



RESEARCH PAPER

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The responses of creatine kinase and lactate dehydrogenase to acute eccentric activity after saffron supplementation in healthy man

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Abstract

The purpose of present study was to investigate responses of creatine kinase and lactate dehydrogenase to acute eccentric activity after saffron and vitamin C supplementation in healthy man. For this purpose 21 subjects attended in this study, they were assigned randomly to three separate group: saffron group, vitamin C group and placebo group and during fourteen days, each group receive their supplement in form of capsule. After fourteen days supplementation subjects were running with their 70% of their VO₂max on treadmill with 10 percent gradient for forty five minutes. Five ml Blood samples were taken from antecubital vein before supplementation, before exercise, immediately after exercise and 1h recovery after exercise. Data Analyzes of between group comparison using repeated measure ANOVA for CK and LDH in done. Results show significant decrease of CK immediately after exercise but LDH didn't show significant changes by saffron supplementation. In general it seems that saffron supplementation during eccentric contraction activity decrease enzymatic activity of CK in comparison with control group.

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Introduction

Many studies have suggested the beneficial effects of nutrients or other agents such as vitamin E, C, green tea extract, melatonin, carbenoxolone, and green propolis, in reducing or preventing cerebral or muscle injury (Hosseinzadeh 2009). *Crocus sativus* L. commonly known as saffron, is a perennial stemless herb of the Iridaceae family and widely cultivated in Iran and other countries such as India and Greece (Rios 1996). Commercial saffron comprises the dried red stigma with a small portion of the yellowish style attached. Compounds considered pharmacologically active and important are volatile agents, bitter principles and dye materials (Rios 1996). In modern pharmacological studies, saffron, or its active constituents, has demonstrated anticonvulsant (Hosseinzadeh 2002), antidepressant (Hosseinzadeh 2004), anti-inflammatory (Hosseinzadeh 2002) and antitumour activities (Abdullaev 1993). Radical scavenger effects as well as learning and memory improving properties (Abe K 1999) and promote the diffusivity of oxygen in different tissues were also reported (Rios 1996). Saffron extract is also chemopreventive and showed protective effects on genotoxins-induced oxidative stress in Swiss albino mice (Premkumar 2003). Recently, Assimopoulou showed that the saffron extract, crocin and safranal exhibited significant radical scavenging activity and thus antioxidant activity (Assimopoulou 2005). There was also a constant decrease in lipoprotein oxidation susceptibility in healthy individuals after administration of 50 mg of saffron twice a day (Verma 1998). Saffron and its constituents have been shown to decrease I/R injury in kidney or brain tissues (Hosseinzadeh 2005). Thus, in this study the effect of saffron extract was evaluated during acute eccentric via analysing of muscle injury factors so creatine kinase and lactate dehydrogenase in the healthy men. Lactate dehydrogenase (LDH) and creatine kinase (CK) enzymes can increase during exercise and not only produce energy and lactate, but play effective roles during inflammatory conditions in muscle cells (Grusard *et al.*, 2003). Therefore, some researchers have attributed LDH and CK levels increment during

physical activity to muscle fibers membrane damage (Chang *et al.*, 2004). In addition, various research have reported that during high-intensity eccentric exercises, blood levels of muscle cells enzymes, protoplasm damage, and acute inflammatory response increase that can result in fatigue, decreased muscle performance, and prolonged recovery. Therefore, it seems necessary to resolve oxidative stress and improve recovery in these people. Furthermore, since the intake of natural antioxidants such as saffron can reduce oxidative stress and its harmful effects, and nullify delayed recovery of oxidative attack through increase of enzymatic and non-enzymatic antioxidants, this research has investigated the impact of saffron supplement and a session of eccentric contraction on the response of LDH and CK.

Materials and methods

Twenty one male students with a mean age of 25.27 ± 1.30 years, weight of 79.88 ± 2.20 kg, and height of 180.5 ± 2.80 cm were randomly selected and divided into three groups of control (placebo), positive control (vitamin C), and experimental (saffron supplement). Before supplementation, blood was sampled from all subjects and stored in refrigerator. Following the first sampling, the subjects consumed capsules containing 100 mg of saffron (experimental group), capsules containing starch (control), and 1000 mg of vitamin C (positive control) for 14 days; the second blood sampling was performed 14 days after ingestion of the capsules. At the next stage, after a 10 minutes warm-up, the test consisting of one session running on a negative slope of 10% and VO_2 max level of 70% was performed for 45 min and immediately after the test, the third blood sample was taken from subjects. A fourth sample was also taken after 60 minutes rest. All samples were transported to the laboratory for measurement of LDH and CK. Normality of all data was approved using the Kolmogorov-Smirnov test. Then to compare the difference of CK and LDH between these groups, the ANOVA repeated test with an inter-group factor of (3 * 4) was carried out. To assess intra-group changes in different groups, the repeated analysis of variance was used and if

significant, the post hoc Bonferroni test was performed to find the location of differences.

Results

The data in table 1 show the mean and standard deviation of CK in supplement onset, before exercise, after exercise and 1 hour after recovery.

Table 1. Mean and standard deviation of CK.

placebo	Vitamin. C supplement	Saffron supplement	group variable
62.2±28.5	52.9±19.9	60.8±19.7	Onset supplement
60.7±35.1	75±23.1	87.1±26.3	Before exercise
74.7±38.7	62.1±29.4	51.8±34.8	After exercise
92±46	56.4±31.8	95.1±28.8	1 hour after recovery

The data in table 2 show mean and standard deviation of LDH in supplement onset, before exercise, after exercise and 1 hour after recovery.

Table 2. Mean and standard deviation of LDH.

placebo	Vitamin. C supplement	Saffron supplement	group variable
187.7±24.6	194.4±41.8	215.4±61.4	Onset supplement
192.4±28.2	194.6±40.1	218.6±58.1	Before exercise
199±22	202±42	227.4±62.8	After exercise
207.6±30.1	208.7±42.3	236.1±54.7	1 hour after recovery

Separate comparison of intra-group changes of CK in saffron supplement group showed a significant change in CK level ($F_{3, 18} = 7.02, p = 0.003$). The post hoc Bonferroni test determined that there was a significant difference between data at supplement onset and before exercise ($p = 0.026$) and between data at 1 hour after recovery and after activity ($p = 0.015$) (Diagram 1.4). Separate comparison of intra-group changes of LDH in saffron supplement group showed no significant change in LDH level ($F_{3, 18} = 14.57, p = 0.000$). The post hoc Bonferroni test determined that there was no significant difference between data at 1 hour after recovery and before activity ($p = 0.048$).

Discussion and conclusion

The present study has investigated the response of CK and LDH to a session of eccentric contraction after saffron supplementation in active men. The impact of saffron supplementation was significant on CK levels in active men ($p = 0.003$), however, no significant changes were observed in vitamin C supplement group ($p = 0.314$). The results showed a significant reduction in CK levels immediately after activity ($p = 0.035$) and an increase in CK levels 2 hours after recovery ($p = 0.002$). Since saffron prevents oxidation of different enzymes by free radicals and reactive oxygen species, its level may remain high and hence reduces CK levels immediately after activity. Eccentric exercise-induced muscular contusion and damage appears to increase 8 and 24 hours following exercise (Stan *et al.*, 2003). Muscles contusion or delayed pain can also be another reason for CK increase during recovery. In contrast to the present results, Sorichter *et al.* (2001) have reported a significant increase in CK of healthy subjects following 20 minutes downhill running with a VO_2 max of 70% immediately after exercise. Similarly, a significant increase was observed in CK levels of subjects following 60 minutes running in a 13.5% slope with a VO_2 max of 75% (Smith *et al.*, 2007). Despite differences between these studies, CK level increases in terms of intensity, duration, and downhill running angle. Increasing levels of circulating CK supports the possibility of skeletal muscle damage in downhill running. Another study examined lipid peroxide concentration through thiobarbituric acid-reactive substances (TBARS) and CK after 45 minutes of downhill running at 12 degrees in a VO_2 max of 75%. Although all variables were increased 6 hours after exercise, no change was found in TBARS concentration immediately after exercise. Six hours after exercise TBARS level has reached to its peak and returned to normal within 72 hours, while increased CK levels remained high until 72 hours after exercise (Morgan *et al.*, 1989). Saffron supplementation showed no significant change in LDH levels of active men in all groups. In other words, saffron supplementation compared with vitamin C and placebo supplementations did not result in a

significant change in LDH levels. Poprzęcki *et al.* (2004) determined the influence of concentric and eccentric muscular work on CK and LDH activity during a graded exercise protocol on 10 students of physical education. All of them performed the treadmill exercise protocol twice (test 1: uphill run, concentric work; test 2: downhill run, eccentric work). The concentric muscular work elicited a higher blood acidosis in comparison to the eccentric one. Both efforts caused a significant increase in plasma CK and LDH activity. After 24 hours of rest, CK activity continued to rise while LDH activity returned to its pre exercise value. After 7 and 24 hours of recovery, plasma CK activity was significantly high following the eccentric exercise. Simultaneously, increase of CK activity during recovery after the eccentric work, pointed at the deterioration of muscle cells (Poprzęcki *et al.*, 2004). Increased activity of CK and LDH after exercise is connected with changes of cell membrane and mechanical damage of the muscle fibers. Changes in myocyte membranes might be caused by the decrease of cellular ATP concentration, cell anoxia, cell lipid peroxidation, ion action disturbances, and intracellular proteins liberation dependent on other particles (Melin *et al.*, 1997; Newham *et al.*, 1986). The intramuscular enzyme activity increases after exercise which could be due to intracellular homeostasis and calcium ion concentration (Saxton *et al.*, 1995). Saffron can inhibit the oxidative stress induced by genotoxic compounds possibly due to its chemical protective effects against genotoxic compounds (Nair *et al.*, 1995), because saffron and crocin, crocetin, and safranal compounds are effective against free radicals and antioxidants (Chen *et al.*, 2008; Asdaq and Inamdar, 2010). In contrast to the present study, Mehdizadeh *et al.* reported a significant decrease in serum LDH and CK-MB and lipid peroxidation after 8 days consumption of saffron supplement. Brown *et al.* (1997) have evaluated eccentric exercise-induced muscle damage indicators in a session of eccentric exercise. Serum CK and LDH activities were increased after an eccentric exercise, while serum alkaline phosphatase activity was unchanged throughout the study. The results of the present study showed a

significant reduction in CK levels soon after activity by saffron complement and increase in CK levels at the onset of supplementation, before activity, and 2 hours of recovery in all groups. Saffron supplementation had no significant change in the levels of LDH at the onset of supplementation, before exercise, after exercise, and 2 hours of recovery in all groups. Saffron can inhibit the oxidative stress induced by genotoxic compounds possibly due to its chemical protective effects against genotoxic compounds, because saffron and crocin, crocetin, and safranal compounds are effective against free radicals and antioxidants. However more research is needed in this field.

References

- Abe K, Sugiura M, Ymaguchi S, Shoyama Y, Saito H.** 1999. Saffron extract prevents acetaldehyde-induced inhibition of long-term potentiation in the rat dentate gyrus in vivo. *Brain Research* **851**, 287–9.
[http://dx.doi.org/10.1016/S0006-8993\(99\)02174-5](http://dx.doi.org/10.1016/S0006-8993(99)02174-5)
- Abdullaev FJ.** 1993. Biological effects of saffron. *Biofactors* **4**, 83–6.
- Armstrong RB.** 1990. Initial events in exercise-induced muscular injury. *Medicine and Science in Sports and Exercise* **22(4)**, 429-435.
- Asdaq SMB, Inamdar MN.** 2010. Potential of *Crocus sativus* (saffron) and its constituent, crocin, as hypolipidemic and antioxidant in rats. *Applied biochemistry and biotechnology* **162(2)**, 358-372.
<http://dx.doi.org/10.1007/s12010-009-8740-7>
- Assimopoulou AN, Sinakos Z, Papageorgiou VP.** 2005. Radical scavenging activity of *Crocus sativus* L. extract and its bioactive constituents. *Phytotherapy Research* **19**, 997–1000.
<http://dx.doi.org/10.1002/ptr.1749>
- Cervellin G, Comelli I, Lippi G.** 2010. Historical

- background clinical, diagnostic and therapeutic features. *Clinical Chemistry and Laboratory Medicine* **48**(6), 749 – 75.
- Chen Y, Zhang H, Tian X, Zhao C, Cai L, Liu Y, Jia L, Yin HX, Chen C.** 2008. Antioxidant potential of crocins and ethanol extracts of *Gardenia jasminoides* ELLIS and *Crocus sativus* L.: A relationship investigation between antioxidant activity and crocin contents. *Food Chemistry* **109**, 484-92.
<http://dx.doi.org/10.1016/j.foodchem.2007.09.080>
- Choung BY, Byun SJ, Suh JG, Kim TY.** 2004. Extracellular superoxide dismutase tissue distribution and the patterns of superoxide dismutase mRNA expression following ultraviolet irradiation on mouse skin. *Experimental Dermatology* **13**(11), 691-9.
<http://dx.doi.org/10.1111/j.0906-6705.2004.00209.x>
- Goldfarb AH, Bloomer RJ, et al.** 2005. Combined antioxidant treatment effects on blood oxidative stress after eccentric exercise. *Medicine and science in sports and exercise* **37**(2), 234.
<http://dx.doi.org/10.1249/01.MSS.0000152887.87785.BE>
- Groussard C, Rannou-Bekono F, Machefer G, Chevanne M, Vincent S, Sergent O, et al.** 2003. Changes in blood lipid peroxidation markers and antioxidants after a single sprint anaerobic exercise. *European Journal of Applied Physiology* **89**(1), 14-20.
<http://dx.doi.org/10.1007/s00421-002-0767-1>
- Hosseinzadeh H, Karimi Gh, Niapoor M.** 2004. Antidepressant effects of *Crocus sativus* stigma extracts and its constituents, crocin and safranal, in mice. *Acta Horticulturae* **650**, 435-45.
- Hosseinzadeh H, Khosravan V.** 2005. Anticonvulsant effects of aqueous and ethanolic extracts of *Crocus sativus* L. stigmas in mice. *Archives of Iranian medicine* **5**, 44-7.
- Hosseinzadeh H, Modagheh MH.** 2009. *Crocus sativus* L.(Saffron) extract and its active constituents (crocin and safranal) on ischemia-reperfusion in rat skeletal muscle. *Evidence-Based Complementary and Alternative Medicine* **6**(3), 343-350.
<http://dx.doi.org/10.1093/ecam/nem125>
- Hosseinzadeh H, Modagheh MH, Saffari Z.** 2009. *Crocus Sativus* L. (Saffron) Extract and its Active Constituents (Crocins and Safranal) on Ischemia-Reperfusion in Rat Skeletal Muscle. *Evidence-based Complementary and Alternative Medicine* **6**(3), 343-350.
<http://dx.doi.org/10.1093/ecam/nem125>
- Hosseinzadeh H, Sadeghnia HR.** 2005. Protective effect of aqueous saffron extract (*Crocus sativus* L.) and crocin, its active constituent, on renal ischemia-reperfusion-induced oxidative damage in rats. *The Journal of Pharmaceutical Sciences* **8**(3), 387-393.
- Hosseinzadeh H, Younesi HM.** 2002. Antinociceptive and anti-inflammatory effects of *Crocus sativus* L. stigma and petal extracts in mice. *BMC Pharmacology* **2**, 1-8.
- Melin B, Bourdon L, Jimenez C, Charpenet A, Bernard O.** 1997. Plasma myo sin and creatine kinase time-course after a concentric-eccentric field exercise. *Archives of Physiology and Biochemistry* **105**, 27-31.
<http://dx.doi.org/10.1076/apab.105.1.27.13149>
- Nair SC, Kurumboor S.** 1995. Saffron chemoprevention in biology and medicine: a review. *Cancer Biotherapy & Radiopharmaceuticals* **10**(4), 257-264.
<http://dx.doi.org/10.1089/cbr.1995.10.257>

Newham DJ, Jones DA, Edwards RH. 1986. Plasma creatine kinase changes after eccentric and concentric contractions. *Muscle and Nerve* **9**,59-63. <http://dx.doi.org/10.1002/mus.880090109>

Premkumar K, Abraham SK, Santhiya ST, Ramesh A. 2003. Protective effects of saffron (*Crocus sativus* L.) on genotoxins-induced oxidative stress in Swiss albino mice. *Phytotherapy Research* **17**,614-17. <http://dx.doi.org/10.1002/ptr.1209>

Ríos JL, Recio MC, Giner RM, Máñez S. 1996. An update review of saffron and its active constituents. *Phytotherapy Research* **10**,189-93. [http://dx.doi.org/10.1002/\(SICI\)1099-1573\(199605\)10:3<189::AID-PTR754>3.0.CO;2-C](http://dx.doi.org/10.1002/(SICI)1099-1573(199605)10:3<189::AID-PTR754>3.0.CO;2-C)

Saxton JM, Donnelly AE. 1995. Light concentric exercise during recovery from exercise-induced muscle damage. *International Journal of Sports Medicine* **16**,347-351. <http://dx.doi.org/10.1055/s-2007-973018>

Sinha S, Ray US, Saha M, Singh SN, Tomar OS. 2009. Antioxidant and redox status after maximal aerobic exercise at high altitude in acclimatized lowlanders and native highlanders. *European Journal of Applied Physiology* **106**, 807-14. <http://dx.doi.org/10.1007/s00421-009-1082-x>

Verma SK, Bordia A. 1998. Antioxidant property of saffron in man. *Indian Journal of Medicine Science* **52**, 205-7.