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Effects of oral administration of thyme (*Thymus vulgaris*) aqueous extract on delayed muscle soreness in Inactive women

Ali Aalizadeh[1], Zahra Vispour*[2], Saeed Changizi-Ashtiyani[3], Fakhrosadat Hashemi[4], Jamalaldin Alvani [5] and Hossein Fatemikia[6]

[1]*Exercise Physiology Research Unit, Bushehr University of Medical Sciences, Bushehr, Iran*
[2]*Exercise Physiology Research Unit, Bushehr Islamic Azad University, Bushehr, Iran*
[3]*Department of Physiology, Arak University of Medical Sciences, Arak, Iran*
[4]*Faculty Member of Bushehr Islamic Azad University, Bushehr Iran*
[5]*General Practitioner and Faculty Member of Marvdasht Islamic Azad University, Shiraz-Iran*
[6]*Department of Physiology, the Medical School, Bushehr University of Medical Sciences, Bushehr, Iran*

ABSTRACT

The antibacterial, antifungal and anti-oxidant properties of the Garden thyme herb are well known. The aim of this study was to examine the effect of thyme aqueous extract on the serum levels of creatine kinase (CK) and lactate dehydrogenase (LDH) in Inactive women following a single session of exhaustive exercise. subjects used in this semi experimental study included 32 female students, mean age of 21.6± 1.41 years, height of 172.66± 4.63 c m and weight of 80.25±11.77 kg. They were randomly divided into 4 groups including: the control, experimental group 1 receiving 200 mg/kg thyme aqueous extract, the experimental group 2 receiving 400 mg ibuprofen and the experimental group 3 receiving both the extracts and ibuprofen. Ibuprofen tablet and the extract were orally administered 8 hours after exhaustive exercise. Physical activity program for inducing muscle soreness was 70 contractions of bicep muscles; each repetition was 40-60% of a maximum contraction. The CK and LDH parameters were measured both before as well as 24, 48 and 72 hours after the exhaustive exercise. According to the results, there were no significant differences in CK and LDH serum levels among different groups at 24, 48 and 72 hours after exhaustive exercise. The results of this study suggest that the scope and extent of muscular damage during sport activities depends on a number of factors, including the severity, duration and type of exercise as well as the type, degree of purity, the amount and the time of supplement consumed.

Keywords: Thymus vulgaris, exhaustive exercise, creatine kinase, lactate dehydrogenase

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INTRODUCTION

Despite various known benefits of physical activity and exercise for athletes and general public , these activities can cause different injuries as well. Based on the level of physical fitness and the exercise conditions, extend of Such Injuries may vary significantly [1].One important consequence of exercise can be Muscle soreness.

As a common injury, muscle soreness is independent of individual’s physical fitness and occurs repeatedly during the life of a person. Based on the severity and the causing factors, it is divided into two types: the acute Muscle soreness and the delayed onset Muscle soreness (DOMS) [1]. The latter often occurs following medium, severe and long term unusual activities as well as exercises that mostly include eccentric contractions [1]. Some signs of delayed Muscle soreness are decreased range of joints movement, reduction of the muscle power, muscle stiffness and fatigue, inflammation, fine Microscopic injuries, , secretion of creatine kinase (CK) and lactate dehydrogenase (LDH) enzymes into the plasma and Increasing inflammatory responses of plasma [2-4].Symptoms usually appear 12 to 24 hours after exercising and are usually unpleasant for people [5].In addition, due to the mechanical pressure during resistance exercises, especially unusual eccentric contractions, leading to cellular damage, the level of serum creatine kinase may remains significantly higher than natural levels (men 24-195 and women 170-24 international units in liter) up to 8 days after exercising [6].results of Meir et al. (2010) suggest that excessive metabolic and mechanical pressure induced by heavy and long term exercises along with eccentric contractions can increase the fatigue index, cellular damage (such as increasing lactate and enzymes present in the peripheral blood) and delayed muscular soreness [7-9].

The etiology of DOMS (delayed onset Muscle soreness) is an unresolved issue and is discussed in public communities, even in medical societies. In this regard, a number of theories have been proposed, the most important of which are: the theory of inflammation and the theory of tissue rupture [10].The available evidence show that long term and severe activities and trainings lead to an increase in the production of free radicals (very unstable molecules) [11].Another word, as a result of an increase in the mitochondrial oxygen intake and the increase in the electron transfer during severe exercise, production of oxygen free radicals are increased, leading to lipid (Fat) per oxidation, which in turn can damage cell membrane through disturbing membrane organization, and change the activities of membrane enzymes and other proteins [12].

According to various studies, the intensity of DOMS follows inverted U pattern, that is, it reaches its peak approximately 25 to 48Hours after the exercise, then gradually drops down, and completely disappears 5 to 7 days after the exercise [13].

A common method for treating the unfavorable effects of fatigue and stresses resulting from relatively intense exercise is the use of some herbs. One such plant is the garden thyme or *Thymus vulgaris L*., an aromatic plant belonging to the family Lamiacea, native to the mediterranean region [14].Thyme can enhance circulation and act as an agitating stimulus for the whole body [15].The stimulatory effects of this herb on nervous system form the basis for treating physical and mental fatigues, and reducing tension, anxiety and insomnia [15].The leaves and stems of thyme are also used in the treatment of depression or changing mood [15].Moreover, thyme is an excellent source of iron, manganese and vitamin K as well as calcium [14].Its organic constituents are thymol, flavonoids, aognol, aliphatic phenols, carvacrol, as well as lotheoline and saponins [16].Naturally, thymol is the main phenolic component of thymus whereas karoacrol is a minor component.

Despite its side effects, it has been claimed that due to its high antioxidant properties (presence of thymol and flavonoids), thyme supplement can improve athletic performance to some extent [17].Conversely, other studies claim that thyme has no effect on endurance capacity of athletes [18-19].Still other studies have shown that flavonoids can enhance antioxidant capacity and improve swimming performance through delaying fatigue in rats. Moreover, thyme supplements can increase endurance capacity and fat metabolism [20].In addition, determination and treatment of muscle injuries resulting from physical activity is essential for public health. Of course, Muscle soreness and fatigue are more prevalent in non-athletes, particularly inactive women and girls that have to participate in a series of sportive activities of physical education. Measurement of serum enzymes such as CK and LDH seems to be a good indicator of physiological and biochemical performance of muscle as well as muscle soreness and injuries. In the present study, we try to investigate the possible effects of aqueous extract of garden thyme on the enzyme indicators of muscle impairment caused by a single session of exhaustive exercise.

MATERIALS AND METHODS

Eighty girl students from the dormitory of the Bushehr University participated in this semi experimental research, which was approved by the ethical committee of medical Sciences of Bushehr University. Following a call for students wishing to participate In the study, 80 girls volunteered and provided information about their personal profile, history of their health and disease, amount of their physical activity and exercise, their possible sporting history, and their written consents for enrolling in this study through completing a form. Of these volunteers, 32 subjects with a mean age of 21.6 ± 1.41 years, height of 172.66 ±4.63 cm and weight of 80.25 ±11.77 kg were selected. They were randomly divided in 4 groups including: the control group, experimental group 1 receiving 200 mg/kg thyme aqueous extract, the experimental group 2 receiving 400 mg ibuprofen and the experimental group 3 receiving both the extracts and ibuprofen. Based on written and oral instructions, the subjects were banned from doing any intense exercise and consuming any food products and supplements 72 hours before the beginning of the experiment.

Exercise plan

Before exercising, the warming up program was carried out for 15 minutes. Members Of all four Groups in turn put the elbow of their weaker hand on a half a meter long table and performed 70 bicep muscle contractions, each contraction amounted to 40-60 percent of a maximum contraction. The duration of each contraction was 3 seconds, and between two contractions, there was a 10 second rest. In addition, between each period which included 10 contractions, there was one minute break. A metronome was used for timing the contractions [17].The profile of ibuprofen Chemical formula of ibuprofen is 2 O18H13C. In the present research, we used tablets containing 400 mg ibuprofen made by ARIA pharmaceutical company (Iran).

Preparation and consumption of thyme aqueous extract

Following confirmation of thyme herb by agricultural research center of Jihad-agriculture in Bushehr province, it was transferred to the research lab at the medical sciences of Bushehr University and dried in complete darkness. Young leaves were selected and milled into powder [22].Fifty gram of leaf powder was mixed with 100 ML of distilled water and placed on a rotator to be slowly mixed for 72 hours at room temperature. Then, the mixture was filtered and the initial extract was prepared. The solvent was evaporated from the extract under vacuum and at 80 °c for one hour [23].After the exhaustive exercise protocol, 200 mg of concentrated aqueous extract was administered to each subject once every 8 hours for 3 days.

To measure CK and LDH, blood samples were taken during a break in the presence of a general practitioner. Sampling was conducted before exercising as well as 24, 48, and 72h after the last programmed exercise, and all 32 subjects were fasting (12 to 14 hours after the last meal). Blood samples were transferred to the laboratory under the standard condition.

Biochemical evaluations

To assess the serum levels of CK and LDH, 5 Ml blood was taken from the right arm vein **[24].**Serum level of CK was measured by chemical colorimeter through jaffe reaction with sensitivity of 1 U/L and changing coefficient of 1.6% (Colorimetric CK kit, Pars azmon Inc., Tehran, Iran). Similarly, the activity of LDH was determined by enzymatic colorimeter with sensitivity of 5U/L and changing coefficient of 1.2 % (colorimetric LDH kit, Pars azmon Inc., Tehran, Iran). The unit of measurement was 1.l, which was determined by an auto Analyzer (Hitachi, model 902, Japan).

Statistical analysis

Data were statistically analyzed using SPSS software (version 19.0) and presented as mean ±standard deviation (SD). Differences were considered statistically significant at $p < 0.05$.

RESULTS

The results showed that the level of LDH in the experimental group 1 (receiving thyme extract) decreased at 24, 48 and 72 hours after exhaustive exercise compared to the control, but this reduction was insignificant (Table 1). However, in the experimental group 2 (receiving ibuprofen), the level of LDH increased significantly at 24 and 48 hours after exhaustive exercise, but decreased insignificantly at 72 hours compared to the control group. Also, there were no significant differences in the level of LDH in the experimental group 3 (receiving both the extract and ibuprofen) at different times after exhaustive exercise compared to the control group. Similarly, the levels of CK in all experimental groups and at 24, 48 and 72 hours after exhaustive exercise showed no significant differences in comparison with its levels before the exercise (Table 1).

Table 1. The serum levels of lactate dehydrogenase (LDH) and creatine kinase (CK) in the experimental and control groups

Group / Variable		Control	Thyme	ibuprofen	Thyme + ibuprofen
Lactate dehydrogenase (LDH)	Before exercise	496.33±92.34	510.83±106.38	493.66±80.84	500.16±162.1
	24h after exercise	488.17±118.6	483±132.55	537.50±134.29	501.83±115.77
	48h after exercise	454.17±74.21	499±112.78	532.67±118.23	440.67±92.87
	72h after exercise	454.5±50.63	454.33±100.52	477.33±96.75	443.33±24.59
Creatine kinase (CK)	Before exercise	77±20.14	90.33±36.69	74.33±19.75	80.33±24.59
	24h after exercise	79.67±16.61	106±57.09	81.83±15.39	84.67±34.13
	48h after exercise	75.68±15.88	90.33±40.45	73.5±16.06	75±10.77
	72h after exercise	76.83±22.12	90±23.58	70.16±17.04	75.66±15.12

DISCUSSION AND CONCLUSION

The findings of the present investigation showed that there are no significant differences in the serum concentrations of CK and LDH of the experimental groups receiving thyme supplement, ibuprofen as well as combination of both extract and ibuprofen at, 24, 48 and 72 hours after exhaustive exercise compared to subjects before the exercise (table 1). The effects of natural plant drugs on the sport performance and health has recently attracted much attentions [25].However, scientific data in this field is very limited and determining the mechanisms involved in the interactions of plant supplements or medicinal herbs with processes of physical activity and exercise is not possible. the results of this research is consistent with Matsus et al. (2006) who found no significant increases in the levels of LDH and CK enzymes after a session of load-less resistance exercise with 10 repetitions and 1 minute rest [26],while they are in sharp contrast with the findings of Kuipers (1985) who reported an increase in the levels of these enzymes [27].It seems that the kind of exercise, the recovery time and the intensity of exercise are involve in the release of LDH and CK enzymes, and various exercises cause different degrees of muscle injuries [27].

Some studies have shown that flavonoids can enhance antioxidant capacity and improve swimming performance through delaying fatigue in rats [20].Thymol is the main natural phenolic component of thymus herb whereas karoacrol is a minor one [17].Thus, in addition to its possible side effects, due to its high antioxidant properties, one can claim that thyme supplement might improve athletic performance to some extent [17].On the other hand, some researchers claim that thyme has no significant effect on endurance capacity of people [18-19].Moreover, Thyme supplements can enhance endurance capacity, and also affect fat metabolism [20].It should be recalled that researchers consider the elevation of creatine kinase secretion into the serum as a sign of muscle injury; thus, any agent capable of preventing or reducing the secretion of this enzyme is in fact, alleviates the severity of muscle injuries [28].In a cross-sectional study, the effect of 1500 mg/g ibuprofen on symptoms of muscle soreness in six healthy nonathletic males has been studied [29].These subjects received the drug 24 hours before and 72 hours after the eccentric activity. The results showed that ibuprofen had no effect on the development of delayed muscle soreness, levels of CK and the signs of histological muscle damage.

In addition, eccentric contractions cause more muscle damage than other kinds of contractions [29].Injuries resulting from continuous contractions lead to structural disorder in myofibrils [30].This disorder is reflected more in the structure of myofibril z-lines [27].and signs of muscle injuries increase after exercise [29].Also, it has been shown that performing severe and long term exercise regardless of suitable recovery time cause damage to muscle fibers, and internal destruction of skeletal muscle and the connective tissues during contractions, followed by an inflammatory response, and infiltration of macrophages along with the release of myocytoplasmic enzymes of muscle fibers, including CK and LDH, leading to signs of pain, motor limitation, biochemical changes and spasm of muscle fibers [30,31].Hence, in some studies, the increase in the serum levels of CK and LDH has been used as a measure to assess the damage to the muscle cells after exercise [30].Some of the reasons for the inconsistency among different studies include methods for preparation of supplements, the amount and timing of consumption, type of herb and extract, degree of purity, sport program (the intensity, duration and type of activity, etc).

The results of Mcanulty et al. (2005) showed that a session of resistance activity has no significant effect on the parameters of muscular injuries in male athlete [32].In contrast, the results of Atashak and Batura (2012, 2013), and Hurley et al. (1959) indicate that resistance activity induces muscle damage leading to an increase in the levels of CK and LDH [33, 34].Perhaps, the contradictory results of recent studies are related to differences in the intensity of physical activities used.

Another probable mechanism involved in oxidative stress induction by resistance exercise is the theory of "damage reinjection-ischemia" [35].According to this theory, severe muscle contractions may cause a temporary reduction in blood flow in the muscle thereby limiting oxygen availability, culminating in ischemia. After contractions, muscle expansion phase, reinjection of blood into muscle supplies abundant oxygen, resulting in the production of free oxygen radicals [35].These free radicals can damage muscular fibers, especially cell membrane, leading to the leakage of cytoplasmic enzymes such as CK. Under natural condition, CK is kept within the cellular space, unless cell membrane is harmed in a way. Thus, researchers usually consider the serum level of CK as a strong indicator of muscle injury [36].The results of this study revealed that the levels of CK In the experimental group receiving thyme extract and ibuprofen show no significant changes in different periods following the exhaustive exercise (Table 1). On the other hand, Milesi (2009) investigated the impact of ibuprofen on CK and the intensity of delayed muscle soreness after hamstring muscle contractions. They found a significant reduction in the level of CK in the group receiving ibuprofen [37]; they concluded that 400mg ibuprofen is an appropriate dose for controlling muscle damage.

The reason for the inconsistencies observed among various studies is likely to be related to several factors, including the severity, duration and type of physical exercise, and the level of individual's fitness [37].Indeed, changes in the serum level of CK enzyme depend on muscle mass, and intensity, duration and volume of physical activities as well as the individual's sex [36].The normal range of this enzyme for men and women are 38-174 unit/L and 96-140 unit/L respectively [36].In general, since there were no significant differences in the concentrations of CK and LDH as the main indicators of muscle damage, it can be concluded that the consumption of 200 mg of thyme aqueous extract cannot prevent muscle soreness and undesirable release of CK and LDH enzymes in non-athlete girls after a session of resistance exercise. It seems that two factors, the period of extract administration and duration of exercise, have played important roles in the results of the present investigation.

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