ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Research Article / Araştırma Makalesi DOI: 10.5152/VetSciPract.2022.220409

What is the Combined Effects of Ginkgo Biloba Extract (EGb761) and Acute Exhaustive Exercise on the Thiol-Disulfide Homeostasis in Rats?

Sıçanlardaki Tiyol-Disülfit Dengesi Üzerine Ginkgo Biloba Özü ve Akut Yorucu Egzersizin Birlikte Uygulanmasının Etkileri Nelerdir?

Ruhi KABAKÇI[1]
Yasemin ÇINAR[2]
Miyase ÇINAR[3]

[1]Department of Physiology, Faculty of Veterinary Medicine, Kırıkkale University, Kırıkkale, Turkey
[2]Department of Physical Medicine and Rehabilitation, Faculty of Medicine, Afyonkarahisar Health Sciences University, Afyonkarahisar, Turkey
[3]Department of Biochemistry, Faculty of Veterinary Medicine, Kırıkkale University, Kırıkkale, Turkey

* This study prepared from the findings of Master Thesis of Yasemin Çınar.

Received/Geliş Tarihi: 10.02.2022
Accepted/Kabul Tarihi: 17.08.2022
Publication Date/Yayın Tarihi: 12.10.2022

Corresponding author/Sorumlu Yazar: Ruhi KABAKÇI
E-mail: ruhikabakci@kku.edu.tr

Cite this article as: Kabakçi R, Çinar Y, Çinar M. What is the combined effects of ginkgo biloba extract (EGb761) and acute exhaustive exercise on the thiol-disulfide homeostasis in rats? *Vet Sci Pract*. 2022; 17(2), 35-40.

Atıf: Kabakçı R, Çınar Y, Çınar M. Sıçanlardaki tiyol-disülfit dengesi üzerine ginkgo biloba özü ve akut yorucu egzersizin birlikte uygulanmasının etkileri nelerdir? *Vet Sci Pract*. 2022; 17(2), 35-40.

ABSTRACT

This study aimed to evaluate the alone and combined effects of ginkgo biloba extract (EGb761) and acute exhaustive exercise on thiol-disulfide homeostasis (TDH) in male rats. Four experimental groups were formed with total 32 rats. Animals in the control and exercise groups received 2 mL/kg of 0.9% saline solution 5 days a week for 4 weeks, while the others in the ginkgo biloba and ginkgo biloba plus exercise groups received 100 mg/kg of EGb761 for the same duration. At the end of this period, rats in groups E and GB+E were made to run on a treadmill at 25 m/min at 5% incline until exhaustion. All plasma samples were evaluated for malondialdehyde (MDA) and nitric oxide (NO) levels, superoxide dismutase activity (SOD), and TDH. The findings of the study showed that exercise significantly increased MDA levels ($P < .001$) and SOD activity ($P < .05$) in group GB+E, while only MDA levels were decreased significantly ($P < .05$) in group GB. On the other hand, neither exercise nor EGb761 could affect the NO levels and the parameters related to TDH ($P > .05$). Although EGb761 can prevent oxidative stress by reducing MDA, its usage in exercise still needs to be further investigated, especially in terms of TDH that is a new important oxidative stress marker.

Keywords: Acute exercise, ginkgo biloba, oxidative stress, thiol-disulfide homeostasis

ÖZ

Bu çalışma Ginkgo Biloba özü (EGB761)'nün ve akut yorucu egzersizin tek ve birlikte uygulanmasının ratlardaki tiyol-disülfit homeostazı (TDH) üzerine etkilerini değerlendirmeyi amaçladı. Toplam 32 sıçan ile 4 deneysel grup oluşturuldu. Kontrol (C) ve egzersiz (E) gruplarındaki hayvanlara, 4 hafta boyunca haftada 5 gün 2 mL/kg %0.9 salin solüsyonu verilirken, Ginkgo Biloba (GB) ve Ginkgo Biloba artı Egzersiz (GB + E) gruplarındaki diğerlerine aynı süre zarfında 100 mg/kg EGB761 verildi. Bu dönemin sonunda, E ve GB+E gruplarındaki sıçanlar, %5 eğimde 25 m/dk hızda yoruluncaya kadar koşturuldu. Tüm plazma örnekleri malondyaldehit (MDA) ve nitrik oksit (NO) seviyeleri ve süperoksit dismutaz (SOD) aktivitesi ve TDH için değerlendirildi. Çalışmanın bulguları, egzersizin GB+E grubunda MDA seviyelerini ($P < ,001$) ve SOD aktivitesini ($P < ,05$) önemli ölçüde arttırırken, GB grubunda yalnızca MDA düzeylerinin önemli oranda azaldığını ($P < ,05$) göstermiştir. Öte yandan, ne egzersiz ne de EGB761 uygulaması NO seviyesini ve TDH ile ilgili parametreleri etkilemiştir ($P > ,05$). EGB761, MDA'yı azaltarak oksidatif stresi önleyebilse de, egzersizdeki kullanımının, özellikle yeni bir oksidatif stres belirteci olan TDH açısından ilerde incelenmesi gerekmektir.

Anahtar Kelimeler: Akut egzersiz, ginkgo biloba, oksidatif stres, tiyol-disülfür homeostaz

INTRODUCTION

Living organisms need nutrients such as carbohydrates, fats, and proteins to maintain their survival functions, in which energy stores are released by oxygen. Although oxygen is required for many physiological functions in the body, those kinds of metabolic events may cause free radicals (FR) or reactive

oxygen species (ROS) formation, as oxygen is a common electron acceptor.[1] Active oxygens derived from FR are also called oxidants which may change the structures or functions of molecules in the target cells, resulting in cell damage due to the disturbance in ribonucleic acid, deoxyribonucleic acid, and diverse enzymatic events.[2] On the other hand, there are various antioxidants, a kind of defense system, that save the cells against the adverse effects of oxidants in the body, which are usually in the form of enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) or non-enzymatic antioxidants such as some vitamins, minerals, and carotenoids. The impairment of the oxidant–antioxidant balance is considered oxidative stress (OS).[3,4]

Thiols, which contain alcohol-derived sulfhydryl groups connected to carbon atoms, are a pool of organic molecules consisting of albumin and protein thiols and other low-molecular-weight thiols like glutathione, cysteine, and homocysteine.[5] They play an antioxidative role in order to preserve the organism from the harmful effects of oxidants. In the case of OS, these compounds are oxidized via oxidants to covalent disulfide bonds. These reversible bonds may be reduced back to thiols. Therefore, there is dynamic thiol-disulfide homeostasis (TDH) between them, which indicates the oxidative status.[6] Recently, a novel method developed by Erel and Neselioglu[7] is commonly used to measure TDH colorimetrically.

During exercise, the production of FR and ROS is increased in the skeletal muscle due to enhanced physical activity and oxygen consumption.[8] Although it is necessary for many kinds of cellular metabolism and functions such as signaling, gene expression, ion transportation, or apoptosis, large amounts of ROS production may lead to adverse effects on lipids, proteins, nucleotides, and many other cellular molecules.[9] It has been confirmed that exercise leads to OS in the body, depending on the exercise type, duration, or intensity.[10] Liu, Zhou[11] showed that ROS level in plantaris muscles of adult rats was increased following acute exhaustive treadmill exercise. It may also increase malondialdehyde (MDA) levels in serum and several other tissues.[4,12-14] Exercise may also be reported to affect the plasma TDH in rodents[15,16] and humans in few studies.[17] Furthermore, it has been reported in some experimental studies that irregular acute exhaustive exercise reduced the antioxidant activities of serum GPX, Taysi, Oztasan,[4] heart SOD, GRD, and GPX,[18] and kidney SOD and NOS[13] in rats.

Ginkgo biloba is a well-known aboriginal tree in China. It has been used in traditional medicine for thousand years due to its beneficial effects on health. The standardized leaf extract of ginkgo biloba (EGb761) is also commonly used worldwide for its antioxidative, anti-inflammatory, and immune-modulatory features, as well as for its low cost and negligible side effects.[19,20] Ginkgo biloba extract acts as antioxidant by 2 mechanism: one of them is directly scavenging FR and the other is indirectly preventing FR formation. While EGb761 can scavenge many ROS, it can also increase the activities of different antioxidants such as SOD, CAT, and GPX.[21] Atashak[22] demonstrated that although acute aerobic exercise increased MDA levels thus resulting in OS, short-term EGb761 supplementation could decrease the exercise-induced OS by increasing total antioxidant capacity in inactive women. It has been also reported that treatment with EGb761 to physically active men during exercise increased blood non-enzymatic antioxidant capacity and aerobic performance.[23] On the other hand, Wang, Zhou[31] reported that there are no favorable effects between exercised and exercise plus EGb761 supplemented patients with peripheral arterial disease. In addition, Sadeghinejad, Soltani[25] have suggested consuming EGb761 with caution in young athletes due to the reducing effects on neurotrophins in the brain and capacities of learning and memory in exercised rats. There are many other studies investigating its relationship to neural, cardiovascular, or behavioral systems.[25-27] However, there are still limited data on the effects of EGb761 on OS status in exercise. Therefore, the current study was designed to investigate the effects of EGb761 on OS in terms of TDH in acutely exhaustive exercised rats.

MATERIAL AND METHODS

The protocol of this study was approved by the Local Ethical Committee of Kırıkkale University (Date: March 27, 2020, Decision no: 2020/02-11). A total of 32 male Wistar Albino rats (2-3 months old) were purchased from the Gulhane Laboratory Laboratory Animals Production and Research Center, Ankara, Turkey, and were housed in a plastic standard cage at temperature between 22°C and 25°C and humidity between 55% and 60% with 12 hours : 12 hours light–dark cycle. All the animals were fed with standard dry pellet rat food and water *ad libitum* throughout the study. Following 2 weeks of acclimation before the experiments, rats were randomly divided into 4 groups: control (C), ginkgo biloba (GB), exercise (E), and ginkgo biloba + exercise (GB+E) with 8 rats in each group.

The standardized ginkgo biloba (EGb761) tablets (Tebokan intens, Abdi İbrahim, Turkey) were crushed and dissolved in 0.9% saline solution (SS) at the concentration of 100 mg/2 mL via vortex. The rats of groups C and E were orally gavaged 2 mL kg/body weight (b.w.) of 0.9% SS for 5 days/week for 4 weeks, while the rats of groups GB and GB+E were orally gavaged 100 mg kg/b.w. EGb761 in 2 mL of 0.9% SS for the same duration. All the applications were performed between 09:00 AM and 12:00 PM during the experiment.

Acute exhaustive exercise (AEE) for rats was set up according to our previous protocol.[28] The animals in groups E and GB+E were familiarized with the 5-lane rodent treadmill (MAY, TME 0805, Missouri, USA) by completing 10-minute running sets for 3 days a week during the drug administration period (4 weeks). For this purpose, rats were run 10, 15, 20, and 25 min/day for 3 days in the first, second, third, and fourth weeks, respectively. While rats were running at the speed of 25 m/min and 5% incline under the induction of electrical shock, they were considered exhausted in case if it touches the electrified grid at the back side of treadmill 5 times within 2 minutes.[14] The exhaustion time for the exercised rats was recorded and it varied between 70 and 127 minutes. The rats in the C and GB groups were not forced to exercise. They only received drugs (0.9% SS or EGb761, respectively) and were put in cages with the standard conditions until surgery.

On the last day of the experiment, all the rats in the exercised groups (E and GB+E) and unexercised groups (C and GB) were killed by cardiac puncture following the ketamine/xylazine (90/10 mg/kg) anesthesia and whole blood was collected. Blood samples collected into heparinized tubes were centrifuged at 3000 rpm at 4°C for 10 minutes to obtain plasma which was used for further biochemical analysis. In the plasma samples, MDA levels were measured according to the protocol of Buege

and Aust[29] and nitric oxide (NO) levels were determined by the method of Miranda, Espey[24] with some modification, while SOD levels in plasma were detected using commercial assay kit (Rel Assay Diagnostic kits, Gaziantep, Turkey) according to the manufacturer's guidelines.

Furthermore, TDH in plasma samples was analyzed by a new colorimetric method established by Erel and Neselioglu.[7] In brief, total thiol (TT) and native thiol (NT) levels of plasma samples were determined spectrophotometrically via auto-analyzer (Mindray BS400, Shenzhen, China). Firstly, NT groups were measured by an Ellman's reagent. Secondly, dynamic disulfide (DD) bonds in the plasma were reduced by using sodium borohydride (NaBH4) to form the free functional thiol groups that provide the TT level together with NT. Total thiol levels were measured by the reaction of 5,5′-dithiobis-2-nitrobenzoic acid (DTNB). Then, residuals of reductant NaBH4 were consumed and removed completely by formaldehyde to stop extra-reduction of DTNB and further reduction of disulfide bonds formed after the DTNB reaction. The concentration of DD bonds was calculated as half of the difference obtained by subtracting NT from TT, since the reduction of a disulfide bond generates 2 distinct thiol groups. Additionally, the percent ratios of DD/TT, DD/NT, and NT/TT were calculated by using previously measured amounts of TT, NT, and disulfide.

Statistical Analyses

All the data obtained from the present study were expressed as mean ± standard error of mean ($\bar{x} \pm$ SEM). Descriptive and statistical analyses of the results were performed using Statistical Package for the Social Sciences version 18.0 (SPSS Inc., Chicago, Ill, USA) package program for Windows.

The normality of all data was checked by Shapiro–Wilk test. The exhaustion times of the rats in groups E and GB+E were compared using Student's *t*-test. One-way analysis of variance (ANOVA) was used for the statistical analysis of normally distributed data of the OS parameters obtained from all experimental groups, while Kruskal–Wallis test was performed for nonparametric data. Multiple comparisons between groups were performed with Duncan test. *P* values less than .05 were considered statistically significant.

RESULTS

The rats in groups E and GB+E were run at the speed of 25 m/min at 5% incline until exhaustion. The exhaustion times were 101.3 ± 5.5 and 101.9 ± 20.3 minutes for groups E and GB+E, respectively, and were not significantly different between acute exercise groups ($P > .05$), according to statistical analysis.

Acute exhaustive exercise significantly increased the plasma MDA levels of rats in group E compared to group C ($P < .001$). Although the animals in group GB have the lowest level of MDA which is significantly different from other experimental groups ($P < .001$), supplemental EGb761 to exercised rats in the group GB+E did not decrease MDA levels which were statistically similar level with group E ($P > .05$) but significantly higher than that of groups C and GB ($P < .001$). Plasma NO levels of animals in each group were not affected by exercise or EGb761 treatment ($P > .05$). The lowest SOD activity was measured in the group C which was statistically similar to group GB, while the highest SOD activity was measured in group GB+E that was not different from the group E but was significantly ($P < .05$) higher compared to groups C and GB (Table 1).

Table 1. Plasma Oxidant and Antioxidant Levels in Ginkgo Biloba Treated and Exercised Rats

	Group C (n = 8)	Group GB (n = 8)	Group E (n = 8)	Group GB+E (n = 8)	
	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE	*P*
MDA (μmol/L)	1.41 ± 0.08^{b}	0.54 ± 0.16^{c}	1.91 ± 0.14^{a}	1.86 ± 0.19^{a}	<.001
NO (μmol/L)	19.53 ± 1.23	17.06 ± 1.39	22.02 ± 1.97	20.94 ± 0.87	>.05
SOD (U/mL)	138.63 ± 66.20^{b}	144.71 ± 54.70^{b}	159.13 ± 56.26^{a}	161.75 ± 57.19^{a}	<.05

MDA, malondialdehyde; NO, nitric oxide; SOD, superoxide dismutase; x̄, mean; SE, standard error of mean.

After the measurement of TDH parameters via new colorimetric method, the ratios of DD/TT (%), DD/NT (%), and NT/TT (%) were calculated using the previously measured TDH parameters. Nonparametric data of DD and DD/TT (%) were analyzed using Kurskal–Wallis test, while ANOVA was used for parametric data of TT, NT, DD/NT (%), and NT/TT (%) of all groups.

As shown in Table 2, there were no statistical differences in the parameters of TDH between the groups ($P > .05$). Lowest concentration of DD indicates that the OS was in group C, and highest concentration of DD was in group E; however, the differences among them were not significant ($P > .05$). There were also non-significant ($P > .05$) alterations in the ratios of DD/TT and DD/NT, and NT/TT, which were highest in group E and group C, respectively.

DISCUSSION

During the last few decades, exercise has been recommended to save people from health problems caused by an increasing inactive lifestyle along with the technological and other industrial developments that make life easier. However, excessive exercise may lead to harmful effects on health status such as OS. This study sought to investigate the effects of EGb761 administration on plasma OS status, especially in terms of TDH in rats with acutely exhaustive exercise.

In the present study, there was no statistical significance between exhaustion times of exercised groups (E and GB+E). Our results were highly in agreement with the findings of the study conducted on rats by Acikgoz, Aksu.[30] Similarly, Liu, Yeo[14] showed that exhaustion times varied, ranging from 20 to 110 minutes for acute running exercise in rats. Moreover, the administration of EGb761 in this study did not extend the exhaustion times of rats in group GB+E. Wang, Zhou[31] have also reported that ginkgo biloba treatment in the exercise training group did not prove more favorable effects compared to the group that exercised alone.

It has been well defined in many studies that moderate exercise can maintain a healthy oxidant status by increasing antioxidant activity and even overproduction of ROS. This situation can easily

Table 2. Plasma Thiol-Disulfide Homeostasis Parameters in Ginkgo Biloba Treated and Exercised Rats

	Group C (n = 8)	Group GB (n = 8)	Group E (n = 8)	Group GB+E (n = 8)	
	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE	$\bar{x} \pm$ SE	*P*
TT (μmol/L)	247.43 ± 93.52	249.43 ± 94.28	225.75 ± 79.81	238.00 ± 84.15	>.05
NT (μmol/L)	150.71 ± 56.96	137.17 ± 52.05	101.00 ± 35.71	$128.1\,3 \pm 45.30$	>.05
DD (μmol/L)	48.36 ± 18.28	55.85 ± 21.11	62.38 ± 22.05	54.94 ± 19.42	>.05
DD/TT (%)	33.23 ± 12.56	42.48 ± 16.06	83.70 ± 29.59	51.26 ± 18.12	>.05
DD/NT (%)	19.63 ± 7.42	21.76 ± 8.22	26.56 ± 9.39	23.52 ± 8.32	>.05
NT/TT (%)	60.75 ± 22.96	56.48 ± 21.35	46.87 ± 16.57	52.96 ± 18.73	>.05

DD, dynamic disulfide; NT, native thiol; SE, standard error of mean; TT, total thiol; x̄, mean.

be affected by the time, duration, or intensity of exercise. Kayacan, Cetinkaya[15] have reported that moderate-intensity exercise is more effective in reducing OS than low- and high-intensity exercise. In another study, although low- and high-intensity exercise could not change the plasma protein carbonyl (PC) and total antioxidant capacity (TAC), incremental exercise significantly increased PC and TAC levels in the plasma.[32] However, acute or intensive exercise leads to excessive ROS production over the balancing capacity of antioxidant systems in the body, which results in OS. According to findings of this study on plasma OS biomarkers, AEE markedly ($P < .001$) increased MDA levels of rats in group E. Similar results have been reported in some previous studies that acute exhaustive treadmill exercise set for 18-35 m/min at 0%-20% incline range enhanced the MDA levels in the serum,[12] kidney,[13] and liver[4,14] tissues of adult rats. On the other hand, Gul, Demircan[18] showed that cardiac MDA levels did not change after AEE on the treadmill for 35 m/min at 10% incline. In addition, it has been reported in rats that there were no statistical differences in thiobarbituric acid reactive substances (TBARS) levels of plasma[32] and different parts of brain[30] between C and E groups. These reports are contradictory to previous reports that oxygen consumption and ROS production are increased during acute exercise, in which lipid peroxidation occurs with elevated MDA levels.[10,33,34] Interestingly, administration of EGb761 to rats in group GB+E could not reduce the increased MDA levels by exercise, while the lowest level of MDA was observed in group GB in this study. In addition, although it has been reported that strenuous exercise or EGb761 application increased the release of NO and inhibited the degradation of NO in blood endothelium[35] or liver, spleen, and bone marrow cells,[36] exercise, EGb761 treatment, or combination of them did not affect the plasma NO level of rats in each group of present study.

Ginkgo biloba is a native tree whose leaves are used in traditional health therapy for years in China and even all over the world.[19,37] It belongs to herbal supplements with rich polyphenols such as quercetin, hesperidin, and epigallocatechin-3-gallate, and its major active ingredients are flavonoids and terpenoids.[23] These phenolic supplements are able to improve antioxidant defense system while reducing ROS production.[38] It has been reported that quercetin attenuated exercise-induced OS by decreasing MDA level and increasing antioxidant enzyme activities.[39,40] Estruel-Amades, Massot-Cladera[38] also showed that hesperidin, another flavanone glycoside, inhibited an increase in ROS production and a decrease in SOD or CAT activities resulting from exhausting exercise in intensively trained rats. Previous studies have revealed that standardized EGb761 decreased MDA levels in rat brain and mice liver with endurance exercise.[37] EGb761 was also used for its antioxidative properties, which were able to increase the activities of SOD, CAT, GPX, and glutathione reductase (GR) in several regions of rat brain.[41,42] Zhou, Yu[20] showed that EGb761 application reduced the elevated MDA levels and increased SOD activity in colitis-induced rats. It has also suppressed the doxorubicin-induced toxicity in rat testes by decreasing lipid peroxidation and increasing SOD and GPX activities and/or glutathione level.[43] However, exercise and EGb761 treatment plus exercise significantly increased the plasma SOD activity in rats compared to sedentary group rather than the usage of EGb761 alone in the present study. These findings are compatible with the results of Bing and Zhaobao's[37] study, in which endurance exercise and EGb761 administration increased SOD activity in mice liver. Moreover, treatment of mice with lemon peel flavonoids enhanced the activities of SOD and CAT and reduced MDA levels induced by acute exhaustive swimming exercise.[44] On the other hand, in another study, 6-week EGb761 supplementation and exercise did not change the enzymatic antioxidant capacities of SOD, CAT, and GR in volunteers, except for GPX activity which was decreased after 1 hour of second trial.[23] As understood above, even though the antioxidative effects of EGb761 were well defined in many cases such as disorders and toxicities, its usage during exercise remains unclear.

Thiols, which serve as non-enzymatic antioxidants in the plasma, reversibly transform disulfides by oxidation via oxidants. Thiol-disulfide homeostasis, as a novel biomarker, gives information about exogenous and endogenous-induced OS in the biologic systems.[6,45] According to our findings, the concentrations of TT and NT were significantly lower in the exercise group than in the other groups. In addition, EGb761 treatment did not cause statistical changes in the concentrations of neither TT nor NT in group GB+E. Although it is not significant, DD concentration and DD/TT and DD/NT percentage ratios were highest in group E according to other groups in this study, which indicate that acute exercise may induce OS. The administration of EGb761 was able to slightly decrease disulfide-related parameters in group GB+E. Studies on the alterations of TDH in exercise are very limited, and the present studies in the literature offer contradictory reports. For example, Kayacan, Yazar[16] have indicated that 10-week exercise, a kind of regular exercise, did not change the TT and DD concentrations and percentages of reduced thiol, oxidized thiol, and thiol oxidation–reduction, except NT concentration in rats. It has also been reported that 60-min walking exercise applied to obese individuals for 12 weeks did not affect any parameters of thiol-disulfide.[17] In contrast, Gol, Özkaya[46] have revealed that thiol and disulfide levels were markedly increased in people with regular exercise compared to sedentary. In another study conducted on rats, unlike low- and high-intensity exercise, only medium-intensity exercise decreased the TT and DD concentrations.[15] In the present study, the differences seen in all the measured parameters related to TDH, which is the premarker of radical-mediated protein oxidation, with AEE and/or EGb761 treatment were not statistically significant compared to control group. This likely resulted from the protocol of applied exercise not leading to protein oxidation to affect the TDH. Our previous findings[28] may support this inference, in which albumin and total protein levels were not altered by AEE, while glucose and triglyceride were decreased.

In this study, we have demonstrated that AEE increased the MDA level and SOD activity in rats. In addition, although the usage of EGb761 significantly decreased MDA levels compared to other groups, EGb761 combined with exercise significantly enhanced the concentration of MDA and SOD activity in group GB+E. However, neither exercise nor EGb761 and their combination altered the values of NO and the other parameters related to TDH.

There is no doubt that acute, intense, or irregular exercise may lead to OS, and TDH plays an important role in the regulation of OS by enzymatic reactions, antioxidant systems, detoxification, or apoptosis.[45] Additionally, EGb761 has antioxidative effects and other beneficial properties. However, further experimental studies are still required to elucidate how TDH changes after exercise and possible protective effects of EGb761 on exercise-induced OS.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Kırıkkale University (Date: March 27, 2020, Decision No: 2020/02-11).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - R.K., Y.Ç.; Design - R.K., M.Ç.; Supervision - R.K.; Data Collection and/or Processing - R.K., Y.Ç.; Analysis and/or Interpretation - R.K., Y.Ç., M.Ç.; Literature Search - R.K., Y.Ç., M.Ç.; Writing Manuscript - R.K., Y.Ç.; Critical Review - R.K., M.Ç.

Acknowledgments: The authors also thank Research Assistant Ali ŞENOL for his kind support during experimental processes, and Kendra Clark for her kind support in English editing of our manuscript.

Declaration of Interests: The authors have no conflicts of interest to declare.

Funding: This study was supported by the Kırıkkale University Scientific Research Projects Coordination Unite (project number: 2020/057).

Etik Komite Onayı: Bu çalışma için gerekli etik izin Kırıkkale Üniversitesi Etik Kurulundan alınmıştır. (Tarih: 10 Şubat 2022, Karar No: 2020/02-11).

Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir - R.K., Y.Ç.; Tasarım - R.K., Y.Ç.; Denetleme - R.K.; Veri Toplanmasıve/veya İşlemesi - R.K., Y.Ç.; Analiz ve/veya Yorum - R.K., Y.Ç., M.Ç.; Literatür Taraması - R.K., Y.Ç., M.Ç.; Yazıyı Yazan - R.K., Y.Ç.; Eleştirel İnceleme - R.K., Y.Ç.

Teşekkür: Yazarlar deneysel süreçlerdeki nazik desteği için Araştırma Görevlisi Ali ŞENOL'a ve İngilizce redaksiyonundaki nazik desteği için Kendra Clark'a teşekkür eder.

Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Finansal Destek: Bu çalışma Kırıkkale Üniversitesi Bilimsel Araştırma Projeleri Koordinasyon Birimi tarafından desteklenmiştir (proje numarası: 2020/057).

REFERENCES

1. Kohen R, Nyska A. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicol Pathol*. 2002;30(6):620-650. [CrossRef]
2. Vujčić V, Radić Brkanac S, Radojčić Redovniković I, et al. Phytochemical and bioactive potential of in vivo and in vitro grown plants of Centaurea ragusina L.–detection of DNA/RNA active compounds in plant extracts via thermal denaturation and circular dichroism. *Phytochem Anal*. 2017;28(6):584-592. [CrossRef]
3. Halliwell B. Antioxidant defence mechanisms: from the beginning to the end (of the beginning). *Free Radic Res*. 1999;31(4):261-272. [CrossRef]
4. Taysi S, Oztasan N, Efe H, et al. Endurance training attenuates the oxidative stress due to acute exhaustive exercise in rat liver. *Acta Physiol Hung*. 2008;95(4):337-347. [CrossRef]
5. Turell L, Radi R, Alvarez B. The thiol pool in human plasma: the central contribution of albumin to redox processes. *Free Radic Biol Med*. 2013;65:244-253. [CrossRef]
6. Jones DP, Liang Y. Measuring the poise of thiol/disulfide couples in vivo. *Free Radic Biol Med*. 2009;47(10):1329-1338. [CrossRef]
7. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. *Clin Biochem*. 2014;47(18):326-332. [CrossRef]
8. Radak Z, Zhao Z, Koltai E, Ohno H, Atalay M. Oxygen consumption and usage during physical exercise: the balance between oxidative stress and ROS-dependent adaptive signaling. *Antioxid Redox Signal*. 2013;18(10):1208-1246. [CrossRef]
9. Waris G, Ahsan H. Reactive oxygen species: role in the development of cancer and various chronic conditions. *J Carcinog*. 2006;5:14. [CrossRef]
10. Ji LL. Antioxidants and oxidative stress in exercise. *Proc Soc Exp Biol Med*. 1999;222(3):283-292. [CrossRef]
11. Liu J, Zhou G, Mei Y, Xie WJ, Li PF, Yang F. Mechanism of oxidative stress in skeletal muscle of rats induced by acute exhaustive exercise. *Zhongguo Ying Yong Sheng Li Xue Za Zhi*. 2020;36(1):17-22. [CrossRef]
12. Liu W, Luo R, Tang C, Zhao X, Zeng S. Serumal oxidative stress status of acute exhaustive exercise rats following sleep deprivation. *Chin J Tissue Eng Res*. 2007;11:7710-7713.
13. Lin X, Qu S, Hu M, Jiang C. Protective effect of erythropoietin on renal injury induced by acute exhaustive exercise in the rat. *Int J Sports Med*. 2010;31(12):847-853. [CrossRef]
14. Liu J, Yeo HC, Overvik-Douki E, et al. Chronically and acutely exercised rats: biomarkers of oxidative stress and endogenous antioxidants. *J Appl Physiol (1985)*. 2000;89(1):21-28. [CrossRef]
15. Kayacan Y, Çetinkaya A, Yazar H, Makaracı Y. Oxidative stress response to different exercise intensity with an automated assay: thiol/disulphide homeostasis. *Arch Physiol Biochem*. 2021;127(6):504-508. [CrossRef]
16. Kayacan Y, Yazar H, Cerit G, Ghojebeigloo BE. A new oxidative stress indicator: effect of 5-hydroxytryptophan on thiol-disulfide homeostasis in exercise. *Nutrition*. 2019;63-64:114-119. [CrossRef]
17. Celik H, Kilic T, Kaplan DS, et al. The effect of newly initiated exercise training on dynamic thiol/disulphide homeostasis in sedentary obese adults. *An Acad Bras Cienc*. 2019;91(4):e20180930. [CrossRef]
18. Gul M, Demircan B, Taysi S, et al. Effects of endurance training and acute exhaustive exercise on antioxidant defense mechanisms in rat heart. *Comp Biochem Physiol A Mol Integr Physiol*. 2006;143(2):239-245. [CrossRef]
19. Shinozuka K, Umegaki K, Kubota Y, et al. Feeding of Ginkgo biloba extract (GBE) enhances gene expression of hepatic cytochrome P-450 and attenuates the hypotensive effect of nicardipine in rats. *Life Sci*. 2002;70(23):2783-2792. [CrossRef]
20. Zhou YH, Yu JP, Liu YF, et al. Effects of Ginkgo biloba extract on inflammatory mediators (SOD, MDA, TNF-α, NF-κBp65, IL-6) in TNBS-induced colitis in rats. *Mediators Inflamm*. 2006;2006:1-9.
21. Mahadevan S, Park Y. Multifaceted therapeutic benefits of Ginkgo biloba L.: chemistry, efficacy, safety, and uses. *J Food Sci*. 2008;73(1):R14-R19. [CrossRef]
22. Atashak S. Effect of Ginkgo biloba L. on total antioxidant capacity and malondialdehyde after aerobic exercise in inactive women. *Iran J Med Aromat Plants*. 2018;34:601-611.
23. Sadowska-Krępa E, Kłapcińska B, Pokora I, Domaszewski P, Kempa K, Podgórski T. Effects of six-week Ginkgo biloba supplementation on aerobic performance, blood pro/antioxidant balance, and serum brain-derived neurotrophic factor in physically active men. *Nutrients*. 2017;9(8):803-904. [CrossRef]
24. Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide*. 2001;5(1):62-71. [CrossRef]
25. Sadeghinejad M, Soltani Z, Afzalpour ME, Khaksari M, Pourranjbar M. What is the combined effect of intense intermittent exercise and Ginkgo biloba plant on the brain neurotrophic factors levels, and learning and memory in young rats? *Pharmacol Rep*. 2019;71(3):503-508. [CrossRef]
26. Oken BS, Storzbach DM, Kaye JA. The efficacy of Ginkgo biloba on cognitive function in Alzheimer disease. *Arch Neurol*. 1998;55(11):1409-1415. [CrossRef]
27. Sierpina VS, Wollschlaeger B, Blumenthal M. Gingko biloba. *Am Fam Phys*. 2003;68(5):923-926.
28. Cinar Y, Kabakci R, Senol A. Effect of Ginkgo biloba extract (Egb761) supplementation on hemato-biochemical parameters following acute treadmill exercise in rats. *Fırat Univ Sağlık Bilimleri Vet Derg*. 2021;35:98-102.
29. Buege JA, Aust SD. Microsomal lipid peroxidation. In: *Methods Enzymol*. 1st ed Abelson J., Simon M., Verdine GL., Pyle AM, eds. USA: Academic Press. 1978;52:302-310. [CrossRef]

30. Acikgoz O, Aksu I, Topcu A, Kayatekin BM. Acute exhaustive exercise does not alter lipid peroxidation levels and antioxidant enzyme activities in rat hippocampus, prefrontal cortex and striatum. *Neurosci Lett*. 2006;406(1-2):148-151. [CrossRef]
31. Wang J, Zhou S, Bronks R, Graham J, Myers S. Supervised exercise training combined with ginkgo biloba treatment for patients with peripheral arterial disease. *Clin Rehabil*. 2007;21(7):579-586. [CrossRef]
32. Kawamura T, Fujii R, Li X, Higashida K, Muraoka I. Effects of Exhaustive exercises, with different intensities, on oxidative stress markers in rat plasma and skeletal muscle. *Sci Sports*. 2018;33(3):169-175. [CrossRef]
33. Davies KJ, Quintanilha AT, Brooks GA, Packer L. Free radicals and tissue damage produced by exercise. *Biochem Biophys Res Commun*. 1982;107(4):1198-1205. [CrossRef]
34. Vincent HK, Morgan JW, Vincent KR. Obesity exacerbates oxidative stress levels after acute exercise. *Med Sci Sports Exerc*. 2004; 36(5):772-779. [CrossRef]
35. Mehlsen J, Drabaek H, Wiinberg N, Winther K. Effects of a Ginkgo biloba extract on forearm haemodynamics in healthy volunteers. *Clin Physiol Funct Imaging*. 2002;22(6):375-378. [CrossRef]
36. Qian ZM, Xiao DS, Ke Y, Liao QK. Increased nitric oxide is one of the causes of changes of iron metabolism in strenuously exercised rats. *Am J Physiol Regul Integr Comp Physiol*. 2001;280(3):R739-R743. [CrossRef]
37. Bing Y, Zhaobao W. Effects of Ginkgo biloba extract on free radical metabolism of liver in mice during endurance exercise. *Afr J Tradit Complement Altern Med*. 2010;7(4):291-295. [CrossRef]
38. Estruel-Amades S, Massot-Cladera M, Garcia-Cerdà P, et al. Protective effect of hesperidin on the oxidative stress induced by an exhausting exercise in intensively trained rats. *Nutrients*. 2019;11(4): 783. [CrossRef]
39. Gergerlioglu HS, Gokbel H, Okudan N, Gergerlioglu N, Demir EA. Quercetin and caffeic acid phenethyl ester (CAPE) attenuate acute exercise-induced oxidative stress. *Prog Nutr*. 2015;17:41-49.
40. Leelayuwat, S. Laddawan, Y. Kanpetta, et al. Quercetin enhances endurance capacity via antioxidant activity and size of muscle fibre type 1. *J Pharm Nutr Sci*. 2012;2(2):160-164. [CrossRef]
41. Bridi R, Crossetti FP, Steffen VM, Henriques AT. The antioxidant activity of standardized extract of Ginkgo biloba (EGb 761) in rats. *Phytother Res*. 2001;15(5):449-451. [CrossRef]
42. Naik SR, Pilgaonkar VW, Panda VS. Evaluation of antioxidant activity of Ginkgo biloba phytosomes in rat brain. *Phytother Res*. 2006; 20(11):1013-1016. [CrossRef]
43. Yeh YC, Liu TJ, Wang LC, et al. A standardized extract of Ginkgo biloba suppresses doxorubicin-induced oxidative stress and p53-mediated mitochondrial apoptosis in rat testes. *Br J Pharmacol*. 2009;156(1): 48-61. [CrossRef]
44. Bao G, Zhang Y, Yang X. Effect of lemon peel flavonoids on anti-fatigue and anti-oxidation capacities of exhaustive exercise mice. *Appl Biol Chem*. 2020;63:1-11.
45. Şimşek E, Erel O, Bicer CK, Çarlıoğlu A. A novel method for determining the relation between nasal polyposis and oxidative stress: the thiol/disulphide homeostasis. *Acta Otolaryngol*. 2016;136(11):1180-1183. [CrossRef]
46. Gol M, Özkaya B, Yildirim C, Bal R. Regular exercise, overweight/obesity and sedentary lifestyle cause adaptive changes in thiol–disulfide homeostasis. *An Acad Bras Cienc*. 2019;91(2):e20180547. [CrossRef]