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Effects comparison of the short-time Mellissa Officinalis L supplementation and downhill running on the blood factors in young swimmers

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Abstract. The purpose of this study was to research the effects of downhill running and short-time Mellissa Officinalis supplementation on the blood elements in male athletes. For this purpose reason, 20 healthy male swimmers were chosen randomly and were divided into two groups of 10 members. The amount of hemoglobin, hematocrit and leukocytes was measured in the basic level, before and after supplementation. After data collection, the variance analysis test was done for evaluation of time series and also Bonferroni test and the independent t-test were used significantly for evaluation of the differences among groups. Also, the significant level of $p<0.05$ was used. Evaluation of research findings has shown that after supplementation the blood elements increased less in the group of Mellissa Officinalis L than the other group as a result of downhill running. In general, we conclude that this herbal supplement can decrease the hemoglobin, hematocrit and leukocytes caused by downhill running.
***Key words**: Mellisa Officinalis L, hemoglobin, hematocrit, leukocytes, delayed soreness, downhill running.*

Introduction

Downhill running like coming down from a mountain or stairs with eccentric actions lead to muscle soreness than other muscle contractions (1, 2). The main characteristic of this type of contraction is the applying extra stress on the muscle fibers and smooth tissues causing damages on skeletal muscles, stronger inflammatory responses and bigger share of oxidative stress in comparison with other contractions (3). Delayed muscle soreness is an unpleasant mood which is accompanied with muscle pain, spasm, weakness and stiffness (2). The delayed muscle soreness usually reaches its peak activity about 24-72 hours after its first stage of exercise and then after 5-7 days it disappears (4).

There are many hypotheses that describe the performance of the delayed muscle soreness. Among these hypotheses we can mention the followings: lactate accumulation, inflammation, muscle spasm, muscle damage, connective tissues, damage and increase in muscle temperature (5, 6).

The findings have shown that there is a relationship between muscle soreness and inflammatory response. Moreover the inflammatory events, after eccentric exercise, may lead to decrease in the muscle power (7).

There are many ways to decrease the delayed muscle soreness including massage, cryotherapy, ultra-sound, taking anti-inflammatory drugs like aspirin, and taking natural and artificial supplements (8, 11). The results have shown that intaking of sports supplements with anti-inflammatory effects prevent delayed muscle soreness, effectively.

So, some of the researchers believe that taking the amino-acid supplements decrease the long-time damage and muscle inflammation caused by eccentric running (7, 11). It seems that using anti-oxidants can prevent the inflammation and improve the soreness and feeling of pain effectively.

Rebuilding process of the muscle fibers which have been damaged mechanically includes inflammatory process and leads to muscle pain, stiffness and lack of muscle strength specially 2-5 days after exercise. The general idea is that the free radicals can play an important role during the inflammatory processes and anti-oxidants can reduce the time and intensity of the muscle pain (2).

There are many nutritional supplements that can prevent or improve the muscle soreness quickly but the researches in this field are still incomplete (12). Goldfarb et al. (2004) have used a combination of anti-oxidants (Vitamins E, C and Selenium) for 14 days before eccentric exercise and two days after that.

They have shown that after this period, all indices of cellular damage have been reduces and less amount of pain and blood creatine kinase has been reported among the respondents (13,14). But the movement range and the isometric contraction have been decreased at the same extent in both practical and control groups. Shafat et al (2004) have used a higher dozen of vitamins E and C for a longer period of time (37days) and they have found out that the decrease of the peak power have became lesser (15). In this study, the inflammatory and the oxidative-stress' indices were not measured. Bloomer (2006) has shown that the supplementation of vegetables and fruits for 2 weeks before aerobic exercises decreases the oxidative stress in women and men and acts like the supplementation of vitamins E and C (15).

Moreover than the vegetables and fruits, scientists have studied the anti-oxidant characteristics of other herbal plants like saffron, green tea, cinnamon etc. (16, 17). Among these anti-oxidative herbal plants, Melissa Officinalis and Thymus Vulgaris L can be mentioned. Rostami et al. (2011) have compared the anti-oxidative impacts of Melissa Officinalis with vitamin C. They have found out that it has a similar effect to vitamin C and it can be used as a natural anti-oxidant (18). Melissa Officinalis and Thymus Vulgaris L are two aromatic plants from Lamiaceae family and they usually grow in south and central Europe, North of Africa, Mediterranean areas and North of Iran. The local name of Melissa Officinalis in Iran is Varnje bo and Ferenj Moshk and its full of anti-oxidant compounds (18). The anti-oxidative power of Melissa Officinalis and Thymus Vulgaris L. has been measured by experimental methods and in the recent studies, the anti-oxidative effects of Melissa Officinals has been studied among the radiology employees, aluminum company workers, patients with fatty liver disease (19-22) and also young athletes (11). So, some researchers have tried to evaluate the effects of downhill running (30 minutes of running on treadmill with 65% intensity of peak aerobic power and a slope of 10%) and the short-term supplementation effects of Melissa Officinalis and Thymus Valgaris L. (1.5 gr teabag, two times a day and for a period of 14 days) on active protein C (acute inflammatory index), feeling of pain, anti-oxidative capacity, creatine kinase, blood factors and compare these two extracts among young swimmers. The purpose of this study was to compare the effects of running in negative slope with and without short term supplementation of Melissa Officinalis on blood factors in young athlete's swimmers.

Material and Method

Participants. This study was done after ethics committee approval in the Islamic Azad University, Boroujerd Branch, in the field of quasi-experimental designing. The participants of the study were 20 healthy male athletes, swimmers, who had a regular exercise schedule. These people were non-smoking, had no history of cardiac, kidney, liver and physical disease and had pronounced their impassibility to Mellisa Officinalis supplementation and blood taking. The participants volunteered to participate and have filled out the consent forms. So, the participants were chosen after a complete introduction over the purpose and methodology of the study in the case of age, body mass indicator, fat percentage, exercise routine and having no history of illness and injury. Based on the previous studies, the sample volume was chosen as 10 for each group as a significant level of 5% and the power level was equal to 2%. 10 people were chosen randomly for each group of supplementation and quasi-drug.

Data collection. Before starting the experiment, the purpose, details and the potential risks were analyzed for all participants and then a written testimonial was gotten from them. In this meeting their height was recorded with the accuracy of 1% with the weight gauges made in Iran. The fat percentage of their body was evaluated by the measuring of the subcutaneous fat layer thickness (chest, abdominal and thighs) by using the American Caliper Lafayette and substitution in body fat estimating equation which was given by Jackson and Pulak (23, 24).

First blood samples were taken from the elbow vein, 10 days before eccentric aerobic activities for evaluation of the basic amount of the indicators. The second blood taking was done immediately after completing the exercise protocol (before supplementation). One week before that the aerobic and anaerobic power of the participants was measured. These two groups of supplementation and quasi-drug took Mellissa Officinalis and dextrose for 14 days, respectively.

After the period of 14 days, participants started to complete the protocol and afterward the third blood-taking protocol took place. Before each stage of blood taking, the participants were asked to fill out the dietary recall questionnaires. Moreover, they were supposed to avoid all kinds of medication, cigarette-smoking, anti-oxidative supplementation and anti-inflammatory drugs.

Eccentric exercises. All participants ran on the treadmill for 30 minutes with the 65% intensity of maxim oxygen consumption (VO_2max) and -8.5 degrees of slope which is equal to 15%.

Basic heart rate of all people was measured and recorded after 10 minutes resting by using a Polar stethoscope. Moreover, the maximum heart rate was recorded during Bruce test by using the treadmill screen. On the other hand, for controlling the activity intensity of 65%, Karvonen method was used. Before starting the protocol, the participants stretched for 5 minute and then ran on treadmill (with 0 slope) for 3 minutes. After that, the slope and speed of the treadmill was increased within 2 minutes to increase the heart rate. All people ran on a treadmill with a slope of 15% for 30 minutes and heart rate of 65%. The heart rate, slope and speed of treadmill were controlled until the end of exercise testing by the observer.

Measuring the blood elements and other research variables. All the research steps were done in the average moisture of 55%, the temperature of 25 degrees and from 8 to 10 o'clock in the morning, and 5ml blood were taken from each participant. First Blood samples centrifuged for 10 to 15 minutes then serum separated, hematologic indexes (used in current paper) were measured using cell counter system (Sysmax 100, Japans Technology) in standard conditions. Hematocrit percent and Serum hemoglobin mg/dl and Serum leukocytes (10^9/L) were reported by laboratory expert staff.

Statistical analyses. After data and blood factors submission, first the general characteristics of data were analyzed as a chart and a table by using Excel 2007. Then, the research hypotheses, the comprehensive statistics, repetitive variance and Bonferroni test were all used (after confirmation of the normality of the data distribution by using Kolmogorov-Smirnoff test and the variance homogeneity) to compare the difference in different time series. Moreover, the interacting effect between and inside two groups was specified significantly and the t-test was used to show the difference between the two groups. All the tests were evaluated significantly ($p<0.05$) by using SPSS17. The characteristics of the subjects are given in table I and other specifications are given in tables II and III, and figures 1-3.

Results

Leukocyte. Based on table II and figure 1, the amount of blood leukocytes in both groups had no significant difference in all three steps of measurement. As it can be seen in figure 2, the blood leukocyte was less in group of Mellissa Officinalis than the quasi-drug group, while this decrease wasn't statistically significant. So, as it can be seen in table I, the results of variance analysis have shown a significant difference in the amount of blood leukocytes in each measurement step. It has been shown by using Bonferroni test that the amount of blood leukocytes increases significantly in response to eccentric aerobic exercising. This indicator decreased more after supplementation in Mellissa Officinalis group than the quasi-drug one, but the difference wasn't statistically significant.

Table I. Somatic and physiological characteristics of the researched groups

Study indicators	Group	Mean	Standard Deviation
Age (year)	Mellissa Oficinalis	15.70	1.25
	Placebo	15.90	1.20
Weight (kg)	Mellissa Oficinalis	61.85	9.103
	Placebo	58.10	8.949
Height (cm)	Mellissa Oficinalis	176.10	5.405
	Placebo	174.10	5.446
Body mass indicator (kg/m^2)	Mellissa Oficinalis	20.589	2.394
	Placebo	20.559	2.308
Fat percentage (%)	Mellissa Oficinalis	11.607	0.247
	Placebo	11.711	0.130
Maximum oxygen consumption (ml/kg/min)	Mellissa Oficinalis	50.00	4.761
	Placebo	49.80	2.936

Hemoglobin. Based on table II and figure 2, the amount of blood hemoglobin in the basic level doesn't have any significant difference with that of pre-supplementation but after that this difference increases in blood serum. As it can be seen in figure 2, the blood hemoglobin increases less in group of Mellissa Officinalis than the quasi-drug group. Also, as it can be seen in table II, there is a big difference between the results of variance analysis in different series of hemoglobin measurement. It has been shown by using Bonferroni test that the amount of blood hemoglobin increases significantly in response to eccentric aerobic exercising in both groups.

This indicator decreased more after supplementation in Mellissa Officinalis group than the quasi-drug one, as a response to eccentric exercising, but the difference wasn't statistically significant.
Hematocrit. Based on table II and figure 3, the amount of hematocrit in both groups had no significant difference in the basic and pre-supplementation levels. As it can be seen in table III, the results of variance analysis in different stages of hematocrit measurement have shown a significant difference. It has been shown by using Bonferroni test that the amount of hematocrit increases significantly in response to eccentric aerobic exercising. This indicator increased less after supplementation in Mellissa Officinalis group, than the quasi-drug one. So, the results are follow-up test are given in table III.

Table II. Serum hemoglobin, hematocrit and leukocytes (mean ±SD) pre- and postexercise supplementation in researched groups

Variables	Groups	Basic amount	Post-exercise (pre-supplementation)	Post exercise (post-supplementation)
Serum hemoglobin (mg/dL)	Mellissa Oficinalis	12.54±0.954	14.7±0.616	14.87*!±0.914
	Placebo	12.94±0.865	14.75±0.705	14.71±0.730
Hematocrit (%)	Mellissa Oficinalis	41.48±1.72	46.28±2.52	44.71*#± 1.77
	Placebo	40.77±1.61	43.99±1.88	44.05±1.88
Serum leukocytes (10^9/L)	Mellissa Oficinalis	6.3±0.50	9.5±1.61	8.5*#± 1.64
	Placebo	7.08±0.86	10.02±1.89	9.66±1.54

**=stands for the inter-group significant difference (p<0.05); !=stands for significant difference proportional to the basic level (p<0.05);#=stands for significant difference proportional to the post exercising and pre-supplementation level (p<0.05).*

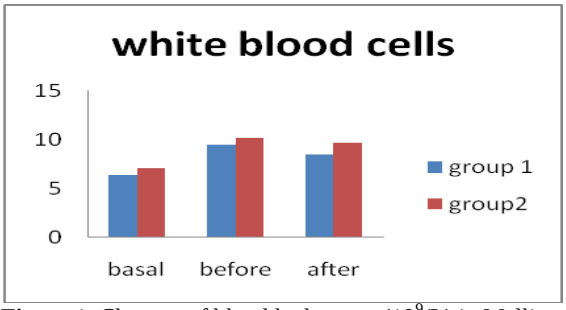

Figure 1. Changes of blood leukocytes (10^9/L) in Mellissa Officinalis and placebo groups during different stages

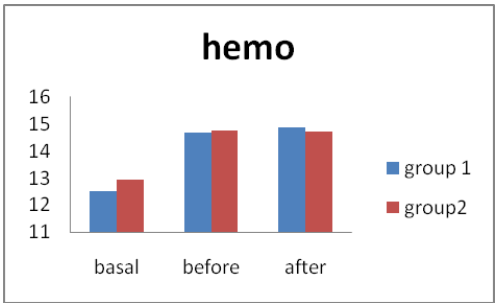

Figure 2. Changes of hemoglobin (mg/dL) amount in Mellisa Officinalis and placebo group during different stages

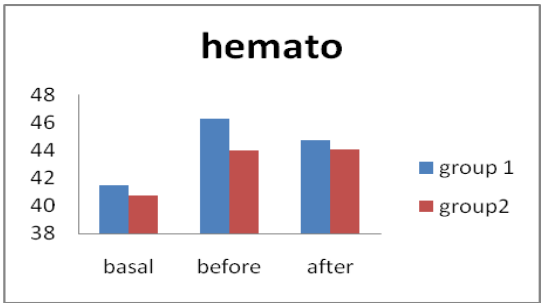

Figure 3. Changes of hematocrit (%) in Mellissa Officinalis and placebo group during different stages

Discussion and conclusion

The findings show that the short-time downhill running and Mellisa Officinalis consumption can significantly affect the amount of blood hemoglobin after supplementation and the short-time supplementation of Mellissa Ofiicinalis can stop increasing the amount of hemoglobin in blood of male athletes significantly right after downhill running and supplementation. This increase in hemoglobin after

downhill running was more significant in the quasi group than the group that has received Mellissa Officinalis. Based on the results of the study, hemoglobin was increased significantly after exercising and 2-weeks after Mellisa Officinalis supplementation in was decreased significantly. Zoldinaro et al (2006) showed that heavy activities with maximal oxygen consumption of 50% on a bicycle ergometer, right after and 60 minutes after the activity can increase the number of white blood cells and its sub units (36), Robertson et al (1998), Robio et al (1996) showed that one exercising session can increase the hemoglobin in the blood environment. Moreover, some researchers claim that the hemoglobin can decrease and remain unchanged (26) as a response to activities, but many studies have shown that the blood hemoglobin increases and hypoxia is caused by activities like interval running, high jogging and endurance running (25). So, immediate increase in blood hemoglobin, right after the exercise and before the supplementation in the current study can be caused by the hematocrit increase, liquid change or movement, plasma volume decrease, and blood concentration increase. Based on the results that show the hemolysis as a result of doing exercises, it seems that increase in plasma hemoglobin is related to structural disadvantages of red blood cells. So, Mellissa Officinalis supplementation can stop the proportional increase of blood hematocrit and hemoglobin which is the same as what Allen and Parkinson et al have found (36). Since they didn't use supplements, the Hematocrit and Hemoglobin increased in second step and before supplementation but after taking a 14-day supplementation period, a proportional decrease has been seen in Hemoglobin amount of Mellissa Officinalis group while it increased in the other group. So, that's why some researchers agree that Mellissa Officinalis can decrease the inflammation to prevent the muscular injuries that lead to increase in blood hemoglobin.

The findings show that the short-time downhill running and Mellisa Officinalis supplementation can significantly affect the amount of hematocrit after supplementation and the short-time supplementation of Mellissa Ofiicinalis can stop increasing the amount of hematocrit in blood of male athletes significantly right after downhill running. Based on the athlete supplementation, it can be said that when the body activity increases, the body need to take oxygen increases as well. When the complete blood having hemoglobin and hematocrit be centrifuged, the space that would be occupied by packed red cell volume would be called hematocrit and it can be defined as the range of RBC (red blood cells) percentage to the complete blood having a PCV (packed cell volume). Moreover, the hematocrit can be known as packed red cell volume. The amount of hematocrit is proportional to amount of hemoglobin and number of RBCs. In this study the amount of reticulocytes' production showed a significant difference in practical level which shows that the speed of blood-production is increased as a result of hematocrit and hemoglobin increased.

Andro et al and Allen et all studied the liver and blood factors of body builders who have used steroid during doing exercise and found out that the factors increased proportionally to the intake dosage (26,36). The results of this study are similar to Parkinson and Allen's findings about changes in amount of blood factors like hematocrit and hemoglobin. When a complete blood having anti-coagulation be left for a long time the red blood cells would be separated from the blood and will precipitate. The distance that red blood cells travel to precipitate after one hour is called RBC precipitation or ESR (erythrocyte sedimentation rate). ESR is a non-specific response to inflammation and tissue injury and reminds us about the illness but it doesn't show the severity of the sickness.

Some authors claimed that blood pressure is caused by significant increase in lipoprotein level of serum, amount of RBCs and liver function and the results were similar to what Allen has found (26). So, Mellissa Officinalis supplementation can prevent ESR and stop the proportional increase of blood hematocrit and hemoglobin which is the same as what Allen and Parkinson et al have found. Since they didn't use supplements, the hematocrit and hemoglobin increased in second step of the experiment. The results of the current study showed that the short-time Mellissa Officinalis supplementation and downhill running can both affect the muscular pain. The amount of this indicator increased significantly in both groups right after downhill running but the short supplementation of Mellissa Officinalis prevented the proportional increase of pain feeling in the male athletes after downhill running.

Adenosine is the main factor of pain caused by injuries (27). The adenosine receptors can be seen in most of the body tissues (22). So it can be said that quasi-drug actions can block the related adenosine receptors.

Receptors A1 and A2a that are located at the end of the sensory neurons of skeletal muscles stimulate pain receptors and Mellissa Officinalis can decrease the feeling of pain by blocking the adenosine receptors (31). Moreover, it has been shown that Mellissa Officinalis having indomethacin can prevent the production of prostoglandins (23, 31). As it has been said, this research is the first paper studying the effects of Mellissa

Officinalis on muscular soreness and for more details we need to study farther. As it was mentioned in the introduction part, Melissa Officinalis and is a kind of aromatic plants from Lamiaceae family. It's one of the most important herbal medicines that is used in cosmetic, nutritional and medical industries because of its pleasant flavor (20, 32).

For the evaluation of the exact effects of Mellissa Officinalis farther studies have to be done with different dosages and for different time periods to show if the supplementation of this plant can reduce the blood factors and soreness caused by eccentric exercising. Also, other factors measuring dehydrongease the inflammation, oxidation and soreness like lactate and malon dyaldehyde must to be studied.

The findings show that the short-time downhill running and Mellisa Officinalis supplementation can significantly affect the amount of blood leukocytes. After downhill running, the amount of blood leukocytes increased more in quasi-drug group than the group receiving Mellissa Officinalis supplementation. The results of this study are the same as what was found by Vimercati et al (2008), and Varani et al (2005) (33, 34). All these researchers have found that running with eccentric contractions can increase the blood leucocytes. For example, in the study by Vimercati, the local blood leukocytes increased 60 minutes after running on a treadmill with a peak oxygen consumption of 65% in both groups (33). Most studies have shown that the increase in white blood cells during and after doing activities is caused by increase in the number of neutrophils and lymphocytes, although the number of monocytes can increase as well (35). Zoldivaro et al (2006) showed that heavy activities with maximal oxygen consumption of 50% on a bicycle ergometer, right after and 60 minutes after the activity can increase the number of white blood cells and its sub units (36). Klose et al (2004) claimed that running on the treadmill with a small slope increase and maximum oxygen consumption of 65% can significantly increase the white blood cells some researchers believe that the increase in White blood cells as a result of eccentric activities is caused by muscle injury and Mellissa Officinalis can decrease the white blood cells by decreasing the inflammation caused by eccentric activities (37).

General findings. This research was the first paper evaluating the effectiveness of short-time Mellissa Officinalis supplementation on the blood factors and the results showed that this herbal supplement can decrease the amount of leukocytes, hematocrit and hemoglobin. But before giving a supplementation schedule to the athletes more biochemical studied must be done to evaluate the other inflammatory factors and enzymes and to prevent the movement and power reduction. This increase in hemoglobin after downhill running was more significant in the quasi group than the group that has received Mellissa Officinalis. Based on the results of the study, hemoglobin was increased significantly right after exercising and 2-weeks after Mellisa Officinalis supplementation in was decreased significantly. Zoldivaro et al (2006) showed that heavy activities with maximal oxygen consumption of 50% on a bicycle ergometer, right after and 60 minutes after the activity can increase the number of white blood cells and its sub units (36).

Moreover, some researchers claim that hemoglobin can decrease and remain unchanged and as a response to activities, but many studies have shown that the Hemoglobin increases and hypoxia is caused by activities like interval running, high jogging and endurance running and (25, 32, 34). So, immediate increase in blood hemoglobin, right after the exercise and before the supplementation in the current study can be caused by the hematocrit increase, liquid change or movement, plasma volume decrease, and blood concentration increase. Based on the results that show hemolysis as a result of doing exercises, it seems that increase in plasma hemoglobin is related to structural disadvantages of red blood cells. On the other hand, the significant reduction of hemoglobin is caused by an anaerobic activity session affecting the response of some rheology factors of blood after supplementation that is a result of hematocrit and blood concentration reduction and increase in plasma volume.

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