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Protective Effect of Prolonged Quercetin Supplement on Oxidative Stress and Antioxidant Enzyme Activities in Boxers

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ABSTRACT This study was planned in order to investigate the protective effect of prolonged quercetin supplement on oxidative stress and antioxidant enzyme activities in boxers. It included 20 voluntary male boxers. The athletes were separated into two groups as the control group (n = 10) and Quercetin group (n = 10). The athletes were supplemented with 500 mg quercetin for 30 days before their exercise programs. Blood samples were drawn from the athletes before and after exercising. MDA, SOD, CAT and GSH analysis were performed. According to the findings, there were an increase in the MDA levels of the control group ($P<0.01$) and a significant decrease in the MDA levels of quercetin group ($P<0.001$) when they were compared after being given quercetin. The levels of antioxidant enzymes SOD, CAT and GSH significantly increased in quercetin group ($P<0.001$). As a conclusion; it can be said that quercetin supplement has protective effect against oxidative stress by decreasing the amount of MDA, which is the end-product of lipid peroxidation and increasing the activity levels of the antioxidant enzymes SOD, CAT and GSH.

INTRODUCTION

Quercetin is the most common and widely distributed flavonoid in the plant kingdom, and it is especially abundant in apples (ranging from 2.1 to 7.2 mg/100 g) and onions (ranging from 28.4 to 48.6 mg/100 g). According to epidemical survey, the daily media intake of quercetin with a typical Western diet and in China was estimated to 10 mg and 5.96 mg/day, respectively (Tang et al. 2016). Human body naturally produces antioxidants, such as superoxide dismutase, catalase, and glutathione peroxidase; however, during strenuous exercise, the antioxidant defense systems in the body are over whelmed by the increased level of free radical generations. Therefore, certain nutrients from diet can help to augment the body's defense systems against free radicals. Proper antioxidant supplementation under conditions of strenuous exercise may enhance performance by preventing the exercise-induced tissue damage and increasing the recovery process (Leelayuwat et al. 2012; Goldfarb et al. 2005). Emerging evidence from our and other groups has shown that quercetin exerts hepatoprotective effects due to its antioxidant capacity, anti inflammatory activity, and gene regulatory properties (Lin et al. 2014).

There is also evidence that quercetin can provide a number of health and performance benefits due to its antiviral, anti-inflammatory, anti-carcinogenic, cardio protective, psycho stimulating, and neuro protective properties (Bjelakovic et al. 2007; Davis et al. 2009). In regard stop physical performance, the potential for quercetin to be ergogenic lies in the electron donating capacity of it sphenolic hydroxyl groups, reducing the effects of reactive oxygen species (ROS) (Nieman 2008). There are also findings that the formation of free radicals and reactive oxygen species (ROS) increases especially during heavy exercise, and oxidative damage forms in muscles, liver and other tissues (Konig et al. 2001; Urso et al. 2003). The heavier the exercises are, the more free radicals form (Göktepe and Günay 2014). In connection with the oxygen consumption, increased free radicals are neutralized by a defense mechanism including both enzymatic and non-enzymatic antioxidants. Quercetin's action mechanisms with regards to free radicals are diverse. Quercetin hydroxyl radical shows

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the highest level of anti-radical characteristics against peroxyl and superoxyde anion when compared to other flavanoids. Quercetin inhibits its superoxyde anion generation through xanthine oxydase, and cleans singlet oxygen as well as hydroxyl radicals (Casagrande et al. 2006).

Exercise causes an imbalance called oxidative stress between ROS and antioxidants. The primary antioxidant enzymes effective at cellular level include SOD (superoxide dismutase), CAT (catalase) and GSH (glutathione) (Göktepe and Günay 2014). It is reported that an acute exercise can directly affect the activities of these enzymes. Scholten and Segreev (2013) put forward that 6 weeks of quercetin supplement and exercise significantly decreased the level of MDA in a study they carried out. Quercetin was found out to increase performance and stamina in clinical studies (Alia et al. 2006; Gaining 2006). In yet another study carried out, it was found out that quercetin intake had a performance-increasing effect by restraining the level of lactate as well as a protective effect against free radicals by decreasing the amount of MDA, the end-product of lipid peroxidation, and supported the antioxidant defense mechanisms of cells by increasing the levels of antioxidant enzymes SOD, CAT, GPx and GST (Göktepe and Günay 2014). In line with these information, this study was planned in order to investigate the protective effect of prolonged quercetin supplement on oxidative stress and antioxidant enzyme activities in boxers.

MATERIAL AND METHODS

Participants

This study included 20 voluntary licensed male boxers of 61-64 kg weight and 161-170 cm height between the ages of 18 and 21, who were documented to have no inconvenience for exercising with doctor's report. Conducted in line with the relevant directive specified in Helsinki Declaration. The voluntary athletes were informed in detail with regards to the rules to be followed, the supplementary materials to be used as well as the tests to be carried out within the scope of the study. Applications were performed at the Physiology laboratory of Kafkas University, Institute of Health Sciences, and the study protocol was approved by the Local Ethics Committee of Kafkas University Medical Faculty.

Exercise Protocol

The boxers having participated in the study were separated into two groups. The first group was evaluated as the control group, while the second group was evaluated as quercetin group. An exercise program of two hours and 80-90 percent intensity was applied to the athletes three times a week for one month. It was ensured that the athletes did not take any medicine within 15 days before the test, which may affect antioxidant defense, and their diets were standardized. Quercetin group was supplemented with 500 mg quercetin for 30 days, 15 mins. before exercises.

The Exercise Program Applied

The program was begun with 20 minutes of general warm-up exercises. After warming up, the athletes had 3 rounds of gloves or punching bag exercise, 1 round of shadow boxing as well as 1 round of rope jumping and walking. Using all box techniques, the athletes repeated the techniques stated below in 3 sets with maximal load. Techniques: having grade position and walking forward, backward, rightward and leftward in grade position as well as adjusting distance. The exercise was finished with the following: left and right crosses in the chin, defense against left and right crosses, left and right crosses in the chin and body and defense against them, left and right hooks in the body and defense against them, shadow boxing using the left and right hooks learned.

Collecting the Blood Samples

Blood samples were drawn from all the sathletes for two times, one being before the exercise and the other after the 30th day of the exercise. After the blood samples drawn centrifuged at 4000 rpm for 15 mins. in cooling centrifuge machine, the parts with plasma left at the top were put into polypropylene tubes, and stored in deep freezer at - 20^0C until their MDA, SOD, CAT ve GSH analyses were carried out.

The Determination of the Biochemical Parameters

Measurement of Malondialdehyde (MDA) Level

The level of MDA a product of the lipid peroxidation was measured spectro photometrical-

ly by the absorption of the pink-red color at 532 nm formed as result of the reaction with TBA at 95^0C. Plasma MDA level was measured by the use of Uchiyama and Mihara (1978) method. The sample absorbance was first multiplied with a dilution factor of 10 and then with a factor obtained from the Standard graph which gave the amount of MDA in mmol/l.

Measurement of Superoxide dismutase (SOD) Level

Total (Cu–Zn and Mn) SOD (EC 1.15.1.1) determination is based upon the conversion of the superoxide radicals generated by xanthine oxidase into H2O2 which reduces nitroblue tetrazolium (NBT) into a blue colored formazan which gives an absorbance peak at 560 nm The data are given in U/l (Uchiyama and Mihara 1978).

Measurement of Catalase (CAT) Level

Measurement of catalase activity (CAT) was performed using the method defined by Aebi (1984). The method mainly includes observing the catabolisation of H_2O_2 substrate at 240 nm.

Measurement of Glutathion (GSH) Level

GSH determination was made spectro photometrical by monitoring the peak at 410 nm corresponding to the product formed by the reaction of the Elman reagent with sulp hydrile groups using the method developed by Fairbanks and Klee (1986). Then the absorbance of the samples was multiplied by the factor obtained from which give the activity of GSH in μmol/l.

Statistical Analysis

The statistical analysis of the data was conducted on the SPSS 17.0 software. The data were expressed as mean ± Standard deviation. The study data was statistically analyzed using the independent group t-test and the one-way analysis of variance (ANOVA) test. P value slower than 0.05 were regarded as significant.

RESULTS

The study was participated by 20 elite boxers with ages of 18-21 years, heights of 161-170 cm, body weights of 61-64 kg and Body Mass Index (BMI) 21-23 kg/m^2 (Table 1). There was no statistical difference between the groups in terms of physical characteristics.

Table 1: Comparison of some of the physical characteristics of the participants

Parameters (n=20)	*Control group Mean±SD*	*Quercetin group Mean±SD*	*P value*
Age (Years)	19.00± 1.05	18.90± 1.10	.343
Height (cm)	167.50± 2.83	167.20± 3.22	.215
Weight (kg)	62.70± 1.15	63.00± 1.05	.081
Body mass index BMI (kg/m)	22.56± 1.06	22.37± 0.80	.733

$p>0.05^*$

There was no significant difference detected between the groups when they were compared before exercise; however, there were an increase in the MDA levels of the control group ($P<0.01$) and a significant decrease in the MDA levels of quercetin group ($P<0.001$) when they were compared after being given quercetin (Table 2).

When the groups, which were supplemented with quercetin and then exercised, were observed before and after the exercise; there was no significant difference detected in SOD, CAT and GSH levels of the control group ($P>0.05$),

Table 2: Comparison of the groups' SOD, CAT and GSH levels before exercise and after quercetin supplement

Parameters	*Quercetin group*			*Control group*		
	Before exercise X±SD	*After exercise X±SD*	*P*	*Before exercise X±SD*	*After exercise X±SD*	*P*
SOD (U/l)	1.85± 0.00	1.86± 0.00	.081	1.84± 0.00	1.95± 0.01	.001
CAT (U/ml)	23.18± 0.88	22.43± 0.95	.105	22.35± 0.99	25.55± 1.52	.001
GSH (ìmol/l)	1.43± 0.01	1.44± 0.11	.061	1.43± 0.00	1.50± 0.02	.001
MDA (mmol/l)	25.33± 1.63	27.34± 2.15	.001	26.65± 1.85	20.14± 1.23	.001

$P>0.05$, $P<0.001^{***}$ (SOD) Süperoksit dismutaz, (KAT) Catalaz, (GSH) Gulutatyon, (MDA) Malondialdehyde

while there was a significant increase observed in the levels antioxidant enzymes SOD, CAT and GSH ($P<0.001$) of quercetin group (Table 2).

DISCUSSION

With this study, it was aimed to investigate the protective effect of prolonged quercetin supplement on oxidative stress and antioxidant enzyme activities in boxers. According to our findings, there was no significant difference detected between the groups when they were compared before exercise; however, there were an increase in the MDA levels of the control group and a significant decrease in the MDA levels of quercetin group when they were compared after being given quercetin. In a study they carried out, Scholten and Sergeev (2013) demonstrated that 6 weeks of quercetin supplement was associated with a significant decrease in serum MDA levels. In another study carried out (Göktepe and Günay 2014), MDA levels of control and study groups were measured before quercetin supplement during rest and exercise, and after quercetin supplement during exercise and recovery; MDA levels of the rats supplemented with quercetin and put through exercise were found to be significantly lower than that of the control group. These reports support our results. In a study conducted (2016), intensive exercise following quercetin (100 mg / kg BW) was administered daily for 5 weeks. As a result, it was observed that quercetin administration increased SOD activity while decreasing the MDA level (Tang et al. 2016).

The heavier the exercises are, the more free radicals form (Palmer et al. 2003). Production of free radicals accelerates in parallel with the increase in oxygen consumption during exercise. Therefore, lipid peroxydation started by free radicals cause's damages in membrane lipids (Viitala 2003; Uysal 1998). The most important endproduct of lipid peroxydation is MDA. MDA is off from the peroxydation of fatty acids containing three or more double bonds (Mercan 2004). However, quercetin inhibits free radical generation in cells, and provides protection against lipid peroxydation (Crespy et al. 1999). In a study carried out (Young et al. 1999), it was found out that there was a significant decrease in the MDA levels of subjects given quercetin with fruit juice for one week. In another study, it was found out that quercetin applied to rats caused decrease in MDA levels (Phachonpai et al. 2010). Similarly, it was found out by Singh et al. that 6 days of quercetin application decreased the MDA production in rats (Singh et al. 2011). In yet another study carried out, it was observed that two weeks of quercetin application caused decrease in the MDA levels of rats (Sriraksa et al. 2012). In the view of these information, it can be suggested that quercetin has an inhibitor effect on the release of reactive oxygen species, and suppresses oxydative stress by inhibiting lipid peroxydation.

Excessive production of ROS during exercise may seriously inhibit antioxidant defense, and cause changes in cellular hemostasis; in this way it can start the oxydative stress, which affects lipids, proteins as well as nucleic acids and cause different cellular damages. These ROS released can be neutralized by a defense mechanism including enzymatic and non-enzymatic antioxidants. In addition to suppress the oxydative stress caused by exercise, trainings also stimulates antioxidant production (Turgut et al. 1999; Schröder et al. 2000). It can be suggested that the increasing activity of such antioxidant enzymes as SOD, CAT and GP_x due to regular training which stimulates mitochondrial biosynthesis and the increase in the amount of free radicals eliminates the harmful effects of oxidative stress (Karolkiewicz et al. 2003). According to we findings, when the groups, which were supplemented with quercetin and then exercised, were observed before and after the exercise; there was no significant difference detected in SOD, CAT and GSH levels of the control group, while there was a significant increase observed in the levels antioxidant enzymes SOD, CAT and GSH of quercetin group. It was reported that in a study carried out, application of quercetin and exercise caused significant increase in the levels of SOD, CAT and GPx enzymes (Adewole et al. 2007). Similarly, in the study conducted by Göktepe and Günay (2014), it was detected that the antioxidant enzyme levels of rats supplemented with quercetin and put through exercise increased significantly compared to the control group. These reports are in parallel with our findings. In a study conducted, it was detected that the activity of SOD, CAT and GPx enzymes increased significantly in rats given quercetin (Phachonpai et al. 2010). It was also found out that two weeks of quercetin application caused an increase in the levels of SOD, CAT and GPx

(Sriraksa et al. 2012). In yet another study conducted by Çiftçi et al., it was observed that quercetin application influenced the levels of SOD, CAT and GPx antioxidant enzyme activities (Çiftçi et al. 2011). Similarly, at the end of the study after six week it was observed that the MDA values of swimmers showed a statistically significant decrease and their GSH and SOD activities showed a statistically significant increase (Kafkas et al. 2013). Therefore, it can be concluded that maximal level of exercise performed with quercetin application eliminates the harmful effects of oxydative stress by increasing the activities of such antioxidant enzymes as SOD, CAT and GSH.

CONCLUSION

Thanks to its action mechanism with regards to free radicals, it can be said that Quercetin hydroxyl radical shows the highest level of antiradical characteristics against peroxyl and superoxyde anion when compared to other flavanoids. Moreover, while quercetin decreases oxiydative stress through SOD, CAT and GSH, it is also possible to say that applying quercetin with exercise has a protective effect. As a conclusion; it can be said that prolonged Quercetin supplement has protective effect against oxidative stress by decreasing the amount of MDA, which is the end-product of lipid peroxidation and increasing the activity levels of the antioxidant enzymes SOD, CAT and GSH.

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