European Online Journal of Natural and Social Sciences 2016; www.european-science.com
Vol.5, No.1 pp. 241-248
ISSN 1805-3602

The Effect of Short-Term Supplementation of Melissa Officinalis Extract on the Level of Serum Malondialdehyde and Total Antioxidant Capacity after Aerobic Activity at a Negative Slope

Arezoo Adelifar[1*], Pezhman Motamedi[2], Yengi Maleki Gholam Hossein[3]
[1]Exercise Physiology, University of Tehran, Iran; [2]Kharazmi University of Karaj, Iran; [3]Exercise Physiology, Kharazmi University of Karaj, Iran
*E-mail: arezooadeli100@yahoo.com

Received for publication: 08 November 2015.
Accepted for publication: 28 February 2016.

Abstract

The aim of this study was to investigate the effects of downhill running and short-term supplementation of Melissa officinalis on the level of serum Malondialdehyde and total antioxidant capacity in male athletes. In this study, 20 healthy male athletes were randomly divided into two groups of 10 subjects (supplement and placebo). The level of serum Malondialdehyde and total antioxidant capacity were measured before and after supplementation. After data collection, repeated variance analysis test for investigating time series and if significant, Bonferroni post hoc test was used and independent t-test was used to assess the differences between groups. The level of significance of ($p \leq 0.05$) was used. The study findings showed that the level of serum Malondialdehyde in the supplement group was increased less than placebo. Also, the total antioxidant capacity rate in the supplement group was more. In general, it is concluded that this herbal supplement can prevent the increase of serum malondialdehyde level and increase the total antioxidant capacity.

Keywords: Melissa officinalis, malondialdehyde, total antioxidant capacity, extroverted activity

Introduction

Downhill running such as coming down from the mountain or stairs with extroverted contractions cause more muscle soreness than any other muscle contractions (Masayoshi, Toshio, 2000; Afshar, Etemadian, and Bashiri, 2010). The main feature of this type of contraction is double pressure on muscle fibers, soft tissue and finally, muscle damage during the performing these activities. Although the extroverted contraction in terms of metabolism needs less energy than other activities, this type of contraction leads to small skeletal muscle damage, the stronger inflammatory response as well as the larger proportion of oxidative stress compared to other contractions (Ghanbari, Tayebi, & Hassan, 2011).

Shafat et al (2004) by using high doses of vitamin E and C in the longer term (37 days) concluded that the reduction of peak power was observed less (Shafat, Butler, Jensen, & Donnelly, 2004). In this study, indictors of inflammatory and oxidative stress were not measured.

Bloomer (2006) in his study showed that the supplementation of vegetables and fruits for 2 weeks before aerobic training reduces oxidative stress in men and women and act like vitamins E and C supplementation (Bloomer, Goldfarb, McKenzie, 2006). In addition to fruits and vegetables, today, scientists have studied the antioxidant properties of medicinal plants such as saffron, cinnamon, green tea, etc. (Ahmed Rahim, 2012). Melissa officinalis is an medicinal herb with anti-oxidant property. Rostami et al (2011) examined the antioxidant effect of Melissa officinalis with

vitamin C and concluded that it has the same effect with vitamin C and suggested that it could be named as natural antioxidants (Rostami, Momeni, & Behnam Rasouli, 2011).

Melissa officinalis is an aromatic plant from Lamiaceae family and mainly grows in central and southern Europe, North Africa, Mediterranean area, and in northern parts of Iran. The indigenous name of this plant in Iran has been Melissa officinalis, Varanjbou and Blue skullcap which is enriched with antioxidant compounds. Antioxidant power of this plant is measured by common and laboratory methods (Akhondzadeh, Noroozian, Mohammadi, Ohadinia, Jamshidi, & Khani, 2003) and in previous studies the antioxidative impact of this plant on radiology staff (Zeraatpishe, Oryan, Bagheri, Pilevarian, Malekirad, & Baeeri, 2011) aluminum workers (Malekirad & Pilehvarian, 2012) and fatty liver diseases (Malekirad et al, 2012) is checked and given that any study has not been conducted on the effect of aqueous extract of this plant on athletes. Therefore, the aim of this study was to investigate the effects downhill running (running 30 minutes on the treadmill at 65% of peak aerobic power and with a negative slope of 8.5 degrees) and short-term supplementation of Melissa officinalis (1.5 grams in T-Bag form per day in two shifts and for 14 days) on the level of serum Malondialdehyde and total antioxidant capacity after aerobic activity at negative slope on male athletes.

Research Methodology

Subjects

The research after ethics committee approval in research of the Islamic Azad University of Boroujerd, in form of quasi-experimental design was conducted. The sample consisted of 20 healthy male athletes with regular exercise. They were not smokers and did not have any medical history of heart, liver, kidney, physical, etc. and declared their insensitivity to Melissa officinalis using or bloodletting. Participants after completing the consent form were willing to cooperate voluntarily. Therefore, after full introduction of the subject, objectives and methodology, healthy subjects by taking into account criteria of age, weight, body mass index, body fat percentage, sports history and no history of diseases and injuries were selected by using healthy questionnaire. The sample size was determined 10 subjects for each group based on previous studies, at significance level (alpha or Type I error) of 5% and power (beta or type II error) of 0.2 by using Medcal software with 10.0.2.0 version. In each group, 10 subjects were randomly replaced into two groups of complementary exercise (Melissa officinalis) and placebo.

Data collection

Before starting the test, the objectives, details as well as possible risks of activity implementation were described for participants and then they were written consent form. At the same meeting, the height (cm) of subjects was recorded with an accuracy of 0.1 by height gauges made in Iran. The body fat percentage of participants was estimated by measuring the thickness of the fat layer under the skin of thoracic, abdominal and thigh area by using calipers Lafayette made in America, and by substituting in the equations for estimating body fat percentage proposed by Jackson and Pollock (Jackson & Pollock, 1978; Jackson & Pollock, 1985).

The first blood samples to determine baseline values of intended indices was taken from the elbow vein ten days before extroverted aerobic activity. The second blood samples were taken immediately after the implementation of exercise protocol (before consumption of supplement). The aerobic power and anaerobic power of the subjects were measured a week before the implementation of exercise protocol. Each of the supplement and placebo groups, respectively, used Melissa officinalis (daily intake of 1.5 mg per kg body weight) and dextrose (daily intake of 1.5 mg per kg body weight) for 14 days. After 14 days, subjects again implemented the protocol and immediately after that, the third blood samples were taken from participants. Before taking blood

samples in each stage subjects were asked to complete the 24-hour dietary questionnaire. In addition, they were asked to avoid to take any medication, smoking, antioxidant supplements and anti-inflammatory complementary such as ibuprofen, ginger, etc. during the project.

Extroverted exercises

Each of the subjects ran on a treadmill for 30 minutes at 65% maximum oxygen consumption and slope of -8.5 degree (15%). The base heart rate of each individual after 10 minutes of rest (sitting status) with Polar pulse meter was recorded. Also, the maximum heart rate during the implementation of Bruce test were recorded through the screen of treadmill. On the other hand, Karonon method was used to control the intensity of activity of 65% of maximum heart rate. Before the implementation of the Protocol to warm up, participants did stretch exercise for 5 minutes and then ran 3 minutes on a treadmill with no slope. After this stage, the slope and speed of the treadmill in order to achieve target heart rate (65% heart rate reserve) during two minutes was increased. Each of the subjects by approaching to 65% heart rate reserve and 15% negative slope ran on treadmill for 30 minutes. Heart rate, slope and speed of the treadmill until the end of the exercise was controlled by the researcher (Afshar, Etemadian, & Bashiri, 2010).

Measurement of blood indices and research variables

All stages of research under standard conditions with relative humidity of 55%, temperature of 25 ° C were done between 8 to 10 am. Base blood sample was taken from all subjects bout five millimeters. Then, blood sample was placed for 30 minutes at laboratory temperature of 22 to 25 degrees to clot. After that, serum was separated by centrifugation device and by using kits of Pars Company, the activity rate of serum Malondialdehyde and total antioxidant capacity by autoanalyzer (Alcyon 300) were measured.

Statistical analysis methods

After enumerating the data and serum index, at first, the general characteristics of the subjects and research data were presented in the form of charts and tables and by using software EXCEL2007 was investigated descriptively. Then, to investigate the research hypotheses (after confirming the normality of the data: results of Kolomograve- Smirnov and homogeneity of variances) the repeated inferential statistical variance analysis after Bonferroni test was used to compare the difference in various time series. Also, interactive effect between groups and within groups (two-sided analysis) was specified and if significant, the t-test was used to determine the difference between the groups and all tests at a significance level of 5% by using SPSS software version 19 were investigated. In addition, the effectiveness of each of the independent variables were determined by using Chi Eta.

Results

Characteristics of the subjects are presented in Table 1 and other specifications are listed in Table 2 and Figure 1.

The total antioxidant capacity level

Results of variance analysis of pain and fatigue (measurement steps and differences between groups) indicate that both short-term supplementation of Melissa officinalis and downhill running influence the total antioxidant capacity changes. Bonferroni post hoc test results indicate that the amount of this index in both groups immediately after downhill running and without supplementation was reduced significantly and almost equally (Figure 1). However, it should be noted that the mean of total antioxidant capacity immediately after the exercise protocol and using supplementation in the group of Melissa officinalis was significantly higher than the group receiving placebo and therefore, the scope of these changes was greater for the second group so that the share

of effect (Chi Eta) in the placebo group was 0.014 and in Melissa officinalis supplement group was 0.522.

Table 1: Mean and standard deviation of anthropometric and physiological indices

Indicators under study	Group	Mean	SD
Age	Mean	15/70±1/25	1/25
	Placebo	15/90	1/20
Weight (kg)	Melissa officinalis	61/85	9/103
	Placebo	58/10	8/949
Height (cm)	Melissa officinalis	176/10	5/405
	Placeb	174/10	5/446
Body mass index (kilograms per square meter)	Melissa officinalis	20/589	2/394
	Placebo	20/559	2/308
Fat percentage(%)	Melissa officinalis	11/607	0/247
	Placebo	11/711	0/130
Maximum oxygen consumption (ml / kg / min)	Melissa officinalis	50/00	4/761
	Placebo	49/80	2/936

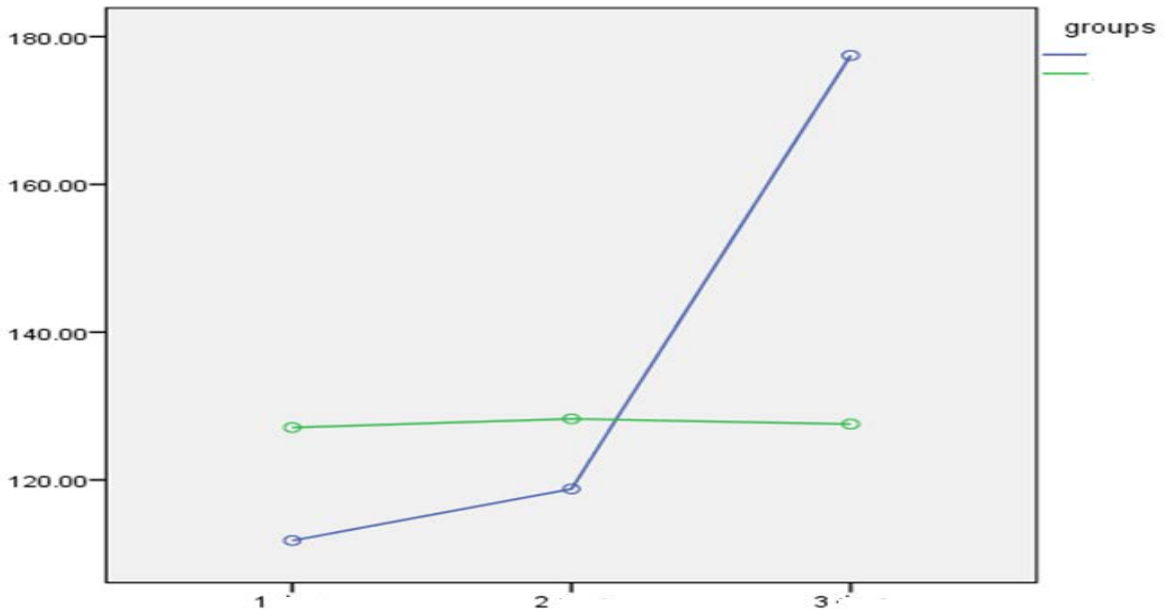

Figure 1: Changes in total antioxidant capacity of Melissa officinalis and placebo groups during different stages (Green line refers to placebo and blue line refers to Melissa officinalis)

Malondialdehyde

The variance analysis results of pain and fatigue (measurement steps and differences between groups) demonstrate that both short-term supplementation of Melissa officinalis and downhill running affect the Malondialdehyde changes. (Table 2) Bonferroni post hoc test results indicate that the amount of this index in both groups immediately after downhill running and without using supplementation was significantly and almost equally increased (Figure 2). It should also be pointed out that the mean of Malondialdehyde immediately after the implementation of exercise protocol and using supplementation in the group of Melissa officinalis was significantly lower than the group receiving placebo and thus, the scope of these changes was greater for the

second group so that the share of effect (Chi Eta) in the placebo group was 0.369 and in Melissa officinalis supplement group was 0.336.

According to the results of the independent t-test and the scope of MDA changes, it can be said that short-term supplementation of Melissa officinalis significantly prevents the relative enhancement of Malondialdehyde (MDA) of male athletes immediately after downhill running (Table 2 and Figure 2).

Table 2: The level of total antioxidant capacity and Malondialdehyde (MDA) (mean ± SD)

Variables	Groups	After the activity (after supplementation)	After the activity (before supplementation)	The amount of base
Malondialdehyde	Melissa officinalis	28.58±11/55*‡†	31.65±15/64†	18.66±2/41
	Placebo	30.47±15/54†	30.45±15/81†	18.29±2/30
Total Antioxidant	Melissa officinalis	15.50±38/24*‡†	123.52±32/48†	119.44±28/91
	Placebo	127.56±19/87	128.27±22/10	127.09±23/42

*: Represents a significant difference between groups (P <0.05). †: represents a significant difference compared to baseline P <0.05. ‡: represents a significant difference compared to after exercise before supplementation (P <0.05).

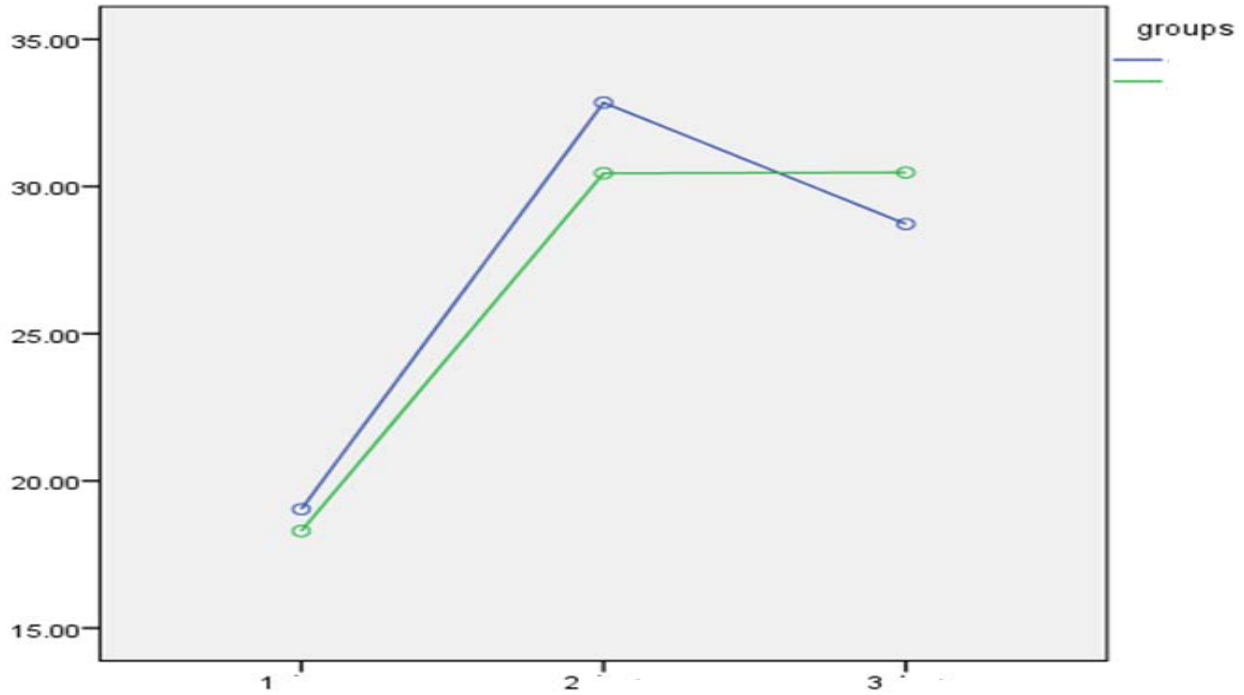

Figure 2: Changes in Malondialdehyde in Melissa officinalis and placebo groups during different stages (Green line refers to placebo and blue line refers to Melissa officinalis)

Discussion

In this study, brewed Melissa officinalis consumption by reducing free radicals on the one hand and on the other hand, the enhancement of the total antioxidants lead to the reduction of oxidative stress and damages caused by it like lipid peroxidation. By measuring malondialdehyde level of blood as a measurement criterion of the lipid peroxidation rate, the amount of oxidation in the cells is specified. The study results showed that the amount of MDA was increased in both

groups after exercise protocol. Although there was no significant between-group difference, this increase was higher in the placebo group. Many studies indicate antioxidant activity of different extracts of this plant in condition and show that extracts of this plant can cleanse free radicals such as Diphenyl Peykril Hydrazyl (DPPH), Azinobis, superoxide anions, hydroxyl, lipid peroxide, and nitric oxide and extracts of this plant can also prevent lipid peroxidation (increase of MDA) (Dastmalchi, Ollilainen, Lackman, Boije af Gennäs, Dorman, Järvinen, Yli-Kauhaluoma, & Hiltunen, 2009).

However, studies on the antioxidant properties of this plant is very low and often is in the form of animal experiments (Triantaphyllou, Blekas, & Boskou, 2001). For example, in some animal studies, antioxidant and inhibitory effect of this plant on lipid peroxidation in rats has been proved (Lima, Fernandes-Ferreira, & Pereira-Wilson, 2006). Human studies conducted on this plant is more about its healing properties. These studies sometimes peripherally point out to the antioxidant properties of this plant. In a study which was conducted to evaluate the protective effect of aqueous and methanol extracts of the plant on the nervous system and in ex vivo form on PC12 cell line, the antioxidant and sweeping power of free radicals by these extracts was proved (López, Akerreta, Casanova, García-Mina, Cavero, & Calvo, 2007).

This study was conducted on athletes for the first time and the antioxidant effect of this plant has not been tested on athletes, however, the supplementation effect of other plants was studied and some of them are listed below:

In one study, drinking green tea inhibits lipid peroxidation in blood erythrocytes of mice and increase the levels of non-enzymatic and enzymatic antioxidants including glutathione, vitamin A and E in their blood. In another study, the effect of drinking oak tea on mRNA antioxidant enzymes, lipid peroxidation and antioxidant status was examined in healthy young women (Wojciech, Ewa, & Elzbieta, 2010). Drinking this tea continuously reduces lipid peroxidation and increases the levels of antioxidant enzymes including Superoxide dismutase, catalase and glutathione peroxidase, and increases plasma total antioxidant level. As it can be seen in all these studies, drinking herbal teas reduces oxidative stress indices and increase antioxidant defense system including enzymatic defense that they are consistent with the study. In the current study, total antioxidant level similar to the above researches, was higher in the Melissa officinalis group and shows that Melissa officinalis like tea can reduce oxidation and increase effective antioxidant. It is likely, Melissa officinalis extract reduces free radicals in different ways and thereby reduces lipid peroxidation and DNA damage (Hohmann, Zupkó, Rédei, Csányi, Falkay, Máthé, & Janicsák,1999). The extract of this plant can revive iron ions with giving electron and thereby swept free radicals and also, by combined transition metal prevents the decomposition of hydro peroxides and Fenton reactions and the formation of free radicals. This activity is called Chelating activity. In this respect, the mentioned extract is stronger than the ascorbic acid and gallic acid (Katalinic, Milos, Kulisic, & Jukic, 2006). The mechanism is very important because it is assumed that transition metal ions such as bivalent iron ions contribute to oxidative damage of nervous tissues and Alzheimer and Parkinson diseases and therefore, such a plant extract can be examined in the treatment of these diseases. Also, this ability can be used in the food industry. As mentioned, the ability of this plant in scavenging various radicals is its other mechanism in preventing oxidative damages. For example, superoxide and nitric oxide radicals which have key role in many diseases including diabetes, cancer, Alzheimer, Parkinson, and cardiovascular and respiratory diseases, extract of this plant by cleaning these radicals can be effective in the treatment of these disease (Triantaphyllou, Blekas, & Boskou, 2001). In order to better explanation of the mechanism of brewed Melissa officinalis in the study, the main components of the brewed Melissa officinalis should be examined. Major antioxidant compounds within the brewed Melissa officinalis are phenolic compounds (Katalinic, Milos,

Kulisic, Jukic, 2006). The effect of phenolic and flavonoids acids in reducing lipid peroxidation is well proved (Verma, Vijayakumar, Mathela, & Rao, 2009) Rosmarinic acid, quercetin and luteolin by cleaning hydrogen peroxide radicals and preventing the production of superoxide radicals reduce lipid peroxidation. The most important of them are rosmarinic acid, luteolin-7-O-glycosides, Quercetin3-Rotinozoeid, gallic acid, acid Colorogenic acid, quercetin3-galactoside, ferulic acid, very little values of vanillin, trans-cinnamic acid, trans-4-coumaric acid and hydroxycinnamic acid and among them Rosmarinic acid allocates more than 40% of the content of phenolic within brewed Melissa officinalis and then derivatives of luteolin and quercetin, gallic acid and ferulic acid have the greatest amount (Kulišić- Bilušić, Katalinić, Dragović-Uzelac, Ljubenkov, Kriško, Dejanović, Jukić, Politeo, Pifat, & Miloš, 2008). It seems that the antioxidant effects observed for Melissa officinalis in this study are related to the above phenolic compounds and their antioxidant properties.

Conclusion

This was the first study which investigated the effectiveness of short-term supplementation of Melissa officinalis on the total antioxidant capacity level and Malondialdehyde (MDA). Generally, it is concluded that this herbal supplement can increase total antioxidant capacity and prevent the enhancement of Malondialdehyde level during aerobic exercise. But before any nutritional prescription to athletes, more studies with biochemical approach (measurement of other enzymes and inflammatory factors) should be done.

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