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The Effects of Simultaneous 8 Weeks Astragalus sp/ Euphorbia Cheriradenia Honey Supplementation and Endurance Training on Membrane Lipid Peroxidation of Erythrocytes after a Bout Acute Exhaustive Treadmill Exercise in Rats

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Abstract:

Strenuous exercise is known to induce oxidative stress leading to the generation of free radicals. The purpose of the present study was to investigate the effects of honey, at a dose (1.5 g/kg weight per d) on the MDA and SOD levels of plasma in rats after exhaustive exercise. Male rats (n=48) were divided into four groups, group 1, sedentary with distilled water (Sed -DW n=12), group 2, sedentary control with honey (Sed-H N=12), group 3, exercise trained with distilled water (ET-WD n=12), group 4, exercise trained with honey (ET- H n=12) groups. Four groups were further divided equally into two groups where the rats were studied at rest and immediately after exhaustive exercise. During the training period, groups 2,4 were treated with 1.5 g/kg body weight honey, freshly diluted by distilled water to 50% and the other groups 1,3 were treated with distilled water (1 ml) just before each administration intragastrically tube daily at 8:00 to rats / once daily for 8 weeks. Endurance training consisted of treadmill running 1.5 h day^{-1}, 5 days a week for 8 weeks, reaching the speed of 2.1 kmh^{-1} at the fourth week. For acute exhaustive exercise, graded treadmill running was conducted reaching the speed of 2.1 km h^{-1} at the 95th min, 10% uphill, and was continued until exhaustion. Erythrocyte

MDA level was significantly increased after exhaustion in groups 1,3 but not in groups 2,4 animals compared with the corresponding sedentary rest. Honey treatment caused a significant decrease in MDA levels of groups 2, 4 compared to the groups 1, 3 animals. While acute exhaustive exercise decreased erythrocyte SOD activity in subgroups 1, 3 rats, it increased the activity of this enzyme in groups 2, 4 rats .Treadmill training increased the endurance time in trained rats compared with sedentary rats. The results of this study suggest that honey supplementation may be useful to prevent acute exhaustive exercise-induced oxidative stress in erythrocytes by up-regulating some of the antioxidant enzyme activities and may have implications in exercising humans.

Key words: endurance training, erythrocyte, exhaustive exercise, SOD, lipid peroxidation.

Introduction

Free radical production occurs continuously in all cells as part of normal cellular function (Sen et al. 2000). At moderate concentrations, however, ROS and other related reactive molecules play an important role in the regulation of signalling processes (Stone and Yang 2006), act as secondary messengers to control a variety of physiologic responses. (Stone and Yang 2006) or are survival and repair signals (Roy et al., 2006). Exhaustive exercise is associated with accelerated generation of reactive oxygen species (ROS), which results in oxidative stress (Ji L 1999), inducing adverse effects on health and well-being. The oxidative stress is an imbalance between the generation of ROS and the antioxidant defence capacity of the body (Sen et al. 2000). Even moderate exercise may increase ROS production, exceeding the capacity of antioxidant defences (Alessio 1993). Specific sources of ROS during exercise include leakage of electrons from the mitochondrial electron transport chain, xanthine oxidase reaction, haemoglobin oxidation and activated neutrophils (Ji L 1999). The ROS has been shown to

induce damage in all cellular macromolecules, such as lipids, proteins and DNA (Packer 1997). Lipid peroxidation of cell membranes changes membrane integrity, leads to increased swelling, and reduces the ability of the cell to maintain ion gradients (Merry et al. 1991). This oxidative damage to cell membranes has been associated with tissue inflammation, muscle fatigue, and impaired recovery following high-intensity exercise (Abuja, 2001).

On the other hand, inhibition of lipid peroxidation is associated with a reduction in muscular fatigue and attenuation of inflammation and reduces skeletal muscle degeneration, and enhances muscle function (Messina et al. 2006), whereas a decrease in glutathione oxidation delays muscle fatigue (Matuszczak et al. 2005). Therefore, inhibition of LDL oxidation might be an important step in preventing impaired athletic performance. There is a considerable volume of literature reporting the incidence of oxidative stress in trained and untrained individuals in response to acute bouts of exercise (Konig et al. 2001).

Given the potential involvement of ROS in detrimental cellular processes, research has focused on the potential beneficial effects of antioxidant consumption (Viña et al. 1999). Some studies have indicated that antioxidant supplementation attenuates oxidative stress, but this is not a consistent finding (Nieman et al. 2002; Arrol et al. 2000; Ashton et al. 1999; Thompson et al. 2001; Vider et al. 2001; Kaikkonen et al. 1998). Corrigan and Kazlauskas reported that vitamins (non- and antioxidant) are the first and most commonly used drugs by athletes (Corrigan and Kazlauskas 2003) for the inhibition of lipid peroxidation and other objects. Nevertheless, evidence suggests that many synthetic antioxidant supplementations may in fact attenuate some of the exercise-induced cellular signals that stimulate adaptations in vascular tissue and skeletal muscle (Gomez et al. 2005; Meilhac et al. 2001).

Furthermore, this appears to allosterically decrease cellular and extracellular antioxidant enzyme activity during

exercise (Goldfarb et al. 1994; Reddy et al. 1998) and may blunt some cellular adaptations induced by exercise which directed most of the attention on the naturally occurring antioxidants (Fejes et al. 1998; Tiwari 2001; Fuhrman et al. 2000; Aviram et al. 2002). Honey has been found to contain significant antioxidant compounds including glucose oxidase, catalase, ascorbic acid, flavonoids, phenolic acids, carotenoid derivatives, organic acids, mallard reaction products, amino acids and proteins (Beretta et al., 2005). The amount and type of these depend largely upon the floral source of the honey darker honeys being generally higher in antioxidant content than lighter honeys (Gheldof et al. 2003).

The phenolic compounds contributed significantly to the antioxidant capacity of honey (Gheldof et al. 2002). Flavonoids can prevent injury caused by free radicals in various ways. Here included is the direct scavenging of free radicals (Korkina and Afanas 1997), the inhibiting of nitric oxide (van Acker et al. 1995) and of the xanthine oxidase activity (Shoskes 1998), the immobilization and firm adhesion of leukocytes to the endothelial, for the formation of oxygen-derived free radicals (Friesenecker et al. 1994) and finally acting through interaction with various enzyme systems (Ferrandiz and Alcaraz 1991).

Antioxidant activity of natural honey was previously reported by many researchers. In a first research, the trial persons were given maize syrup or buckwheat honeys with a different antioxidant capacity in a dose of 1.5 g/kg body weight. In comparison to the sugar control, honey caused an increase of both the antioxidant and the reducing serum capacity (Schramm et al., 2003). In the second study humans received a diet supplemented with a daily honey serving of 1.2 g/kg body weight. Honey increased the body antioxidant agents: blood vitamin C concentration by 47%, ß-carotene by 3%, uric acid by 12%, and glutathione reductase by 7% (Al-Waili 2003). Beretta et al. demonstrated the protective activity of a honey of multifloral origin, standardized for total antioxidant power and analytically profiled (HPLC-MS) in antioxidants, in a cultured

endothelial cell line (EA.hy926) subjected to oxidative stress. Cumene hydroperoxide (CuOOH) was used as free radical promoter. Native honey (1% w/v pH 7.4, 10(6) cells) showed strong quenching activity against lipophilic cumoxyl and cumoperoxyl radicals, with significant suppression/prevention of cell damage, complete inhibition of cell membrane oxidation, of intracellular ROS production and recovery of intracellular GSH. They suggested that, through the synergistic action of its antioxidants, honey, by reducing and removing ROS, may lower the risks and effects of acute and chronic free radical induced pathologies in vivo (Beretta et al. 2007). However, there is no report regarding antioxidant effects of chronic oral administration of natural honey against oxidative stress induced by exhaustion exercise; therefore, in the present study, effects of chronic administration of oral honey on oxidative changes induced by a bout acute exhaustive aerobic exercise were investigated in Wistar rat.

MATERIALS AND METHODS

Animal care

Male Wistar rats weighing 210–275 g (n = 48, 12 weeks old) were purchased from Razi Vaccine & Serum Research Institute and were used in this study. All rats were housed in conventional wire-mesh cages, four rats per cage, in a room with the temperature regulated at 23 ±2°C, humidity 50-45% and in daily light / dark cycle (12h) (0700-1900 h dark; 1900-0700 h light), given standard rat chow and tap water ad libitum. All procedures were approved by the Tehran University Animal Care and Usage Committee and followed the guidelines established by American Physiological Society.

Experimental design

The animals were housed for one week prior to any special treatment. In the second-week all the animals were randomly divided mainly into four groups, group1, sedentary

with water (Sed -DW N=12), group2, sedentary control with distilled honey (Sed-H n=12), group3, exercise trained with distilled water (ET-WD n=12), group4, exercise trained with honey (ET- H n=12). Four groups were further divided equally into two groups where the rats were studied at rest and immediately after exhaustive exercise. During the training period, groups2,4 were treated with 1.5 g/kg body weight, honey was freshly diluted by distilled water to 50% and the other groups 1,3 treated with distilled water (1 ml) just before each administration, intragastrically tube, daily at 8:00 to rats / once daily for 8 weeks. Two hours after administration, the animals in the groups3, 4 were run on the treadmill 5 days a week for 8 weeks. Experiments were conducted between 10:00 and 12:00 h.

Training and Acute Exhaustive Exercise

After divided, the acclimated group (ET) rats underwent treadmill exercise through the use of one 15-minute running session on a rodent treadmill at a speed of 10-15 m/min and a 0° grade (1 session a day, 5 days/week). The treadmill was equipped with an electric shock grid on the rear barrier to provide exercise motivation to the animals. Trained exercise began with gradual increases in training speed and time so that rats were running 2.1km h^{-1} in the fourth week. Training continued for 1.5 h day^{-1}, 5 days a week, for 8 weeks. During the eighth week of the training program, the groups (Sed) were also accustomed to treadmill running at 1.0–1.2 km h^{-1}, for 15 min day^{-1}, for 5 days before sample collection. This regimen was used to ensure that untrained rats could also tolerate the acute exhaustive exercise without having a significant training effect (Sen et al. 1992). At the end of the training period and after 3 days at rest, half of all rats were randomly selected into the acute exhaustive exercise group (each group N=6 , totality N=24). In acute exhaustive exercise, running speed was 1.2 km h^{-1} (10% uphill gradient) for the first 10 min; after that the speed was increased gradually to 2.1 km h^{-1}at the 95th min, and kept constant until the rats were exhausted. The loss of the

righting reflex when the rats were turned on their backs was the criterion of exhaustion. To eliminate diurnal effects, the experiments were performed at the same time (10.00–12.00 hours). Immediately after exhaustion exercise, blood samples were obtained from the abdominal aorta of the rats under light ether anesthesia and anticoagulated with sodium heparin (15 U/ml). The other half of all rats (N=24) underwent anesthesia immediately before the acute exhaustive exercise, then blood samples were obtained according to the same program. These samples were used for the measurement of lipid peroxidation, and antioxidant defence system of erythrocytes.

Determination of erythrocyte malondialdehyde (MDA) level and the activities of SOD

At the end of the experiment, the animals were anaesthetized with ketamine-HCl (Ketalar, 20 mg/kg^{-1}, i.p.), and the blood was collected by cardiac puncture after thoracotomy. Blood samples were collected in vacutainer tubes with K_3-EDTA as anticoagulant (1/10, v/v). They were centrifuged at 3000 g for 15 min and plasma was removed using a Pasteur pipette. Then, erythrocytes were washed with 0.9% NaCl solution three times, and washed erythrocytes were haemolysed by dilution with deionized water (50-fold). Haemoglobin (Hb) values of the samples were measured using a GEN-S counter haematology analyser. The haemolysate was kept at –80˚ C until biochemical determinations.

The erythrocyte MDA level was estimated by the method described by Jain et al. (1989) based on thiobarbituric acid reactivity. MDA, an end-product of fatty acid peroxidation, reacts with TBA to form a coloured complex that has maximum absorbency at 532 nm. For this purpose, 0.2 ml hemolysate was suspended in 0.8 ml phosphate-buffered saline (pH 7.4) and 0.025 ml butylated hydroxytoluene. Thirty percent trichloroacetic acid (0.5 ml) was added. Tubes were vortexed and allowed to stand in ice for at least 2 h. They were then

centrifuged at 2000 rpm for 15 min. One millilitre of each supernatant was transferred to another tube, and 0.075 ml of 0.1 mol l^{-1} EDTA and 0.25 ml of 1% TBA were added. Tubes were mixed and kept in a boiling water bath for 15 min. Absorbency was read at 532 and 600 nm (600 nm reading is for preventing haemoglobin interference), after tubes were cooled to room temperature. Butylated hydroxytoluene, an antioxidant, was added to prevent MDA formation during the assay, which could result in falsely elevated TBA activity. Absorbency at 600 nm was subtracted from absorbency at 532 nm. The concentration of MDA was calculated using $1.56 \cdot 10^5$ cm^{-1} mol^{-1}, the absorbency coefficient of the MDA–thiobarbituric acid complex at 532 nm. Superoxide dismutase (SOD) activity was determined by inhibition of the reduction of nitroblue tetrazolium by superoxide anion radicals, which are produced by the xanthine-xanthine oxidase system (Sun et al., 1988).

Statistical analyses

Results given are means (SE). To evaluate differences among the four groups studied, one-way ANOVA with Bonferroni post-hoc test was used to compare endurance times at the end of the study and body weights both at the beginning and at the end of 8 weeks of treadmill training. Paired t-test was also used to compare the body weights before and after 8 weeks of training. To evaluate differences MDA, SOD among the groups studied two-way ANOVA with Scheffe post hoc test was used. Differences were considered statistically significant when $P< 0.05$.

Results

Body weight and endurance time

Body weights among the four groups increased during the 8-week study period (Table 1). There were no significant differences in body weight among the four groups. (Table 1).

Exhaustion time was significantly longer in ET-H group compared with ET-WD and Sed groups. The mean endurance time of treadmill running to exhaustion was 162 ±10, 138 ±16 , 120±17, 112±14 min for group ET-H , ET-WD, Sed-H, Sed- WD respectively. There were significant differences in endurance time among the four groups.

Table 1 Body weights of untrained and trained rats at the beginning and at the end of the study. The results are means (SE)

Parameter Group	Groups			
	Sedentary (Sed)		Exercise Trained (ET)	
subgroups	Sed-DW	Sed -H	T -DW	T-H
N	12	12	12	12
Body weight (g) at the beginning of the study	237.6±20.6	243.4±13.6	240±16.6	233.3±21.2
Body weight (g) at the end of 8 weeks	282.9±11.1#	284.4±12.3#	275.2±10.0#	269±13.6#

Sed, sedentary; ET, exercise trained, DW, distilled water, H, honey.
#P<0.001, paired t-test, difference in body weights before and after 8 weeks
difference in body weights between (Sed) and (ET) groups before and after 8 weeks, *P was < 0.05, one way ANOVA with Bonferroni post hoc test

Malondialdehyde level

Malondialdehyde results are presented in (Fig. 1). Erythrocyte MDA level was significantly increased after exhaustion in the Sed-DW-Exh, ET-DW-Exh groups but not in the Sed-H- Exh, ET-H- Exh groups animals compared with the corresponding sedentary rest($p<0.05$).The honey treatment significantly prevented the increases in Erythrocyte MDA levels in the Sed-H- Exh, ET-H- Exh groups induced by exhaustive

exercise compared to the Sed-DW-Exh, ET-DW-Exh groups animals($p<0.05$).

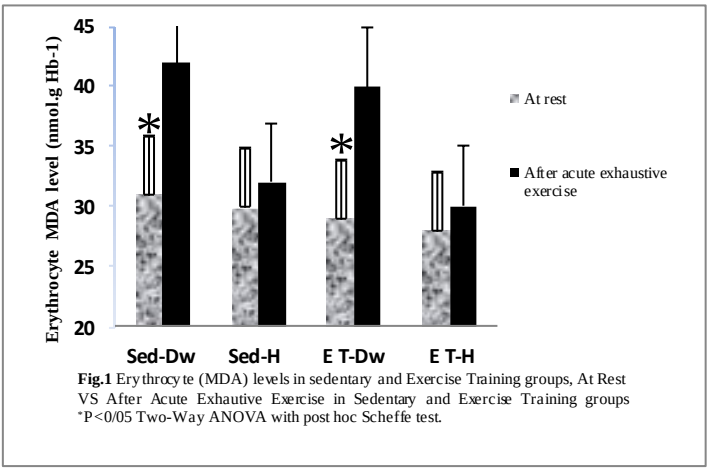

Fig.1 Erythrocyte (MDA) levels in sedentary and Exercise Training groups, At Rest VS After Acute Exhautive Exercise in Sedentary and Exercise Training groups *P<0/05 Two-Way ANOVA with post hoc Scheffe test.

Antioxidant enzymes

The results of erythrocyte SOD enzyme activity in four groups are shown in Figure 2. SOD enzyme activity was significantly decreased in the Sed-DW-Exh, ET-DW-Exh than controls (Sed- Re, ET- Re) ($p<0.05$). Comparing the supplemented groups, Sed-H- Exh, ET-H- Exh groups had significantly increased SOD activities than their controls (Sed-H- Re, ET-H- Re) ($p<0.05$). Comparing the Sed-H- Exh, ET-H-Exh groups, SOD activity of the honey-supplemented groups was significantly higher than Sed- Exh, ET- Exh groups ($p<0.05$).

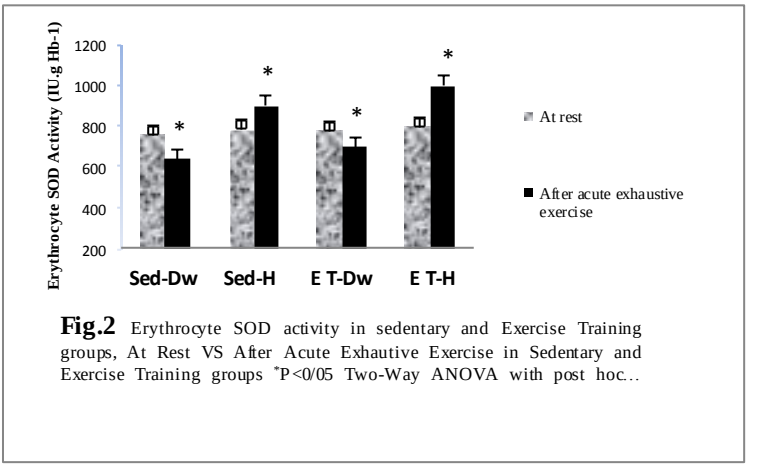

Fig.2 Erythrocyte SOD activity in sedentary and Exercise Training groups, At Rest VS After Acute Exhautive Exercise in Sedentary and Exercise Training groups *P<0/05 Two-Way ANOVA with post hoc…

Discussion

In this study, antioxidant enzymes activities of erythrocytes, and erythrocyte peroxidation were investigated following acute exercise of honey-supplemented and normal diet fed rats. The SOD activities of erythrocytes was significantly increased and decreased in Sed-H-Exh, ET-H-Exh and Sed-Dw-Exh, ET-Dw-Exh groups respectively compared with pre-strenuous exercise at rest in control groups after exhaustive exercise. As well as the erythrocyte peroxidation index significantly and no significant increase in Sed-Dw-Exh, ET-Dw-Exh and Sed-H- Exh, ET-H- Exh groups respectively compared with pre-strenuous exercise at rest in control groups after exhaustive exercise. At the end of 8 weeks, supplemented animals did not differ from non-supplemented rats in body weights. Thus, supplementary honey had no influence on growth rate of the rats. In this research, honey treated groups showed a significant increase endurance time of treadmill running to exhaustion compared with the control groups ($P<0.05$). The results showed that honey supplementation increased performance of exhaustive exercise in rats.

Oxidative stress induced by acute exercise can significantly elevate markers of erythrocytes peroxidative

damage because physical exercise promotes the production of ROS due to a substantial increase in oxygen consumption (Chen et al., 2002). The MDA, a metabolite of phospholipid peroxidation, is a popular index of first condition on living body oxidative damage (Lu et al., 2006). In the present study, the MDA concentration in plasma was significantly increased in the Sed-Dw-Exh, ET-Dw-Exh groups which were exhausted after nearly 2 h of running as compared with pre-strenuous exercise at rest in control groups. This result agrees with Chieh-chung et al., (2005) who reported that MDA in plasma and muscle significantly increased by 0.8 and l.l fold respectively with strenuous exercises caused oxidation damage, increasing lipid peroxidation and decreasing antioxidant. Huang et al., 2008, reported that strenuous exercise elevate MDA, XO and MPO levels of myocardial, muscular, hepatic, pulmonary and renal tissues.

The current study showed that MDA levels of plasma were not significantly increased in the Sed-H-Exh, ET-H-Exh groups compared to pre-strenuous exercise at rest in control groups ($P<0.05$). As per the above findings, it is suggested that fortifying antioxidant defence mechanisms with 8 weeks treatment with honey supplementation results in reduced erythrocyte deterioration in relation to decreased oxidant stress after exhaustive running episodes. This result lends support to previous investigations that show honey, like other antioxidant agents, does protect against damage or injury. This protective effect of honey is partly mediated via amelioration of oxidative stress in plasma. This finding is consistent with the results obtained by Erejuwa et al. 2010; Erejuwa et al. 2011; Blasa et al. 2007; Erejuwa et al. 2011, who reported that the combination of honey with metformin or glibenclamide might offer additional antioxidant effect to these drugs. This might reduce oxidative stress-mediated damage in diabetic kidneys. The animals were treated honey (1.0 g/kg) orally once daily for four weeks. Blasa et al., 2007, claimed that honey flavanoids are able to prevent the production of MDA.

The presence of phenolics in honey might have also contributed to the reduction of oxidative stress. This is so because phenolic content in honey was found to have antioxidant activity and the ability to scavenge free radical activity (Kishore et al. 2011).The antioxidant activity of honey is generally attributed to its phenolic compounds and flavonoids (Beretta et al., 2007; Kishore et al., 2011). The main phenolic and flavonoid compounds in honey include ellagic acid, gallic acid, syringic acid, benzoic acid, cinnamic acid, ferulic acids, myricetin, chlorogenic acid, caffeic acid, hesperetin, coumaric acid, isoramnetin, chrysin, quercetin, galangin, luteolin and kaempferol (Eraslan et al. 2010; Petrus et al. 2011). While some of these bioactive compounds such as alangin, kaempferol, quercetin, isorhamnetin and luteolin are found in most honey samples, others such as hesperetin and naringenin are found in few honey varieties Petrus, K., 2011. Gallic acid is one of phenolic compound in honey, which has been found to be the strongest free radical scavenger. It has aromatic rings with hydroxyl group in its molecular structure, which can decrease or chelate the divalent ions that act as the catalyst towards lipid peroxidation process (Perez et al. 2006).

Other researchers show that honey can determine the amelioration of oxidative stress in tissues such as GIT, liver, kidney, pancreas, eye, plasma, red blood cells and reproductive organs (Gharzouli et al. 2002; Al-Waili et al. 2006; Mohamed et al. 2011; Zaid, et al. 2011; Erejuwa et al. 2010; Kassim, et al. 2010).Furthermore, Astragalus sp/ Euphorbia cheriradenia-honey, which is darker in colour, contains higher amount of antioxidants because studies have revealed that honey with dark colour such as buckwheat and mixed-breed honey have higher antioxidative and scavenging activities against free radicals or active oxygen species (Nagai et al. 2006; Khalil et al. 2011).

On the other hand, honey phenolics are readily available and they are absorbed better through the gut barrier due to its presence in the aglyconic form produced by glycosidases present

in the bee salivary glands (Gheldof et al. 2003), in contrast with other phenolics in foods or beverages such as tea, which are present in glycosidic form that requires absorption by passive diffusion. Available evidence suggests that honey may ameliorate oxidative stress by scavenging both free radicals such as $OONO^{-}$,$O2^{\cdot -}$ (Estevinho et al. 2008) and non-free radicals such as NO (Bilsel et al. 2002). Recently, it has been shown that honey ameliorates oxidative stress by up-regulating Nrf2 moderately, an important intracellular transcription factor (Erejuwa et al. 2009). Evidence also suggests that honey may reduce inflammation as evidenced by the inhibition of NO and prostaglandin E(2) production (Bilsel et al. 2002). This is important because both oxidative stress and inflammation are interrelated (Peake et al. 2007).

It is well known that SOD is regarded as the first line of defence by the antioxidant enzyme system against ROS generated during exhaustive exercise (Huang et al. 2009). The increase in SOD in plasma would indicate an up-regulation of the defence mechanism to try to cope with an enhanced production of super oxide anion radicals. This in turn might help to down-regulate the production of lipid peroxides or oxidative stress (Lee et al., 2009). In the present study, the acute exercise caused a significant decrease in erythrocyte SOD activity in Sed-Dw-Exh, ET- Dw-Exh groups compared to control groups. Conflicting results were reported regarding erythrocyte SOD activities after acute exercise.

Vesovic et al. and Akova et al. have determined that SOD activities decreased in the erythrocytes after acute exercise (Vesovic et al. 2002; Akova et al. 2001). However, Duthie,et al. found that SOD activities did not change after acute exercise in the erythrocytes (Duthie et al. 1990). Regarding animal studies, (Gunduz and Senturk 2003) showed

an increase in SOD activity in the erythrocytes of the untrained rats after exhaustive exercise.

However, Oztasan et al., 2004 reported a decrease in erythrocyte SOD activity and an increase in erythrocyte GPx activity following acute exhaustive exercise in untrained rats. Clearly, these results are mixed and likely depend on the time of sampling, as well as the duration and intensity of exercise, which have varied considerably across studies.

On the other hand, acute exhaustive exercise increased the erythrocyte SOD activity in trained rats in Sed-H- Exh, ET-H- Exh groups, which suggested that honey supplementation was able to up-regulate antioxidant enzymes to protect against oxidative stress-induced injury during exercise. Antioxidant nutritional supplementation improves antioxidant defence of the erythrocytes (Senturk et al. 2001; Peng et al. 2000).

An increase in SOD activity in Sed-H- Exh, ET-H- Exh groups confirmed previous knowledge; honey supplementation prevented the decrease in SOD activity after acute exercise. The ability of honey to modulate antioxidant enzymes activities were supported by other studies (Erejuwa et al. 2010; Petruset et al. 2011; Yao et al. 2011; Erejuwa et al. 2010; Erejuwa et al. 2012; Mohamed et al. 2011). These studies show that honey supplementation significantly restored the activities of SOD in different tissues, organs, body fluids or compartments. These include liver and erythrocytes (Yao et al., 2011), hepatic (Petrus et al. 2011), pancreas (Erejuwa et al. 2010) kidney (Erejuwa et al . 2012) testis (Mohamed et al. 2011).

Conclusion

In conclusion, this study is to directly verify the effect of flavonoids from honey against oxidative stress induced by

exhaustive exercise. The experiment results indicated that honey supplementation increased performance of exhaustive exercise, reduced lipid per-oxidation, and up-regulated antioxidant enzymes to protect against oxidative stress-induced injury during exercise.

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