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EFFECTS OF LITHOSPERMIC ACID SUPPLEMENTATION AGAINST ACUTE EXERCISE-INDUCED OXIDATIVE STRESS IN RATS

Hakkı Çoknaz[1], Esen Sezen Karaoğlan[2], Ufuk Özgen[3]

[1]*Düzce Üniversitesi Spor Bilimleri Fakültesi, Beden Eğitimi ve Spor Anabilim Dalı, Düzce, Türkiye*
[2]*Atatürk Üniversitesi, Eczacılık Fakültesi, Farmasötik Botanik Anabilim Dalı, Erzurum, Türkiye*
[3]*Karadeniz Teknik Üniversitesi, Eczacılık Fakültesi, Farmakognozi Anabilim Dalı, Trabzon, Türkiye*

ABSTRACT

Objective: *The aim of the study was to investigate whether lithospermic acid (LSA), a naturally derived polyphenolic compound, supplementation could prevent acute exercise-induced oxidative damage to skeletal muscle tissue in the rats.*

Material and Method: *Rats were randomly selected and divided into three groups (n= 7/group): Group 1- Control group (non-exercise, UT), Group 2- Exercise group (exercise, T) and Group 3- Exercise+lithospermic acid group (exercise+lithospermic acid, T/LSA). At the end of the exercise protocol, the samples were removed from the right leg quadriceps muscle. Malondialdehyde (MDA), myeloperoxidase (MPO), glutathione (GSH) and superoxide dismutase (SOD) levels were measured in the muscle samples.*

Result: *Compared to the UT group, malondialdehyde (MDA) and myeloperoxidase (MPO) activity levels in the muscle tissue of the T group significantly increased ($p<0.001$). MDA and MPO activity levels significantly decreased in the T/LSA group compared to the T group ($p<0.05$). Glutathione (GSH) and superoxide dismutase (SOD) activity levels significantly decreased in the T group compared to the UT group ($p<0.05$). GSH and SOD activity levels in muscle tissue significantly increased in the T/LSA group compared to the T group ($p<0.05$). No significant difference was found in MDA, MPO, GSH, and SOD levels between the UT group and T/LSA group ($p>0.05$).*

Conclusion: *In conclusion, our study shows that oxidative activity levels in the muscle tissue of the T/LSA group decreased significantly in skeletal muscle-induced acute exercise in rats, so it can be considered that lithospermic acid has a protective effect against oxidative damage.*

Keywords: *Antioxidant, acute exercise, lithospermic acid, oxidative stress, free radical.*

CORRESPONDING AUTHOR: Hakkı Çoknaz Düzce Üniversitesi Spor Bilimleri Fakültesi Konuralp Yerleşkesi 81620 Merkez, Düzce hakkicoknaz@duzce.edu.tr

HÇ https://orcid.org/0000-0001-9495-8946 ESK https://orcid.org/0000-0002-9098-9021 UÖ https://orcid.org/0000-0001-9839-6717

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SIÇANLARDA AKUT EGZERSİZE BAĞLI OKSİDATİF STRESE KARŞI LİTOSPERMİK ASİT DESTEĞİNİN ETKİLERİ

ÖZET

Amaç: Bu çalışmanın amacı, doğal kaynaklı bir polifenolik bileşik olan litospermik asit (LSA) takviyesinin, sıçanlarda akut egzersiz kaynaklı iskelet kası dokusunda oksidatif hasarı önleyip önleyemeyeceğini araştırmaktır.

Materyal ve Metot: Sıçanlar rastgele seçildi ve üç gruba (n=7/grup) ayrıldı: Grup 1- Kontrol grubu (egzersiz yapmayan, untrained (UT)), Grup 2- Egzersiz grubu (egzersiz yapan, trained (T)) ve Grup 3- Egzersiz + litospermik asit grubu (egzersiz yapan + litospermik asit, T/LSA). Egzersiz protokolünün sonunda, sağ bacak kuadriseps kasından örnekler çıkarıldı. Kas örneklerinde malondialdehit (MDA), miyeloperoksidaz (MPO), glutatyon (GSH) ve süperoksit dismutaz (SOD) düzeyleri ölçüldü.

Bulgular: UT grubuna kıyasla, T grubu kas dokusunda, malondialdehit (MDA) ve miyeloperoksidaz (MPO) aktivite düzeyleri anlamlı derecede arttı (p<0,001). T/LSA grubunda, T grubuna oranla, MDA ve MPO aktivite düzeyleri anlamlı derecede azaldı (p<0,05). UT grubuna kıyasla, T grubunda glutatyon (GSH) ve süperoksit dismutaz (SOD) aktivitesi seviyeleri anlamlı derecede azaldı (p<0,05). T/LSA grubunda T grubuna oranla kas dokusundaki GSH ve SOD aktivite düzeyi anlamlı derecede arttı (p<0,05). UT grubu ve T/LSA grubu arasında, MDA, MPO, GSH ve SOD düzeylerinde anlamlı bir fark bulunamadı (p>0,05).

Sonuç: Sonuç olarak, çalışmamız, sıçanlarda iskelet kası kaynaklı akut egzersizde, T/LSA grubunun kas dokusunda oksidatif aktivite düzeylerinin anlamlı derecede azaldığı için litospermik asidin oksidatif hasara karşı koruyucu bir etkisi olduğu düşünülebilir.

Anahtar kelimeler: Antioksidan, akut egzersiz, litospermik asit, oksidatif stres, serbest radikal.

INTRODUCTION

Physical exercise induces many adaptive changes in skeletal muscle, including the metabolism and composition of the tissue.[1] Recently, there has been a great deal of interest in the role of oxidative stress and inflammatory responses as related to tissue damage and fatigue from exercise.[2] During running training, reactive oxygen species (ROS) production has been attributed to high oxygen consumption and as much as 100-200 times the skeletal muscle levels, resulting in substantially increased mitochondrial electron flux.[3,4] Some studies indicate that xanthine oxidase (XO) and Myeloperoxidase (MPO) are the two main sources of extracellular free radicals during strenuous exercise and are also responsible for the tissues damage caused by exhaustive exercise.[2] Since oxidative stress contribute to fatigue, tissue damage, and impaired recovery from exhaustive exercise, much research has focused on supplementation of nutraceutical agents for reducing these effects. Lithospermic acid (LSA) was originally isolated from the roots of Salvia mitiorrhiza, a common herb of oriental medicine. Previous studies demonstrated that LSA has antioxidant effects.[5]

LSA (Figure 1) is a naturally occurring phenolic compound found in various Lamiaceae and Boraginaceae species.[6] LSA has elicited endothelium-dependent vasodilatation, lowered blood pressure, produced antioxidant effects, and has hepatoprotective effect.[5,7] LSA also has been shown a wide range of pharmacological activities such as reducing atherosclerosis, anti-inflammatory, anti-viral, HIV-1 integrase, and hyaluronidase inhibition, aldose reductase inhibition and improvement in uremic symptoms.[6]

In this study, LSA, isolated from the methanolic extract of the aerial parts of Origanum rotundifolium, was investigated in terms of antioxidant activity. The isolation of LSA has been performed using several chromatographic methods.[8]

Origanum genus belonging to Labiatae family is represented by about 22 species and 32 taxa in Türkiye. Origanum species, 21 taxa of them are endemic in Turkish flora, are known as "kekik, mercanköşk, merzengüş" in Türkiye Baser.[9] Origanum species have been used against intestinal pain, toothache, cold, catarrh, and rheumatism in folk medicine in Türkiye.[10]

The goal of the present study was to investigate whether LSA supplementation can prevent oxidative damage of the skeletal muscle tissue as well as fatigue caused by acute exercise in rats.

MATERIAL AND METHOD

Animals

All experiments were approved by the Ethics Committee of the Düzce University, according to the Guidelines for Animal Care and Experimentation (2024/03/5).

Twenty-one 3-month-old male Sprague-Dawley (200-250 g) rats were used. Rats were fed standard rat chow and water ad libitum. The animals were kept on a 12-h light and 12-h dark regime and maintained at 23°C.

Exercise Protocol

The animals were divided randomly into the following groups:

Group 1 (n=7;$\bar{X}$= 227±19 g): Untrained (UT). The UT group has not exercised.

Group 2 (n=7;$\bar{X}$=223±20 g): Trained (T). The rats were subjected to acute exhaustive exercise using treadmill running for 60 min (20 m/min, 0% grade).[11]

Group 3 (n=7;$\bar{X}$=230±20 g): Trained and lithospermic acid (T/LSA). The rats were subjected to acute exhaustive exercise using treadmill running for 60 min (20 m/min, 0% grade).[11] LSA (25 mg/kg, intraperitoneally) was administrated 30 min before running.

Analytical Procedures of Oxidative Stress-Associated Parameters

The right leg quadriceps muscle tissue samples of the anesthetized rats were removed within 30 minutes after the end of the exercise. The muscular tissues were rinsed in ice-cold normal saline, blotted dry and stored at -80°C for further analysis. Biochemical tests of the right leg quadriceps muscle tissue samples of the rats were performed one day later. Rats were sacrificed by cervical dislocation under anesthesia.

The Analysis of the Levels of MDA, GSH, and SOD Activities

The muscle tissues (approximately 200 mg) were homogenized in ice-cold 50 mM Phosphate Buffered Saline (pH 7.4); 1:10 weight/volume (w/v) with a homogenizer (IKA ultra turrax T 25 basic, IKA Labotechnik, Staufen, Germany) at 16000 rpm for 10 min. The homogenates were used to measure the level of GSH level, MDA level, superoxide dismutase (SOD) activity. All procedures were performed at 4°C.

MDA concentration of the homogenates was determined spectrophotometrically by measuring the presence of thiobarbituric acid-reactive substances.[12] Results were expressed as nanomoles per gram (nmol/g) of tissue.

GSH concentration was measured spectrophotometrically using Elman's reaction.[13] Results were expressed as micromoles per milligram (µmol/mg) of tissue.

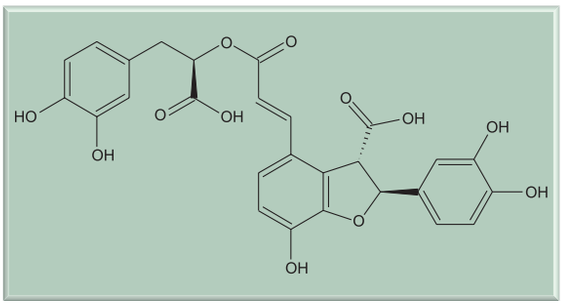

Figure 1. Lithospermic acid

SOD enzyme activity was measured based on the production of hydrogen peroxide from xanthine by xanthine oxidase and the reduction of nitroblue tetrazolium, as previously described.[14] Results were expressed as units per gram (U/g) of tissue.

The Analysis of the Level of MPO Activity

The muscle tissues (approximately 100 mg) were homogenized in ice-cold 50 mM Phosphate Buffered Saline (pH 6) containing 0.5% hexadecyltrimethylammonium bromide (Sigma Chem Corp., St. Louis, Missouri); 1:10 weight/volume (w/v) with a homogenizer (IKA ultra turrax T 25 basic, IKA Labotechnik, Staufen, Germany) at 12000 rpm for 10 min. The homogenates were used to measure the level of Myeloperoxidase (MPO) activity. All procedures were performed at 4°C. Aliquots (0.3 ml) were added to 2.3 ml of reaction mixture containing 50 mM PB (pH 6), o-dianisidine, and 20 mM hydrogen peroxide (H_2O_2) solution. H_2O_2 was used as a substrate for MPO. Oxidized o-dianisidine forms a stable chromophore absorbtion at a wavelength of 460 nm.[15] One unit of enzyme activity was defined as the amount of MPO present that caused a change in absorbance measured at 460 nm for 3 minutes. One unit of enzyme activity was defined as the amount of MPO present that caused a change in absorbance measured at 460 nm for 3 minutes. One unit of MPO acitivty was defined as the amount required to degrade 1 µmol of H_2O_2 per minute at 25°C. MPO activity was recorded as activity per gram of tissue (U/g protein). Protein assays were made by the method of Lowry *et al*.[16]

Statistical Analyses

Differences in measured parametres among the three groups were analysed by the Kruskal-Wallis test. Dual comparisons between groups that presented significant values were evaluated with the MannWhitney U-test. Statistical significance was accepted at a value of $p<0.05$.

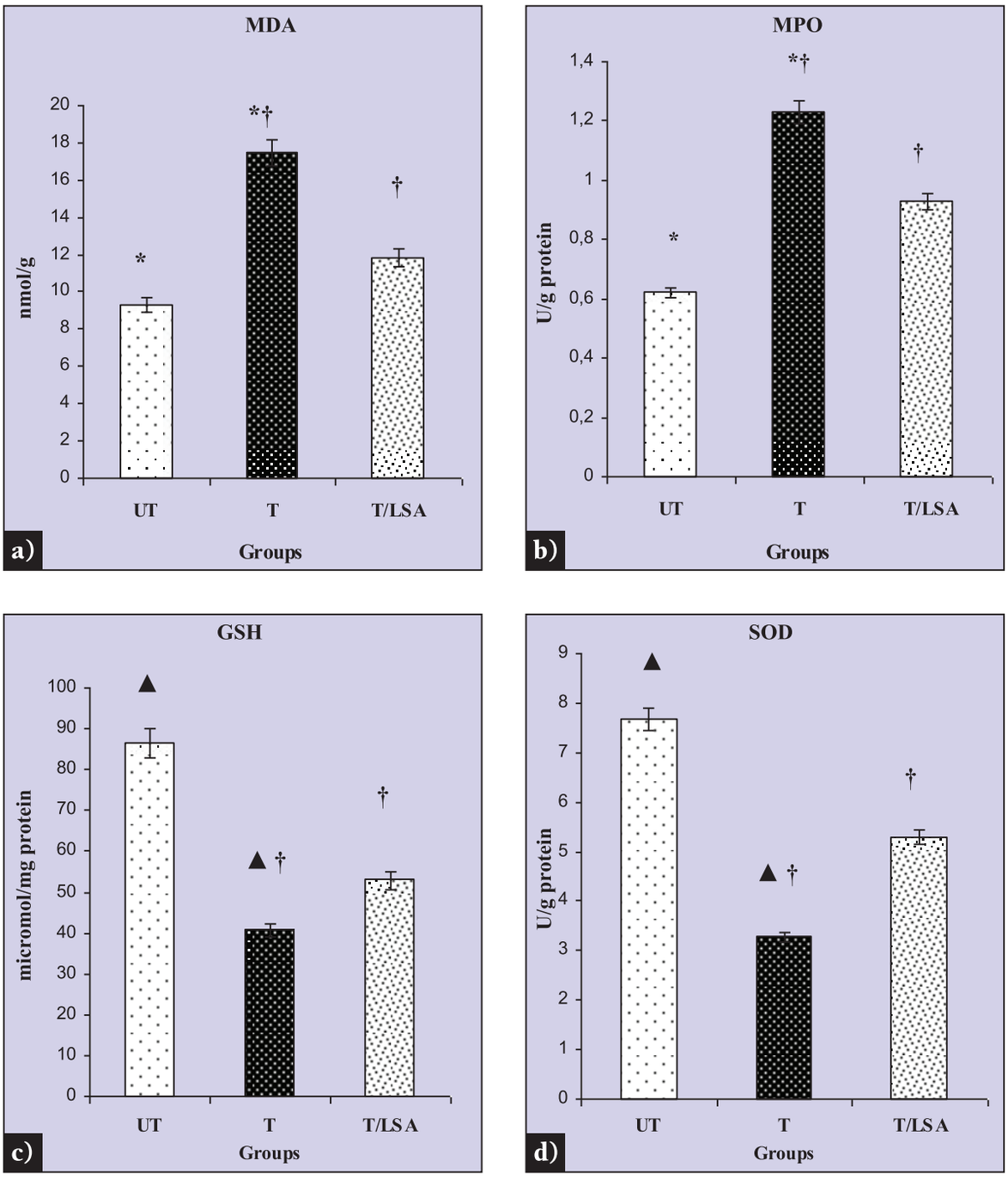

Figure 2. (a) MDA, **(b)** MPO, **(c)** GSH and **(d)** SOD activity in the muscle tissue of UT, T and T/LSA (*p<0.001; ▲, † p<0.05).
MDA: Malondialdehit, **MPO:** miyeloperoksidaz, **GSH:** glutatyon, **SOD:** süperoksit dismutaz, **UT:** untrained, **T:** trained, **LSA:** lithospermic acid, **U/g:** units per gram

RESULTS

The MDA, MPO, GSH and SOD values for the different groups have been shown in Figure 2a, b, c and d, respectively.

The levels of MDA (Figure 2a) and MPO (Figure 2b) activities significantly increased in the muscle tissue of the T group (p<0.001) compared to the UT group. However, MDA (Figure 2a), and MPO (Figure 2b) activity levels significantly decreased in the T/LSA group compared to the T group (p<0.05). The levels of GSH (Figure 2c) and SOD (Figure 2d) activities significantly decreased in the muscle tissue of T compared to UT (p<0.05). The GSH (Figure 2c) and SOD (Figure 2d) activity levels in muscle tissue significantly increased in the T/LSA group compared to the T group (p<0.05). No significant difference was found in MDA (Figure 2a), MPO (Figure 2b), GSH (Figure 2c), and SOD (Figure 2d) levels between the UT group and T/LSA group (p>0.05).

DISCUSSION

Muscle damage can be induced through various mechanisms. Exercise with high metabolic demands or with high repetition rates, such as in cycling and marathon running, can induce ROS formation, which may be detrimental to muscular cell functions.[17-19] We have investigated the potential protective effects of LSA in oxidative damage of the muscle tissue caused by acute exercise in a rat model. Our findings show that LSA reduces oxidative stress in rats' muscle tissue subjected to acute exercise. LSA was used for the first time in a rat model of acute exercise. Therefore, the implications of these findings are important.

Muscle tissue contains many antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) to contrast free radicals damage.[20-22] In addition to, XO and MPO are the two main sources of extracellular free radicals during strenuous exercise and are also responsible for the tissues damage caused by exhaustive exercise.[2] Since an oxidative stress, during the exercise, can be caused by the increase of oxidant compounds and/or the antioxidant decrease.[20,23]

Silva *et al*. investigated that mitochondrial adaptations and oxidative damage after 4 and 8 weeks of running training in skeletal muscle of mice. They suggested that eight weeks of running training are necessary for increases in mitochondrial respiratory chain enzyme activities to occur, in association with decreased oxidative damage.[24]

Pasquini *et al*. evaluated that oxidative stress and recovery times in trained dogs during two different hunting exercises, with reactive oxygen metabolites-derivatives (d-ROMs) and biological antioxidant potential (BAP) tests. A 20-min aerobic exercise and a 4-h aerobic exercise, after 30 days of rest, were performed by the dogs. They result that showed an oxidative stress after exercise due to both the high concentration of oxidants (d-ROMs) and the low level of antioxidant power (BAP). Besides, the recovery time is faster after the 4-h aerobic exercise than the 20-min aerobic exercise. Oxidative stress monitoring during dogs exercise could become an interesting aid to establish ideal adaptation to training.[25]

Miyazaki *et al*. reported that intense endurance training can elevate antioxidant enzyme activities in erythrocytes and decreases neutrophils O_2 production in response to exhausting exercise. Therefore, this up-regulation of the antioxidant defense system would result in a reduction of lipid peroxidation in the erythrocyte membrane.[26]

LSA has been shown to possess strong effects on antioxidative and free radical scavenging, on protecting hepatitis, uremia, and on improving blood circulation.[5-7,27] In addition, LSA has shown to inhibit FBS-induced vascular smooth muscle cells (VSMC) proliferation and LPS-induced VSMC migration and an ameliorative effect on myocardial ischemia reperfusion.[28]

The current study, unlike previous studies, a 60-min aerobic exercise were performed by the rats. After acute exercise, oxidative damage in skeletal muscle was assessed after 15 min rest. The present experimental study, the post-exercise oxidative stress enzymes SOD, GSH, MPO and MDA were studied. The levels of MDA and MPO activity were significantly increased in the muscle tissue of the trained group compared to the untrained group. However, in T/LSA treated group significantly decreased muscle tissue MDA and MPO level. More importantly, in the LSA-treated trained group, muscle GSH and SOD levels were found to be preserved.

CONCLUSION

In the light of these results, our study indicates that the levels of oxidative activity were significantly decreased in the muscle tissue of the LSA-treated trained group, thus protecting from oxidative damage in skeletal muscle induced acute exercise in rats. In conclusion, it can be considered that LSA or LSA contained medicinal plants may be included in the composition of pharmaceutical/nutraceutical products against exercise-induced oxidative damage.

AUTHOR CONTRIBUTION STATEMENT

HÇ literature search, research concepts, design, data acquisition, data analysis, animal studies,
ESK extraction and isolation studies, manuscript writing, research concepts, design,
UÖ structure elucidation, manuscript writing, literature search.

*The authors declare that there are no conflicts of interest.

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