

## **Heart and plasma LDH and CK in response to intensive treadmill running and aqueous extraction of Red *Crataegus pentaegyna* in male rats**

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### **ABSTRACT**

*The purpose of the current study was to investigate the effect of a high intensity treadmill running training (8weeks) with or without aqueous extraction of Crataegus-Pentaegyna on heart and plasma LDH and CK. Thirty-two Wistar male rats (4-6 weeks old, 125-135 gr weight) were used. Animals were randomly assigned into training (n = 16) and control (n = 16) groups and further divided into saline-control (SC, n = 8), saline-training (ST, n = 8), red Crataegus pentaegyna extraction -control (CPEC, n = 8), and red Crataegus pentaegyna extraction -training (CPET, n = 8) groups. Training groups have performed a high-intensity running program 34 m/min on 0% grade, 60 min/day, 5 days/week) on a motor-driven treadmill for 8 weeks. Animals were fed orally with Crataegus extraction and saline solution (500mg/kg body weight/or 10ml/kg body weight) for last six weeks. Seventy- two hours after the last training session, rats were sacrificed; plasma and heart were excised and immediately frozen in liquid nitrogen. LDH and CK levels were measured by colorimetric method. Statistical analysis was performed using a one way analysis of variance and Tukey test. Significance was accepted at P = 0.05. Result showed that consumption crataegus lowers LDH and CK in heart and plasma. Also the heart LDH and CK were lower in the CPET compared to the ST, while plasma LDH and CK in CPET was higher than the ST. The results of ANOVA showed that the due high-intensity exercise and consumption crataegus, there are significant differences between levels of hearth LDH (P<0/001), plasma (P<0/006) and hearth (P<0/001) CK. It appears that high-intensity exercise led to increased tissue damage and inflammatory factors in plasma. In other hand, consumption aqueous extraction of Red Crataegus maybe inhabits these factors and prevents muscle and heart damage.*

**Keywords:** LDH ,CK, *Crataegus pentaegyna*, High-Intensity running

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### **INTRODUCTION**

Hawthorn is the oldest known medicinal plant in European medicine; its actions on the heart were first described by Dioscorides in the first century [1]. Red Crataegus (With different names such as Hawthorn, *Crataegus monogyna*, *Crataegus oxyacantha*) is a valuable medicinal plant of the rose family that is used today to treat cardiac dysfunction and circulation, especially as anti-infection and anti-oxidant. In many European countries, the extract of Crataegus is used to treat congestive heart failure. Clinical efficacy and safety of herbal medicine Crataegus has been shown by several tests [2]. Hawthorn has traditionally been used to treat anxiety, asthma, hypertension, dyslipidemia, hypotension, angina, arrhythmias, heart failure, and indigestion. The most substantial evidence for clinical benefits of hawthorn is its use in chronic congestive heart failure (CHF). Hawthorn substantially increased maximal work load tolerance, increased exercise tolerance, decreased the pressure–heart rate product (an index of cardiac oxygen consumption), and improved symptoms of fatigue and shortness of breath as compared with placebo [3].

Human subjects treated with COC extract after myocardial infarction have shown improvements in heart rate, reduction in blood pressure, and an increase in the left-ventricular ejection volume. Meta analysis of a randomized trial with COC extract showed its beneficial role as an adjunctive treatment for chronic heart failure. It has also been shown that an alcoholic extract of COC promoted improvement in TCA cycle enzyme activity and protected the mitochondria against isoproterenol-induced cardiac injury [4]. Although COC extract has been shown to improve cardiac function, the exact mechanism of action has not yet been elucidated. However it was shown that treatment with COC ethanolic extract improves the cardiac function at both the functional and molecular levels. The COC extract-treated hearts also showed a four-fold decrease in the infarct size compared to I/R group. Reduction in the damage allows the myocardium to recover faster as reflected in the improved contractile functions of COC extract-treated myocardium. This is further supported by the decreased levels of cardiac marker enzymes CK and LDH. A possible explanation could be that administration of COC extract during reperfusion produced a mild negative chronotropic effect, which might have resulted in a reduction of LV pressure leading to a reduction in the pressure overload on cardiomyocytes membrane. On the other hand, the ROS produced can be scavenged directly by the OPCs present in the COC extract thereby preventing lipid peroxidation and helping to maintain membrane integrity. This is further supported by the decreased levels of CK and LDH in the treated group [5].

Normally several enzymes such as LDH, CK, and CKMB are enclosed within the cell but due to the different exercises the possibility of their release in blood increases [6-7]. Lactate dehydrogenase is one of these enzymes which exist in the cell cytoplasm of all of the body tissues with different concentrations. Most of the studies that surveyed the effect of different activities and exercises especially physical strength and endurance sports on the amount of activity of LDH enzyme, reported a significant change in these enzymes [7]. In a way that in a study Franca and et al surveyed 20 healthy male athletes and their responses showed a significant increase in LDH after the marathon competition and even in the recovery period [8]. In addition to activity during the process of producing energy and lactate the LDH enzyme has also an effective role in creating inflammatory conditions for the muscular cells [9-10]. Thus some of the researchers reported that the increase of LDH level along the physical exercise is caused by the membrane damage of muscle fibers [11]. Creatine kinase (CK) is measured as another important enzymes in energy metabolism in the body which saves the high concentrations of intracellular ATP through phosphorylation of creatine as an important index [6-7, 12]. In a way that in the study of Kratz and et al on 37 runners (32 males and 4 females) with the age mean of 49 years old after performing the marathon in the serum marker of cell damage of CK and CKMB a significant increase was observed [13]. This enzyme exists in most of the cells and different isoforms of this enzyme exists in different tissues[14]. CK enzyme increases its activity in a condition which the exercise has tendency toward aerobics. Thus some of the studies considered the increase of activity of this enzyme due to the effect of severe physical training which shows tissue damages and inflammatory conditions [15].

Extreme sports are accompanied by increase of cell and muscle tissue damaging index [9]. Although these blood indices are usually more studied in strength and eccentric sports, in extreme aerobic exercises they also have the ability of increase according to the severity and time of exercise. On the other hand it has been shown that in addition to effects on the cardiovascular system Hawthorn also could control some of the factors related to cell damages. Thus in this research efforts have been made to study the simultaneous effect of consuming the aqueous extract of hawthorn and extreme aerobic exercises on the cell damage indices at plasma level and heart tissues of male Wistar rats.

## MATERIALS AND METHODS

### *Animals*

All experiment involving animals were conducted according to the policy of Iranian convention for the protection of vertebrate animals used for experimental and other scientific purposes; the protocol was approved by the Ethics Committee of the Sciences, University of Mazandaran (UMZ) and Babol University of Medical Sciences (BUMS, Mazandaran, Iran). Thirty-two Wistar male rats (4-6 weeks old, 125-135 gr weight) were acquired from the Pasteur's Institute (Amol, Mazandaran), and maintained in the Central Animal House of the Faculty of Physical Education and Sports Science of UMZ. Five rats were housed per cage (46-L) with a 12-hour: 12-hour light-dark cycle Temperature and humidity were maintained at 22°C ± 1.4°C and 50% ± 5%, respectively. Diets (a pellet form) and water were provided ad libitum. Animals were randomly assigned into control (n = 16) and training (n = 16) groups. Rats were further divided into saline-control (SC) (n=8), saline-training (ST) (n=8), *Crataegus pentaegyna* extraction -control (CPEC) (n=8), and *Crataegus pentaegyna* extraction - training (CPET) (n=8). The control (SC and CPEC) groups remained sedentary; whereas the training groups underwent a high running exercise program.

#### *Exercise training protocol*

At first, the animals were familiarized with the rat treadmill apparatus, every day and for 5 days. The exercise groups were trained for 8 weeks on a motor driven treadmill as previously reported elsewhere (15). The rats were submitted to run at 34 m/min for 60 minutes, 5 d/week (21). The animals were killed 72 hours after the last exercise session. Food but not water was removed from the cages 3 hours before the sacrifices [16].

#### *Plant material and Preparation of the Crataegus extraction*

The ripped fruit samples of *Crataegus-Pentaegyna* were collected from Neka forest in the Mazandaran province of Iran, and well washed. Fruits were dried in oven at 35° C for 4 days. The extraction was prepared according to the Cai *et al* [17]. Briefly, the whole ripped and dried fruit *Crataegus-Pentaegyna* were ground to fine powder. Water extraction: 5 g of the powdered sample was extracted with 100 mL distilled water at 80C<sup>0</sup> for 20 min. After cooling the extract was filtered. Then centrifuged at 3,000 rpm for 17 min and filtered through filter paper. The freshly prepared extracts were cooled and immediately used in the experiments. In last six weeks, all treatments were given in a single daily dose orally using special gavages needles. After training, 500mg/kg /or 10 ml/kg body weight liquid extraction of *Crataegus-Pentaegyna* [18] was orally assigned to the *Crataegus-Pentaegyna* groups and the same amount of saline was fed to saline groups.

#### *Preparation of the GC/MS Analyses*

The whole ripped and dried fruits of *Crataegus-Pentaegyna* were grounded in house electronic grinder to a fine powder, part of which macerated by n-hexane (Merck Co., USA). For 72h at room temperature, extracted by soxhlet, and evaporated using a rotary evaporator. Chromatographic analysis was carried out on HP devices, 6890 series GCMS apparatus combined with a mass selective detector. The capillary column used was a HP-1MS. Helium was used as carrier gas. The fatty acid components of *Crataegus-Pentaegyna* extracts were determined using library search soft-ware from Wiley/NBS Registry Mass Spectral Data and in-house "BASER Library of Fatty Acid Constituents".

#### *Biopsy and plasma collection*

Seventy-two hours after the last training session, rats were anesthetized with intra peritoneal administration of a mixture of ketamine (30– 50mg / kg body weight) and xylazine (3– 5mg / kg body weight). Blood samples are directly collected from the Inferior vena cava and after transferring them to laboratory tubes containing EDTA they were centrifuged in rpm 3000 for 15 minutes and the plasma was collected. After freezing the plasma by the use of liquid nitrogen they were kept in refrigerator at -80°C for measuring collagen 18.

After separating and being washed with saline and being frozen with freeze clamp the heart tissue was immediately powdered by the use of porcelain mortar. After weighing the powdered tissues they were put into the glass tubes and for each 50 milligrams of powdered tissue 1cc PBS buffer were added to it. The combination of tissue and PBS were immediately homogenized by the use of homogenizer machine (in a container full of powdered ice) and again it was centrifuged for 10 minutes. The supernatant was separated and it was put into special tubes and transferred to liquid nitrogen and then it was preserved in the freezer under the temperature of -80°C until the time of measuring.

#### *Measurement of LDH and CK*

LDH and CK levels by colorimetric method using a kit made in France ELITECH GROUP Company was measured.

#### *Statistical methods*

The Kolmogorov-Smirnov test was used to determine the normality of distribution, and variables were found to be normally distributed. All results are expressed as means ± SEM. Statistical analysis were performed using a one way analysis of variance and tukey test. Significance was accepted at P < 0.05. All statistical analysis was performed with SPSS (Version 15; SPSS).

## **RESULTS AND DISCUSSION**

In table 1 the results related to LDH and CK enzymes are provided in the form of indices of mean and standard deviation in different groups. Results show that consuming hawthorn results in the decrease of amount of LDH and CK at cardiac and plasma levels. Also the amounts of cardiac LDH and CK in hawthorn-exercise group were less than the saline-exercise group while the amounts of Plasma LDH and CK in hawthorn-exercise were higher than saline-exercise group.

**Table 1: Mean and SD of LDH and CK in different research groups**

Index	Group	SC	CPEC	ST	CPET
LDH	Heart (U.mg)	91.117±6.729	81.896±3.864	112.052±40.805	91.798±5.546
	Plasma (U.l)	196.382±42.938	152.83±17.994	197.67±15.38	284.57±19.078
CK	Heart (U.mg)	2.061±0.278	2.043±0.297	2.974±0.917	2.423±0.207
	Plasma (U.l)	107.25±23.285	85.83±1.797	105.17±12.303	137±15.21

Variance analysis results showed that a significant difference exists between the amounts of cardiac LDH ( $P<0.001$ ) plasma ( $P<0.006$ ) and cardiac ( $P<0.001$ ) as a results of consuming hawthorn and performing endurance exercises (table 2). The post-hoc Tukey test showed that the level of cardiac LDH in saline-exercise group was more than other groups, and thus it indicates that the hawthorn extract has significantly managed to decrease its level, but no significant difference was observed between the level of plasma LDH ( $P<0.073$ ), the post-hoc Tukey test related to the cardiac and plasma CK showed that the highest amount of CK was related to saline-exercise group, which indicates that the hawthorn extract has significantly managed to also decrease the level of CK.

**Tale 2: Results related to the variance analysis on the amounts of LDH and CK**

		Sum of squares	df	Mean square	F	Sig.
Heart LDH	Between groups	2938.72	3	979.576	26.109	0.000
	Within groups	8932.46	24	327.186		
	Total	11871.19	27			
Plasma LDH	Between groups	60931.65	3	20310.55	2.632	0.073
	Within groups	17891.75	23	777.902		
	Total	78823.40	26			
Heart CK	Between groups	3.608	3	1.203	10.35	0.000
	Within groups	5.496	24	0.229		
	Total	9.104	27			
Plasma CK	Between groups	8806.01	3	2935.34	5.251	0.006
	Within groups	6523.16	23	283.616		
	Total	15329.18	26			

Extreme exercises potentially damage the cardiac function, and essentially they are not safe. This relative risk increases for damaging the heart cells during the extreme exercises and it increases up to one hour after exercising. Exercise-induced cardiovascular disorder in the absence of cardiovascular diseases shows a series of symptoms which are called cardiac fatigue. Extreme exercises are accompanied by the increase of cell and muscle tissue damaging indices, for example we could refer to Lactate dehydrogenase (LDH) and Creatine phosphokinase (CPK) enzymes. LDH and CK are important enzymes participating in transferring the lactic acid to Pyruvate and formation of ATP from ADP in anaerobic systems and they are also known as indicators of oxidative stress. In muscle damages the CK enzyme shows the highest changes and it is the measuring indicator of muscle damage. LDH enzyme changes occur after the CK and usually its amount gradually increases 24 to 48 hours after stimulation [19]. Normally lots of enzymes such as LDH and CK are enclosed within the cell but due to performing different exercises the possibility of them to be released in the blood increases. LDH is one of these enzymes existing in the cell cytoplasm of all of the body tissues with different concentrations. Most of the researches that studied the effect of exercising on the amount of activity of LDH enzyme reported the significant change of this enzyme [12]. CK is another important enzyme in energy metabolism in body with maintaining the high concentration of intracellular ATP through phosphorylation and it is measured in most of the researches as another important index [12]. Thus this possibility exists that cell damages are created due to performing exercises [20]. Although these blood indicators are usually studied in eccentric and strenuous exercises this is possible that the extreme aerobic exercises also increase according to the severity and time of exercise in a way that in addition to exercising in the process of producing energy and lactate, LDH enzyme also has an effective role in creating inflammatory conditions for muscle cells [9-10]. Thus some of the researchers reported the increasing level of LDH along with physical exercises due to the membrane damage in muscle fibers [11]. Along with LDH enzyme, CK also has an important effect on the process of producing energy in anaerobic conditions [14]. This enzyme exists in most of the cells and its different isoforms exist within different tissues [15]. Some of the studies consider the increase of activity of this enzyme due to the effect of extreme physical activity indicating the tissue damages and inflammatory conditions. Due to the increase of these inflammatory factors and damages caused by them in different tissues, the immune system is severely affected and it increases the level of antioxidants in the blood circulation and tissues in order to cope with this situation. In the current research the level of LDH enzyme and CK enzyme due to the severe exercise in both groups is coincided with increase in its activity. Kratz and et al studied 37 runners (32 males and 4 females) with age mean of 49 years old after performing marathon in serum marker of cell damage of CK and CKMB and they observed a significant increase [13].



In a study on 20 healthy athletic males Franca and et al reported a significant increase of LDH in responding to marathon competition after the competition and even in the recovery period [8]. Several factors are effective on the increase of serum level of these indices in aerobic exercises, as such a delay in transferring oxygen to active tissues, organs and muscles due to the lack of equality of required oxygen for activity with its severity at the beginning of starting severe aerobic exercise and also tendency toward anaerobic exercising at the final moments, due to the increase of exercise severity are some of the factors which increase the level of LDH and CK [21]. On the other hand the immune system also increases the activity of antioxidant enzymes for coping with effective factors on the muscle damages. This possibility exist that increase of levels of inflammatory indicators such as LDH and CK affect the immune system and the increase of these enzymes especially CK in the recovery period and resting hours results in the decrease of immune system. LDH enzyme is widely propagated in the body tissues and its high concentration is found in liver, myocardial, kidney, musculoskeletal, red blood cells and other tissues. The amount of LDH the same as CK and other enzymes increases after the muscle damages, but its concentration stays high for a longer period of time. The current research results show that consuming hawthorn results in decreasing the amounts of LDH and CK at cardiac and plasma levels. Also the amounts of cardiac LDH and CK in hawthorn-exercise group was less than saline-exercise group while the amounts of plasma LDH and CK in hawthorn-exercise group were higher than the saline-exercise group. Consuming hawthorn results in the strength of the cell membrane and it considerably prevents the cell failure and increases the mentioned enzymes in interstitial fluid and blood. Red Crataegus is a valuable medicinal plant that is used today to treat cardiac dysfunction and circulation, especially as anti-infection and anti-oxidant [22]. Medicinal importance Crataegus is due of phenolic compounds [23]. Because Red Crataegus contains bioflavonoid and piroantosianins, it is of great importance in the pharmaceutical industry [24]. The herb Red Crataegus is the dominant flavonoid, rosin and Quercetin, there are in some parts there [25]. Flavonoids are compounds having antioxidant properties which prevent the LDL cholesterol oxidation in in vitro situations which may show the anti-adrenergic properties of red extract of hawthorn [9-10]. Flavonoids are compounds preventing from platelet aggregation and their adhesion [11-12]. Flavonoids are compounds preventing from platelet aggregation and their adhesion. Some studies show that antioxidants have the ability to harness ROS including free radicals such as Superoxide, Hydroxyl, and Peroxyl radical and it could have some benefits for treating Ischemia – reperfusion I/R which the mediator for heart damage. Thus there is a need for understanding and recognizing the interferences of appropriate antioxidant for treating I/R which is the reason of tissue damage and disorder in myocardia function [26-27]. COC includes oligomeric, flavonoids and polyphenols that their antioxidant properties are well known. Several studies show that COC extract is effective in removing ROS specially the free radicals [28-29]. In a research Ebrahimzadeh and et al also studied the antioxidant activity of methanol and aqueous extract of Crataegus (Pentagna type) by the use of in vitro experimental systems. Both of the tested extracts showed a high antioxidant activity. Also the extracts showed a high phenolic content [30]. OPC's present in COC extract might scavenge the free radicals ex vivo. This reduces the oxidative stress and allows the cells to recover from reperfusion-induced injury. The O<sub>2</sub><sup>•-</sup> and ROO<sup>•</sup> radicals were significantly reduced in the COC extract-treated group demonstrating the antioxidant activity of COC. Severe tissue damage is known to occur during reperfusion due to massive production of ROS. The possible mechanism could be, since the COC extract is available at the time of reperfusion, it can scavenge the free radicals and can reduce the reperfusion-induced injury to the myocardium. These findings supported by the decreased levels of cardiac marker enzymes CK and LDH indicating less tissue damage in the COC extract-treated myocardium [5]. In this research also the findings are supported by the decrease of CK and LDH in red hawthorn-saline group.

## CONCLUSION

At the end the research findings indicate that high intensity aerobic exercises could lead to increase of numbers of factors related to the muscle and heart damage such as LDH and CK and due to the increase of these inflammatory factors and damages caused by them in different tissues the immune system will be affected severely and the antioxidant levels in blood circulation and tissues increases in order to cope with these situations. On the other hand, this possibility exists that consuming aqueous extract of red hawthorn may result in the strength of the cell membrane and considerably restrain the cell failure and increase of level of mentioned enzymes in the interstitial fluid and blood. Also due to having flavonoid substances in itself the red hawthorn could enhance the antioxidant system and prevent from muscle and cardiac cells damages.

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