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Effects of Spirulina on Some Oxidative Stress Parameters and Endurance Capacity in Regular and Strenuous Exercises

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Abstract

Objective: We examined the effects of spirulina supplementation on oxidative status in plasma, liver and muscle tissue and endurance capacity in moderate and exhaustive swimming exercise in rats.

Method: Animals were divided into six groups: control, spirulina (SP), chronic exercise (CE), chronic exercise with spirulina (CES), exhaustive exercise (E), and exhaustive exercise with spirulina (ES). Spirulina was administered orally to rats in the SP, CES and ES groups at a dose of 750 mg/kg per day for 6 weeks. The chronic exercise groups underwent swimming exercise for 1 hour/day for 6 weeks. Animals from groups E and ES were subject to exhaustive exercise stress. Creatine kinase (CK), CK-MB, lactate dehydrogenase activities, and uric acid levels were determined in the plasma, whereas malondialdehyde levels and MPO, XO, SOD, CAT, GPx and antioxidant activities were measured in plasma, liver and muscle tissues.

Results: Spirulina supplementation attenuated the increase in plasma CK activity induced by exhaustive exercise. Although chronic exercise increased plasma SOD activity, it promoted decreases in liver tissue XO activities and MDA levels as well as muscle tissue MDA levels. Exhaustive exercise reduced liver CAT activities, whereas plasma CAT activities increased. Spirulina supplementation had no effect on endurance capacity in a single session of exhaustive swimming exercise.

Conclusion: We concluded that spirulina platensis, ameliorates increases in the plasma activities of CK, probably by decreasing pre-oxidative MDA levels in skeletal muscle.

KeyWords: Exercise, oxidative stress, antioxidant status, spirulina, muscle damage, endurance capacity

1. Introduction

The production of reactive oxygen species (ROS) is an inevitable result of cellular metabolism. Organisms protect themselves against the harmful effects of ROS via the "antioxidant defense" system that serves to scavenge free radicals. The defense system utilizes several enzymatic and nonenzymatic antioxidant modalities such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), reduced glutathione (GSH), vitamin E, and vitamin C. In stressful situations, ROS production increases with a concomitant decrease in the efficacy of the antioxidant defense system, resulting in "oxidative stress," which is defined as a disturbance of the balance between prooxidative and antioxidative processes in favor of prooxidation (Fisher-Wellman and Bloomer, 2009). Intense physical exercise is a factor that provokes oxidative stress. Exhaustive physical exercise, which dramatically increases oxygen consumption, escalates free radical production, resulting in the lipid peroxidation of polyunsaturated fatty acids in cellular membranes, and reduces antioxidant activities in blood and various tissues (Minato *et al*., 2003; Chang *et al*., 2007; Choi and Cho, 2009;). Malondialdehyde (MDA) and thiobarbituric acid-reactive substances (TBARS) are widely used indicators of exercise-induced tissue injury (Fisher-Wellman and Bloomer, 2009). Exhaustive exercise also leads to increments of plasma and skeletal muscle xanthine oxidase (XO) and myeloperoxidase (MPO) activities (Liu *et al*., 2005). Although a single session of exhaustive exercise can generate excessive amounts of ROS due to intense oxygen consumption (Davies *et al*., 1982), regular exercise has numerous health benefits including reduced risks of cancer, diabetes, and cardiovascular disease (Blair *et al*., 2001; Powers and Jackson, 2008). The antioxidant defense capacity of sedentary people can be easily over burdened under conditions such as acute physical exercise; therefore, the consumption of products containing exogen antioxidants (i.e. foods or supplements) may help sedentary individuals manage oxidative stress during exercise (Bucioli *et al*., 2011).

Spirulina is a spiral-shaped microscopic blue–green alga that lives in both freshwater and saltwater and contains approximately 60%–70% protein. It is widely used as a human and animal supplement because of its high-grade protein, iron, gamma-linolenic acid, carotenoid, vitamin B1, vitamin B2, vitamin B12, provitamin A (beta-carotene), vitamin C, vitamin E, selenium, C-phycocyanin, and flavonoid content. In addition, dietary supplementation of spirulina has been reported to attenuate exercise-induced muscle injury. In a study of spirulina supplementation in 16 college students for 3 weeks, Lu *et al.* (2006) found that spirulina is protective against

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exhaustive exercise-induced skeletal muscle damage, probably by decreasing prooxidative activity. Kalafati *et al*. (2010) administered 6 g/day spirulina to nine moderately trained male athletes and reported that this supplementation boosted fat oxidation and exercise performance while decreasing lipid peroxidation. Spirulina supplementation improves antioxidant capacity and inflammation markers and decreases lipid peroxidation in rats subjected to strength training (Brito *et al*., 2020). It has healing effects on intense exercise-induced muscle damage and inflammation in elite rugby players (Chaouachi *et al*., 2022) and increases the amount of hemoglobin in submaximal exercise with arm cycling. It also improves oxygen consumption and heart rate during exercise (Gurney and Spendiff, 2020), and spirulina supplementation improves plasma lipid profile (Hernández-Lepe *et al*., 2019b), maximal oxygen consumption and body composition in both young sedentary men and overweight obese individuals (Hernández-Lepe *et al*., 2019a). However, the exact mechanism of spirulina in reducing exercise-induced oxidative stress and supporting exercise performance is not entirely clear, and there are some conflicting reports in the literature; no positive effects on plasma lipid peroxidation (Ferreira *et al*., 2021), muscle damage and endurance capacity (Pappas *et al*., 2021), and some metabolic parameters (Mohammad *et al*., 2022). In this context, we evaluated the effects of spirulina on oxidative stress, antioxidant defense activity and endurance capacity due to acute exhaustive and chronic swimming exercise in this study. We aimed to clarify the utility of spirulina as a supplement to protect against exercise-induced oxidative stress.

2. 2.Materials and Methods

2.1. Animals

Forty-eight healthy male Wistar strain albino rats (mean initial weight, 352±27 g) were purchased from Selcuk University Experimental Medicine Research and Application Center. Rats were housed in polycarbonate/stainless steel cages (four rats/cage; base area, 1820 cm2) under controlled temperature (20°C ±2°C) and relative humidity (50% ± 5%), with a 12-h/12-h light/dark cycle. Standard rat chow (Bilyem A.S. Ankara, Turkey) and tap water were provided ad libitum. All experimental procedures were approved by the Selcuk University Experimental Animals Local Ethical Commission.

2.2. Experimental Design and Supplementation

The 48 rats were randomly and equally divided into six groups. The study was organized to last for 6 weeks. All treatments were administered by oral gavage. The control group (C) received 2 mL/day tap water. Spirulina group (SP) was administered orally daily 750 mg/kg spirulina dissolved in tap water. Rats in the chronic exercise (CE) group were subjected to swimming exercise sessions in specially designed pools for 1 h/day for 5 days each week. Rats in the CE group also received 2-mL/day tap water. The chronic exercise + spirulina (CES) group was subjected to swimming exercise similar to the CE group, but unlike the CE group, the animals in this group were given 750 mg/kg/day spirulina. Rats in the exhaustion (E) group received 2-mL/day tap water and performed exhaustive exercise on the last day of the study. The exhaustion + spirulina group (ES) received 750 mg/kg/day spirulina dissolved in tap water during the study period (6 weeks) and performed exhaustive exercise on the last day of the study. High-purity (≥99%) Spirulina platensis powder was purchased from Egert Natural Products Ltd. (Bornova/Izmir). Spirulina, which was dissolved in cold tap water (10°C) using a magnetic stirrer, was freshly prepared before each administration.

2.3. Aerobic Exercise Procedure

We preferred swimming exercise to avoid prolonged exercise-induced muscle injury, electrical shock-stimulated exercise, plyometric contractions because these factors alone can provoke oxidative stress (Misra *et al*., 2009). Endurance exercise was executed in temperature-controlled (30°C) pools (120 × 80 × 80 cm^3). A maximum of four animals simultaneously swam in the same pool. The experiments were conducted at the same time each day. Chronic swimming exercise was performed for 60 min/day for 5 days each week over the course of 6 weeks. Rats were acclimatized for 3 consecutive days prior to testing sessions via swimming for 10, 20, and 30 min, respectively, on the 3 days.

2.4. Exhaustive Exercise Procedure

On the last day of the study, the swimming endurance time test was applied to evaluate the effects of Spirulina platensis on the exercise endurance of rats. Rats in groups E and ES were subjected to a single session of exhaustive exercise, and animals that were not able to keep their noses above water surface for more than 10 s or that lost motor coordination were considered to be exhausted (Bucioli *et al*., 2011).

2.5. Sample Collection

Rats in the C and SP groups were sacrificed at the completion of treatment with tap water or spirulina. CE and CES rats were sacrificed 24 h after the last exercise session, and E and ES rats were sacrificed after exhaustion. In the course of the experiments, five rats (two SP rats, one CES rat, and two ES rats) died as a result of aspiration. Rats were anesthetized with ketamine/xylazine (60 mg/kg /10 mg/kg, i.p.). Cardiac blood (8–10 mL) was obtained from each animal via puncture and poured into EDTA-containing sterile tubes after confirming deep anesthesia. Plasma was acquired by cold centrifugation (NUVE NF 1200R) of blood at 3000 rpm for 10 min. Following exsanguination, the liver and skeletal muscle (gastrocnemius) were excised and gently washed with cold (4°C) distilled water to remove excess blood and residual tissue. Plasma and tissue samples were stored at−80°C (SANYO MDF-U5386S) until analysis.

2.6. Biochemical Analysis

The plasma activities of creatine kinase (CK), the MB isoform of CK (CK–MB), lactate dehydrogenase (LDH), and uric acid (UA) levels were analyzed using an autoanalyzer (Siemens Dimension EXL).CK, CK–MB, and LDH activities were expressed as U/L, whereas UA levels were expressed as mg/dL. MDA levels, XO, MPO, SOD, CAT, and GPx activities and antioxidant activity (AOA) were evaluated to assess tissue and plasma oxidative stress and antioxidant defense status. Tissue lipid

peroxidation was measured with a commercial assay kit, which measures TBARS, according to the manufacturer's instructions (Cayman Chemical Co., Ann Arbor, MI, USA). MDA levels were expressed as µmol/g of wet tissue. Activities of XO, which is a generator of ROS, were measured using a commercial assay kit (Cayman Chemical) based on H_2O_2 production during hypoxanthine oxidation. Tissue and plasma XO activities were expressed as U/mg andµU/mL, respectively. The SOD, CAT, GPX, and AOA analyses were performed using commercial kits (Cayman Chemical) in accordance with the manufacturer's instructions. Tissue and plasma SOD activities were expressed as U/mg protein and U/mL, respectively. Tissue and plasma CAT and GPx activities were expressed as nmol/mg/min protein and nmol/mL/min, respectively. Tissue and plasma AOAs were expressed as mM/g and mM/mL, respectively. Tissue protein was measured by the Lowry method (Lowry *et al*., 1951).

2.7. Statistical Analysis

The results were expressed as the mean±SD. The obtained data were subjected to one-way analysis of variance (ANOVA) and compared by Tukey's test, with $p<0.05$ indicating significance, using SPSS.

3. Results

The initial weights of the animals were similar among the groups (one-way ANOVA, $p>0.05$). The mean weight gain was 12.8% for spirulina-treated groups versus 12.5% for control groups. The smallest weight gain was observed in the CE group (3.8%), but there were no statistically significance differences in weight gain among the groups (one-way ANOVA, $p>0.05$). The mean swimming time for the E and ES groups in the exhaustive swimming test on the final day of the study was 162±8 and 164±9 min, respectively. Despite the fact that the ES group had a longer swimming time, the contribution of spirulina supplementation was not statistically significant ($p>0.05$).

Table 1 shows the effects of spirulina supplementation on plasma creatine kinase, CK-MB, and LDH activities, as well as UA levels, at the end of the six-week study. At the end of the study, there was no significant difference in plasma CK, CK-MB, LDH activities, or UA levels between the CON, SP, CE, and CES groups ($p>0.05$). The exhausting swimming exercise performed on the rats in Group E and Group ES on the last day of the study caused an increase in plasma CK, CK-MB and LDH activities in the rats ($p<0.05$). In addition, spirulina supplementation for 6 weeks caused a significant decrease in plasma CK activity ($p<0.05$), which is the most important finding of our study. Plasma UA levels were found to be increased in E group compared to SP, CE and CES groups ($p<0.05$).

Table 1. Effects of spirulina on biochemical parameters after swimming exercise challenge in the plasma.

	CON	SP	CE	CES	E	ES
CK (U/L)	220,50±127,26 [a,b]	239,67±104,69 [a,b]	161,38±50,13 [a,b]	268,17±183,98 [a,b]	963,57±342,69	610,33±222,69 [b]
CK-MB (U/L)	253,38±94,07 [a,b]	288,67±84,86 [a,b]	199,50±40,59 [a,b]	298,00±136,68 [a,b]	818,57±153,29	658,33±111,98
LDH (U/L)	128,63±59,77 [a,b]	113,83±34,12 [a,b]	96,88±19,93 [a,b]	159,50±125,03 [b]	366,57±122,57	293,17±121,69
UA (mg/dl)	0,70±0,71	0,45±0,08 [b]	0,38±0,07 [b]	0,38±0,04 [b]	1,26±0,43	0,82±0,27

Data are shown as means±S.D. CK= Creatin kinase, CK-MB= Creatin kinase-MB, LDH= Lactate dehydrogenase, UA= Uric acid.

[a] $p<0.05$ versus ES. [b] $p<0.05$ versus E.

The effects of spirulina supplementation on liver and skeletal muscle MDA levels in animals subjected to chronic exercise are presented in Figure 1. Compared with the CON group, liver and muscle MDA levels were lower in the SP, CE, and CES groups ($p<0.05$). Exhaustive exercise did not alter liver MDA levels (data not shown). The effect of chronic exercise on MPO activities in the liver, skeletal muscle, and plasma was not significant ($p>0.05$, data were not shown). Nonetheless, it was discovered that XO activity in the liver tissue of chronically exercised rats (rats in the CE and CES groups) was increased compared to the CON group ($p<0.05$, Table 2). We discovered that XO activity was lower in the CE group than in the CON and SP groups ($p<0.05$) in skeletal muscle. Exhaustive exercise had no effect on plasma or skeletal muscle MPO and XO activities ($p>0.05$), but it did reduce liver MPO and XO activities ($p<0.05$).

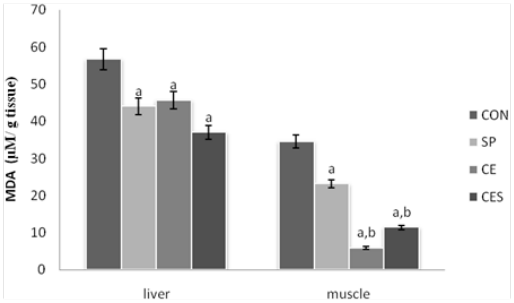

Figure 1. Effects of chronic exercise and spirulina supplementation on MDA concentrations in liver and muscle tissues. CON: control group; SP: spirulina treated group; CE: moderate, long-term exercised group; CES: exercise performed plus spirulina group. Data are expressed as mean ± SD. [a] $p<0.05$ versus CON. [b] $p<0.05$ versus SP.

Table 2. Myeloperoxidase (ng/mg of protein) and xanthine oxidase (U/mg of protein) in liver and muscle tissues after exercise and spirulina supplementation.

		CON	SP	CE	CES	E	ES
MPO	Liver	54,48±15,58	57,40±10,68	41,38±15,03	36,59±16,70	28,58±11,64[a,b]	23,16±6,46[a,b]
	Muscle	12,54±2,29	13,95±7,30	9,55±2,34	12,53±4,83	10,03±2,63	6,80±5,78
XO	Liver	94,82±31,43	64,99±3,62	61,07±22,01[a]	60,40±10,35[a]	52,42±12,03[a]	42,19±5,38[a]
	Muscle	20,66±7,41	22,76±12,00	12,14±1,87[b]	20,53±4,59	18,15±3,62	19,06±4,55

Data are shown as means±S.D. MPO= Myeloperoxidase,XO= Xanthine oxidase.

[a]p<0.05 versus CON. [b]p<0.05 versus SP.

Changes SOD, CAT and GPx activities in liver and skeletal muscle were not significant in the chronic exercise groups (CE and CES groups) compared to the CON group (p>0.05). However, skeletal muscle AOA activity in the CE group was lower than in the CON group (p<0.05). Plasma CAT activities were found to be higher in E and ES groups compared to CON group (Figure 2, p<0.05). Plasma SOD activities were found to be statistically higher in CE and CES groups compared to SP group (p<0.05, Figure 3), while plasma GPx activities were observed to be lower in CES group compared to CON group (p<0.05). **The changes in SOD and GPx activities and AOA levels were not statistically significant (data not shown, p>0.05) in exhaustive exercised rats (E and ES groups).**

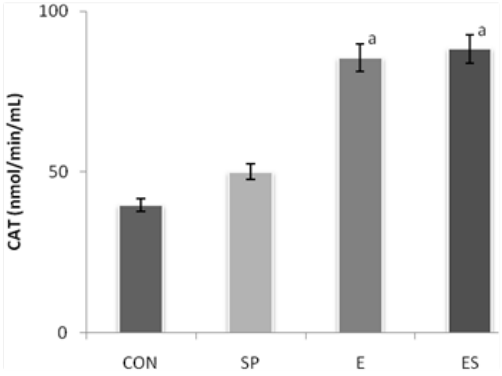

Figure 2. Effects of exhaustive exercise and spirulina supplementation on CAT activities in the plasma. CON: control group; SP: spirulina treated group; E: exhaustive swimming exercise group; CES: exhaustive swimming exercise performed plus spirulina group. Data are expressed as mean ± SD. [a] p <0.05 versus CON and SP

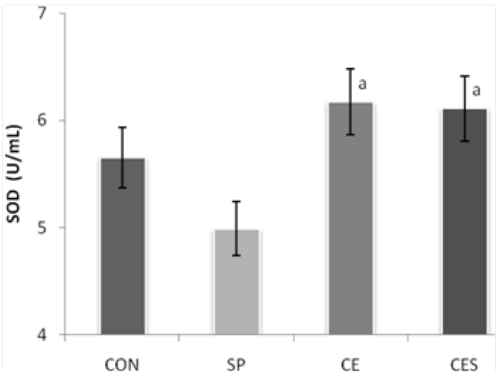

Figure 3. Effects of chronically exercise and spirulina supplementation on SOD activities in the plasma. Data are expressed as mean ± SD. [a] p <0.05 versus SP

SOD and CAT activities in liver tissue were found to be lower in the E and ES groups than in the CON and SP groups, and AOA levels were found to be lower only in the CON group (Table 3, p<0.05). The changes observed in skeletal muscle SOD, CAT and GPx activities and AOA levels after exhaustive exercise were not significant (data not shown, p>0.05).

Table 3. Effects of spirulina supplementation on hepatic antioxidant enzyme activities after exhaustive swimming exercise.

	SOD	CAT	AOA	GPx
CON	3,59±1,01	44,90±6,67	1,34±0,23	1,45±1,34
SP	3,55±1,30	43,70±9,94	1,23±0,27	1,19±1,10
E	2,05±0,61[a,b]	32,92±6,49[a,b]	0,88±0,31[a]	1,14±0,74
ES	1,81±0,41[a,b]	25,98±4,93[a,b]	0,71±0,12[a,b]	2,21±2,44

Data are shown as means±S.D. SOD (U/mg of protein) =Superoxide dismutase, CAT (nmol/min/mg of protein)=Catalase, AOA (mM/g tissue)=Antioxidant activity, GPx (nmol/min/mg of protein)=Gluthatione peroxidase.

[a]p<0.05 versus CON. [b]p<0.05 versus SP.

4. Discussion

The aim of the present study was to examine the effects of long-term/exhaustive exercise and spirulina intake on the oxidative status of plasma, liver tissue, and muscle tissue and endurance capacity in adult male rats. We found that exhaustive exercise alone increased the plasma activities of CK, CK–MB, and LDH. An important finding of our study was that spirulina attenuated the exhaustive exercise-induced increment of CK activities in plasma. In addition, spirulina supplementation and acute or chronic exercise induced changes in antioxidant status and oxidative stress parameters. MDA levels, which are an end product of lipid peroxidation in the liver and skeletal muscle, were decreased by both spirulina supplementation and chronic exercise.

Although the influence of exercise on metabolism is well defined, the weight gain of exercised rats was not different from that of control rats. This finding is in concordance with previous studies (Lima *et al*., 2013; Barcelos *et al*., 2014). Thus, it can be suggested that swimming-based exercise has no major impact on the weight of rats, probably due to the intensity and duration ofthe exercise. In addition, 750 mg/kg/day (p.o.) spirulina supplementation for 6 weeks did not alter the weight of rats compared with that of the control animals. We could not locate any study in the medical literature that reports the direct effects of spirulina on body weight. However, there are several studies consistent with our findings that describe mechanisms by which spirulina influences weight (Khan *et al*., 2006; Ismail *et al*., 2009; Moura *et al*., 2011). Therefore, in summary, it can be deduced that spirulina supplementation has no effect on the weight of rats. Moderate exercise or personal training programs improve pulmonary and cardiac function and decrease the incidence of chronic illness as a primary treatment while enhancing athletic performance (Pedersen and Saltin, 2006). Meanwhile, strenuous exercise has been demonstrated to provoke oxidative injury, particularly in skeletal muscle and the liver, as a result of free radical accumulation due to increased oxygen consumption (Huang *et al*., 2009; Kan *et al*., 2013). Modest amounts of ROS in tissues are essential for homeostatic harmony, but excess ROS production causes oxidative stress. There is bi-directional knowledge in the medical literature about exercise-induced ROS production. A study in laboratory animals revealed that prolonged moderate exercise reduces levels of MDA, which is widely accepted as a marker of oxidative stress (Viboolvorakul *et al*., 2009). Thus the emergence of desirable adaptive responses to chronic exercise-induced oxidative stress is the most feasible explanation of the aforementioned decrement. Intermittent stimulation by modest amounts of ROS promotes exercise-related adaptations. In the present study, significant decreases in both liver and skeletal muscle MDA levels were noted (Figure 1). This result indicates that our exercise protocol is sufficient in intensity and duration to reverse the preoxidative status in skeletal muscle and liver tissues In contrast, provocation of oxidative stress has been reported after regular exercise (Barcelos *et al*., 2014). These diversities in study results may be associated with the usage of varying animal species or strains and the application of different exercise protocols primarily in terms of intensity and duration. Furthermore, in contrast to the aforementioned studies, a weight pendant (expressed as a percentile of body weight) was not attached to tails of swimming rats in our study. However, it is obvious that the increases in ROS and free radical generation in tissues as a result of acute/exhaustive exercise exceed the endogenous defense capacity and lead to oxidative stress, ultimately resulting in damage to multiple tissues. Exhaustive exercise increases whole-body oxygen consumption, resulting in increased electron escape from the mitochondrial transport system and disturbances of prooxidant–antioxidant homeostasis (Ji, 1999). Numerous animal studies reported an increase in lipid peroxidation, as evaluated by MDA levels, following exhaustive exercise (Huang *et al*., 2009; Kan *et al*., 2013). In our study, a single session of exhaustive exercise increased plasma activities of CK, which is frequently used for assessing muscle injury, and previous studies suggested that elevated CK activities are associated with oxidative stress (Huang *et al*., 2009; Kan *et al*., 2013). In our study, we found that spirulina diet regimen ameliorated the exhaustive exercise-induced increase in plasma CK activity (Table 1, $p<0.05$). Similarly, Lu *et al*. (2006) observed that the administration of 7.5 g/day spirulina for 3 weeks decreased CK activities by 28.77%, suggesting that the administered dose of spirulina in this study was adequate to protect against skeletal muscle injury after exhaustive exercise. A recent study of elite rugby athletes reported that muscle damage was prevented as a result of reduced plasma CK and LDH increases due to intense exercise with spirulina supplementation (Chaouachi *et al*., 2022). However, in our study, despite this healing effect of spirulina against muscle damage, no significant difference was found between the groups in the endurance test performed on the last day ($p>0.05$). Kalafati *et al*. (2010) reported that 6 g/day spirulina supplementation for 4 weeks did not significantly change serum CK activities compared with placeboin moderately trained men, but this supplementation increased the time to exhaustion. Consistent with this study, Franca *et al*. (2010) noted that 7.5 g/day spirulina administration to cyclists for 4 weeks did not induce a significant alteration in CK activities, and similar to our findings, did not exert an ergogenic effect. The variance of the results may be associated with the different training grades and exercise protocols used in the different studies.

Antioxidants are radical-scavenging molecules that prevent cellular damage caused by oxidative stress. Controversial results exist in the literature regarding the response of endogenous antioxidant defense mechanisms to exercise. This variance may be a consequence of the differences in exercise protocols used in the studies, as the response is dependent on the type, duration, and intensity of exercise. In the present study, plasma SOD activities were higher in the CE and CES groups than in the SP group (Figure 3). The lack of an increment of SOD activities in the SP group indicates that the stated effect of chronic exercise in the CES group is independent of spirulina. However, no change in CAT activities in the liver tissue was reported in a study utilizing a model of 4 weeks of moderate exercise, but the researchers noted a more significant decrease in CAT activities in the sedentary group following exhaustive exercise relative to that in trained subjects (Choi and Cho, 2007). Aydın *et al*. (2009) reported a decline in GPx and CAT activities after a single exercise session. Yu *et al*. (2012) observed a decrement of skeletal muscle SOD activities following exhaustive swimming exercise. Nevertheless, other studies noted an increment (Saborido *et al*., 2011), maintenance (Caillaud *et al*., 1999), or a decrement (You *et al*., 2010) in SOD activities after exhaustive exercise. In our study, exhaustive exercise resulted in an augmentation of plasma CAT activities relative to the findings in the CON and SP groups (Figure 2). This effect was noted in both the E and ES groups. Taysi *et al*. (2008) reported a decline of CAT activities after exhaustive exercise in liver tissue, whereas Huang *et al*. (2009) stated that the levels of antioxidant enzymes such as SOD, GPx, and CAT were increased as a compensatory response to oxidative stress induced by exhaustive exercise in muscle tissue. However, no changes in the aforementioned enzyme activities in the liver and

kidneys were observed in that study (Huang *et al*., 2009). In humans, spirulina supplementation did not alter GPx activities (Lu et al., 2006), while this supplementation was associated with increased plasma SOD activities (Lu *et al*., 2006) and GSH concentrations (Kalafati et al., 2010), independent of exercise. The results of recent human and animal studies evaluating spirulina supplementation are conflicting. Three different doses of spirulina resulted in an improvement in antioxidant capacity and inflammation, as well as a dose-dependent decrease in lipid peroxidation assessed by MDA (Brito *et al*., 2020), while in another animal study, it was reported that spirulina supplementation improved oxidative stress parameters and strengthened the antioxidant defense system of intestinal tissue in rats subjected to strength training (Araujo *et al*., 2020). Furthermore, it was shown that the increase in the uterine tissue MDA level in female rats due to exercise was statistically reduced by spirulina supplementation, while spirulina improved the total antioxidant activity (Ferreira *et al*., 2021). Spirulina supplementation was shown to improve body composition as assessed by free fat mass and immune system as assessed by IgA in overweight and obese women performing high-intensity interval training, but no positive contribution to exercise performance has been evaluated (Nobari *et al*., 2022). Moreover, the contribution of spirulina to the metabolic functions of individuals with different physical characteristics is noteworthy in some recent studies. Spirulina supplementation has been reported to improve body composition, maximal oxygen consumption and plasma lipid profiles, especially in dyslipidemic obese men and overweight obese women; and spirulina supplementation has a synergistic effect with exercise on these parameters (Hernández-Lepe *et al*., 2019a, 2019b). In order to fully explain the mechanism of these positive contributions reported in human studies, studies in experimental animals and tissue level analyses are significant. In this regard, our study is the first to demonstrate the effect of spirulina on plasma, liver and muscle tissue oxidative status, plasma muscle damage markers and endurance capacity due to regular and exhaustive swimming exercise in rats. However, the main limitations of our study include the fact that spirulina supplementation was not applied at different doses (less than or higher than 750 mg/kg), which could be more effective, and that some inflammation markers were not evaluated.

5. Conclusions

In conclusion, we suggest that Spirulina platensis, a blue-green alga and food supplement, ameliorates increases in plasma CK levels, which are a marker of exhaustive exercise-related muscular breakdown, probably by decreasing preoxidative MDA levels in skeletal muscle. However, it is apparent that spirulina supplementation in adult male rats had no positive effect on antioxidant activity and ergogenic status with our exercise protocol. Few studies have investigated the effects of spirulina supplementation on exercise-induced oxidative stress and exercise endurance capacity, and the mechanism by which spirulina alleviates oxidative stress during exercise is not yet well understood. Future studies are needed for satisfactory comprehension of the mechanism.

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