Lipids in Healthy, Diabetic, and Hyperlipidemic Subjects: Comparison with Dextrose and Sucrose. *J. Med. Food,* **7**: 100-107.

Al-Waili NS (2005). Clinical and mycological benefits of topical application of honey, olive oil and beeswax in diaper dermatitis. *Clin. Microbiol. Infect*., **11**: 160-163.

Asadi-Pooya A, Pnjehshahin MR and Beheshti S (2003). The antimycobacterial effect of honey: An *in vitro* study. *Riv. Biol*., **96**: 491-496.

Bahrami M, Ataie-Jafari A, Hosseini S, Forouzanfar M, Rahmani M and Pajouhi M (2008). Effects of natural honey consumption in diabetic patients: An 8-week randomized clinical trial. *Int. J. Food Sci. Nutr.,* **2**: 1-9.

Baltrusˇaityt V, Venskutonis PR and Cˇ eksteryt V (2007). Radical scavenging activity of different floral origin honey and beebread phenolic extracts. *Food Chem.,* **101**: 502–514.

Bilsel Y, Bugra D, Yamaner S, Bulut T, Cevikbas U and Turkoglu U (2002). Could honey have a place in colitis therapy? Effects of honey, prednisolone and disulfiram on inflammation, nitric oxide, and free radical formation. *Dig. Surg.,* **19**: 306-311.

Brady NF, Molan PC and Harfoot CG (1997). The sensitivity of dermatophytes to the antimicrobial activity of manuka honey and other honey. *Pharm. Sci*., **2**: 1-3.

Chepulis LM (2007). The effect of honey compared to sucrose, mixed sugars, and a sugar-free diet on weight gain in young rats. *J. Food Sci.,* **72**: S224-S229.

Chow J (2002). Probiotics and prebiotics: A brief overview. *J. Ren. Nutr*., **12**: 76-86.

Chowdhury M (1999). Honey: Is it worth rubbing it in? *J. R. Soc. Med*., **92**: 663-664.

Ezz El-Arab AM, Girgis SM, Hegazy ME and Abd El-Khalek AB (1999). Effect of dietary honey on intestinal microflora and toxicity of mycotoxins in mice. *BMC Complement. Altern. Med*., **6**: 1-13.

Gheldof N, Wang XH and Engeseth NJ (2002). Identification and quantification of antioxidant components of honeys from various floral sources. *J. Agric. Food Chem.,* **50**: 5870-5877.

Gheldof N, Wang XH and Engeseth NJ (2003). Buckwheat honey increases serum antioxidant capacity in humans. *J. Agric. Food Chem.,* **51**: 1500-1505.

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

opening at reperfusion protects against ischemia–reperfusion injury. *Cardiovasc. Res.,* **60**: 617- 625.

Hegazi AG and Abd El-Hady FK (2007). Influence of Honey on the Suppression of Human Low Density Lipoprotein (LDL) Peroxidation (*In vitro*). *eCAM*., 1 -9

Jeddar A, Kharsany A, Ramsaroop UG, Bhamjee A, Hafejee IE and Moosa A (1985). The Antibacterial action of honey, An in vitro study. *S. Afr. Med. J*., **67**: 257-258.

Johnson DW, Eps CV, Mudge DW, Joan Wiggins K, Armstrong K, Hawley CM and Campbell SB (2005). Randomized, controlled trial of topical exit-site application of honey versus mupirocin for the prevention of catheter-associated infections in hemodialysis patients. *J. Am. Soc. Nephrol.,* **16**: 1456–1462.

Kim GT, Chun YS, Park JW and Kim MS (2003). Role of apoptosis–inducing factor in myocardial cell death by ischemia reperfusion. *Biochem. Biophys. Res. Commun.,* **309**: 619- 624.

Lusby PE, Coombes A and Wilkinson JM (2002). Honey: A potent agent for wound healing? *J. Wound. Ostomy. Continence. Nurs*., **29**: 295-300.

Meda A, Lamien EC, Millogo J, Romito M and Nacoulma OG (2004). Ethnopharmacological communication Therapeutic uses of honey and honeybee larvae in central Burkina Faso. *J. Ethnopharmacol*., **95**: 103-107.

Molan PC (2001). Why honey is effective as a medicine? The scientific explanation of its effects. *Bee World*, **82**: 22-40.

Moundoi MA, Padila-Zakour OI and Worobo RW (2001). Antimicrobial activity of honey against food pathogens and food spoilage microorganisms. Vol. 1. Geneva, Cornell University, NYSAES, pp.61-71.

Najafi M, Mahdizadeh-Aghdam E, Rafie F and Eteraf Oskouei T (2008a). Effects of pharmacologic preconditioning by natural honey on arrhythmias and infarct size in isolated heart. *Pharm. Sci. J*., **13**: 1-11.

Najafi M, Nazemiyeh H, Ghavimi H, Gharakhani A and Garjani A (2008b). Effects of hydroalcoholic extract of *Cynodon dactylon* (L.) pers. on ischemia/reperfusion-induced arrhythmias. *DARU*, **16**: 233-238.

Najafi M, Garjani A, Maleki N and Eteraf Oskouei T (2008c). Antiarrhythmic and arrhythmogenic effects of l-carnitine in ischemia and reperfusion. *Bull. Exp. Biol. Med*., **146**: 210-213.

Najafi M, Javidnia A, Ghorbani-Haghjo A, Mohammadi S and Alireza Garjani (2010). Pharmacological Preconditioning with L-carnitine: Relevance to myocardial hemodynamic function and glycogen and lactate content. *Pak. J. Pharm. Sci.,* **23**(3): 250-255.

Olaitan PB, Adeleke EO and Ola OI (2007). Honey: A reservoir for microorganisms and an inhibitory agent for microbes. *Afr. Health Sci.,* **7**: 159-165.

Rakha MK, Nabil IZ and Hussein AA (2008). Cardioactive and vasoactive effects of natural wild honey against cardiac malperformance induced by hyperadrenergic activity. *J. Med. Food,* **11**: 91-98.

Schramm DD, Karim M, Schrader HR, Holt RR, Cardetti M and Keen CL (2003). Honey with high levels of antioxidants can provide protection to healthy human subjects. *J. Agric. Food Chem.,* **51**: 1732-1735.

Sewllam T, Miyanaga N, Onozawa M, Hattori K, Kawari K, Shimazui T and Akaza H (2003). Antineoplastic Activity of Honey in an Experimental Bladder Cancer Implantation Model: *In vivo* and *in vitro* studies. *Int. J. Urol*., **10**: 213-219.

Shimazawa M, Chikamatsu S, MorimotoN, Mishima S, Nagai H and Hara H (2005). Neuroprotection by Brazilian green propolis against *in vitro* and *in vivo* ischemic neuronal damage. *eCAM*, **2**: 201-207.

Snow MJ and Manley-Harris M (2004). On the nature of non-peroxide antibacterial activity in New Zealand manuka honey. *Food Chem.,* **84**: 145-147.

Tonks AJ, Cooper RA, Jones KP, Blair S, Parton J and Tonks A (2003). Honey stimulates inflammatory cytokine production from monocytes. *Cytokine*, **21**: 242-247.

Trueblood NA, Ramasamy R, Wang LF and Schaefer S (2000). Niacin protects the isolated heart from ischemia-reperfusion injury. *Am. J. Physiol.: Heart Cir. Physiol*., **279**: H764-771.

Walker MJA, Curtis MJ and Hearse DJ (1988). The Lambeth conventions: Guidelines for the study of arrhythmia in ischemia, infarction and reperfusion. *Cardiovasc. Res*., **22**: 447-455.

White JW (1979). Composition of honey. *In:* Honey: A Comprehensive Survey, London, Heinemann, pp.157-192.

Yaghoobi N, Al-Waili N, Ghayour-Mobarhan M, Parizadeh SMR, Abasalti Z, Yaghoobi Z, Yaghoobi F, Esmaeili H, Kazemi-Bajestani SMR, Aghasizadeh R, Saloom Khelod Y and Ferns GAA (2008). Natural honey and cardiovascular risk factors: Effects on blood glucose, cholesterol, triacylglycerole, crp, and body weight compared with sucrose. *Sci. World J.,* **8**: 463-469.

Zacharowski K, Blackburn B and Thiemermann C (2001). Ranolazine: A partial fatty acid oxidation inhibitor, reduces myocardial infarct size and cardiac troponin T release in the rat. *Eur. J. Pharmacol.,* **418**(1-2): 105-110.

Zaghloul A, El-Shattawy H and Kassem A (2001). Honey, a prospective antibiotic: Extraction, formulation, and stability. *Pharmazie,* **56**: 643-647.

Zalibera M, Stasko A, Slebodova A, Jancovicova V, Cermakova T and Brezova A (2008). Antioxidant and radical-scavenging activities of Slovak honeys – An electron paramagnetic resonance study. *Food Chem.,* **110**: 512-521.

Zumla A and Lulat A (1989). Honey: A remedy rediscovered. *J. R. Soc. Med.,* **82**: 384-385.