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Research Paper

The effects of a 4-week sweet cassava polysaccharides supplementation on oxidative stress and inflammation caused by an acute endurance running

Chung Bing Yang[1], Chin Hsing Hsu[2], Chien Huang Yen[3] and Te Hung Tsao[4]*

[1]Department of Physical Education and Kinesiology, National Dong Hwa University, Hualien, Taiwan.
[2]Department of Recreational Sports and Health Promotion, National Pingtung University of Science and Technology, Pingtung, Taiwan.
[3]Department of Biotechnology, National Pingtung University of Science and Technology, Pingtung, Taiwan.
[4]The Center for Physical and Health Education, National Sun Yat-Sen University, 70 Lienhai Rd., Kaohsiung 80424, Taiwan, R.O.C.

*Corresponding author. E-mail: thtsao@mail.nsysu.edu.tw. Tel: 886-7-7266553. Fax: 886-7-7266553.

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ABSTRACT

This study examined the effect of sweet cassava polysaccharides (SCP) supplementation on an exhaustive running-induced oxidative stress, antioxidant enzymes and inflammatory markers. Sprague-Dawley (SD) rats (five-week-old, n = 32) were randomly divided into four groups, control (Con), exercise (Ex), or two exercise groups with different SCP supplementations (ExSCP 0.5 and ExSCP 1.0). The Ex, ExSCP 0.5 and ExSCP 1.0 groups had a consecutive 6-day running on a rat treadmill with rest in the cage in the 7th day each week. The SCP dosages for the ExSCP 0.5 and 1.0 groups were 0.5 and 1.0 g / kg body weight daily, respectively. After four weeks, an exhaustive running was conducted in the Ex, ExSCP 0.5 and ExSCP 1.0 groups and the blood samples of the rats in all four groups were collected to analyze the antioxidant enzymes and the markers of oxidative stress and inflammation on the completion of the running. An exhaustive running resulted in an increase in oxidative stress in the three exercise groups as compared with the Con. However, the TBARS concentrations in the ExSCP 0.5 and 1.0 groups were significantly lower than those in the Ex by 32 and 38% (P< 0.05), respectively. For antioxidant enzymes, the superoxide dismutase (SOD) concentrations were significantly higher in the ExSCP 1.0 than those in the Ex by 54% (P<0.05) at post-running. Regarding the C-reactive protein(CRP) and interleukin-6 (IL-6) concentrations at post-running, the ExSCP 0.5 and 1.0 groups tended to be lower than the Ex.An increase in antioxidant enzymes by SCP supplementation that possibly played a role in mitigating oxidative stress caused by an endurance running.

Keywords: Thiobarbituric acid reactive substances (TBARS), antioxidant capacity, C-reactive protein (CRP), interleukin-6 (IL-6).

Abbreviations: CAT, catalase; CON: control group; **CRP,** C-reactive protein; **Ex,** exercise group; **ExSCP 0.5,** exercise with daily 0.5 g SCP/kg body weight; **ExSCP 1.0,** exercise with daily 1.0 g SCP/kg body weight; **GPx,** glutathione peroxidase; IL-6: interleukin-6; SCP: sweet cassava polysaccharides; **TBARS,** thiobarbituric acid reactive substances; SOD: superoxide dismutase.

INTRODUCTION

Exhaustive sport or strenuous training results in an obvious elevation in oxidative stress compared with at rest by several times (Tanskanen et al., 2011; Vider et al., 2001), in which the reactive oxygen species (ROS) surpassed the capability of buffering from antioxidant defenses. On the other hand, an increase in inflammation after a strenuous exercise is also associated with exercise-induced oxidative stress (de Lucas et al., 2104; Pedersen and Hoffman-Goetz, 2000). Collectively, elevated oxidative stress and inflammation worsened subsequent sport performance

(Allen et al., 2008; Powers and Jackson, 2008). As a result, the attenuation of oxidative stress and inflammation due to strenuous exercises or trainings by the enhancement of antioxidant and anti-inflammation capacity is an important issue for athletes and coaches.

On the other hand, regular exercise can increase antioxidant capacity, in which the innate antioxidant system can be enhanced through regular training or physical activity (Bloomer, 2008; Goto et al., 2003). In addition to regular exercise, the intake of antioxidant and anti-inflammation substances is able to enhance antioxidant and anti-inflammation capacity, respectively. For elite athletes or regular exercisers, the supplementation of antioxidant substance under routine high-intensity training or intensive exercise program has been focused on for many years, although the results regarding the combination of regular exercise and antioxidant substance supplementation were equivocal (Toldy et al., 2005; Urso and Clarkson, 2003).

In addition to being influenced by oxidative stress and inflammation, sport endurance performance is affected by the change in muscle glycogen content. Studies reported that fatigue displayed or sport performance declined due to a reduction in the amount of muscle glycogen (Arnall et al., 2007; Coyle et al., 1986). As a result, an increase in muscle glycogen via the ingestion of carbohydrate was able to enhance sport performance or delay fatigue (Ivy, 2001). Of substances, sweet cassava is abundant in carbohydrate, which consists of monosaccharides (fructose, arabinose, and galactose) and polysaccharides (Charles et al., 2008). Yen et al. (2013) verified that the supplementation of extracted sweet cassava polysaccharide (SCP) prolonged the running time to exhaustion by increasing muscle glycogen in a six-day of exercise model.

Regarding polysaccharides, several studies reported the attenuated effect of polysaccharides on exercise-induced oxidative stress in addition to increased endurance performance. For example, Yu et al. (2006) and Li et al. (2007) found that the extract *Euphorbia kansui* (Euphorbiaceae) polysaccharides increased antioxidant enzyme activities and prevented lipid peroxidation during strenuous exercise and the extract radix pseudostellariae polysaccharides could enhance endurance performance and decrease oxidative stress in forced swimming rats, respectively. Furthermore, Charles and Huang (2009) reported that the SCP could increase the expression and activity of antioxidative enzymes besides detoxification. Although the above-mentioned studies indicated that the extracted polysaccharides attenuated exercise-induced oxidative stress, few studies have examined the effect of SCP supplementation on endurance running-induced oxidative stress, antioxidant capacity and inflammation. Perhaps, the supplementation of SCP may benefit antioxidant or anti-inflammation capacity in addition to increasing muscle glycogen. Therefore, the purpose of this study was to examine the effect of a 4-week SCP supplementation with regular exercise on oxidative stress, antioxidant capacity and inflammation induced by an acute bout of endurance running. The hypothesis of this study was that the SCP supplementation mitigated oxidative stress and inflammation caused by an exhaustive endurance running.

MATERIALS AND METHODS

Animal

This study was approved by the Animal Studies Committee of National Pingtung University of Science and Technology. Based on the endurance running performance in the study (Yen et al., 2013), there were 32 rats for an 80% statistical power at $P<0.05$. Male Sprague - Dawley (SD) rats (n=32, five weeks old and weighting 180~200 g) were maintained at a temperature of 24 ± 1°C under humidity-controlled conditions (40% ~ 45%) with a 12-h light/dark schedule (lights on at 0600) and were allowed food (Fwusow, Taichung, Taiwan) and water *ad libitum*. After one week of acclimatization, the rats were randomly assigned to four groups, control (Con), exercise (Ex), or exercise with 2 different supplementation dosages, 0.5 g / kg body weight SCP (ExSCP 0.5) or 1.0 g / kg body weight SCP (ExSCP 1.0).

Training program

The experimental period was four weeks. After acclimatization, the Ex, ExSCP 0.5 and ExSCP 1.0 groups began the running program. There were six consecutive days (once daily) for running on a rat treadmill and the 7th day was arranged for staying in rat's cage without any exercise each week. While the rats in the Ex, ExSCP 0.5 and ExSCP 1.0 groups were running in the afternoon (approximately 3-5 pm), the rats in the Con group staying their cages inside were placed beside the treadmills. The velocities and durations were 10-15 m/min for 10 min/each time in the first week, 15-20 m/min for 20 min/each time in the second week, 25 m/min for 30 min/each time in the third week and 25 m/min for 40min/each time in the fourth week.

SCP preparation and supplementation

The sweet cassavas were harvested in Kaohsiung County, Taiwan. After washing, peeling and pelletizing, sweet cassavas were in the procedures for isolating and preparing SCPs. The methods used by Charles et al. (2008) were adopted. Briefly, the mixtures (250 g cassava flour with 500 - 750 g of water) were centrifuged at 14,300 g at 4°C for 20 min and the supernatants removed. Then, crude mucilage was produced when the supernatant was filtered, concentrated, and lyophilized. Crude polysaccharides were fractioned by anion exchange chromatography with elution

by NaCl at different concentrations (0.5, 1.0, 2.0, and 3.0 ml). The SCP was purified by Sephacryl S-400/HR gel filtration chromatography after being pooled, concentrated, desalted, and freeze-dried.

In regard to the supplementation of SCP for the ExSCP 0.5 and ExSCP 1.0 groups, there were two time points each day, in the morning (about 0800-0900) and after the completion of daily running training (1700-1800). The supplementation time points in the day without the treadmill running were the same as those in the training days. The rats in the ExSCP 0.5 and ExSCP 1.0 groups were fed using gastric intubation by a dose of 0.5 and 1.0 g SCP/kg body weight /day, respectively. The dose in each feeding time point was the half of the daily dose (0.25 and 0.5 g SCP/kg body weight for the ExSCP 0.5 and ExSCP 1.0 groups, respectively). The dosage of SCP was determined by the rat's daily weight measurement in the morning and was mixed with the same weight of distilled water.

Exhaustive running

After the completion of a four-week experimental period (no running training in the final day), the Ex, ExSCP 0.5 and ExSCP 1.0 groups proceeded to execute an exhaustive running, in which the speed was 30 m/min with 0% gradient (modified from Brooks and White, 1978). The rats were motivated to run by gentle prodding with a nylon brush to the point of exhaustion, which was defined as the rat could not catch the speed of treadmill despite continuous prodding with a nylon brush. After ascertaining the exhaustion, the rats were moved from the treadmill for anesthetization.

Blood biochemistry

After the Ex, ExSCP 0.5, and ExSCP 1.0 groups completed the exhaustive running, the rats in the four groups were anesthetized by Zoletil 50 (Virbac, France) and blood samples from abdominal aorta were collected. The serum from the centrifugation (1500 rpm for 15 minutes) of the blood samples was analyzed for antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and oxidative stress, and thiobarbituric acid reactive substances (TBARS) using commercial kits (Cayman, MI, USA). The C-reactive protein (CRP) and interleukin (IL)-6 in the serum, the representatives of inflammatory marker, were determined using commercial kits (CRP, Immulite 2000, Los Angeles, CA; IL-6, B&D Systems, Minneapolis, MN). To reduce interassay variations, all variables from the blood samples were performed in duplicate at the same day according to analytical procedures provided in manufacturers' instructions. The intra- and inter-assay coefficients of variation (CVs) were < 7% for the above-mentioned variables.

Statistical analysis

All data were expressed as the mean ± standard deviation and were analyzed by SPSS software (SPSS vers. 20.0, Chicago, IL). The time to complete exhaustive running in the Ex, ExSCP 0.5 and ExSCP 1.0 groups and the other variables in the four groups were analyzed by a one-way ANOVA. If F value showed evidence of significance among the data, Tukey's *post hoc* was used for identification, where a significance existed between groups. The significant level was set at $p < 0.05$.

RESULTS

Regarding rats' body weight prior to the exhaustive running, the Con group (361.8 ± 4.0 g) was significantly heavier than the other three groups (Ex: 317.9 ± 30.0 g, ExSCP 0.5: 323.8 ± 37.2 g, ExSCP 1.0: 331.6 ± 22.8 g, $p<0.05$). The rats in the Ex, ExSCP 0.5 and ExSCP 1.0 groups completed the exhaustive running and the time to complete an exhaustive running was 70.1 ± 10.0 min for the Ex, 104.2 ± 11.7 min for the ExSCP 0.5 and 112.0 ± 8.3 min for the ExSCP 1.0. There was a significant difference between the three groups ($p<0.05$), and *post hoc* analyses showed that both ExSCP 0.5 and ExSCP1.0 groups were significantly longer than the Ex group ($P<0.05$). However, no significant difference for the endurance performance was found between the two ExSCP groups ($p>0.05$).

The TBARS levels in the four groups are shown in Figure 1. Although the TBARS levels in the Ex (120.8 ± 23.0 μM), ExSCP 0.5 (80.5 ± 16.3 μM) and ExSCP 1.0 (75.4 ± 9.7μM) groups were significantly higher as compared with the Con group (56.5 ± 7.7 μM, $p<0.05$) after an exhaustive running, the ExSCP 0.5 and ExSCP 1.0 groups were significantly lower than the Ex group in this variable ($p<0.05$). However, the TBARS levels in the ExSCP 0.5 and 1.0 groups did not significantly differ at post-exhaustive running.

For the CAT (Figure 2) and SOD (Figure 3) concentrations after an exhaustive running, there were significant differences between the groups. Although the CAT concentrations in the Ex, ExSCP 0.5 and ExSCP1.0 groups (1.1± 0.3, 1.4± 0.2, and 1.4± 0.3μmol/ml for Ex, ExSCP 0.5 and 1.0, respectively) were lower than those in the Con group (1.5± 0.1μmol/ml), only the Ex group showed significantly lower than the Con group in this variable. On the other hand, the CAT concentrations in the ExSCP 0.5 ($p=0.067$) and ExSCP 1.0 ($p = 0.083$) tended to be higher than those in the Ex group. For the SOD levels, there was no significant difference between the Con and two groups with SCP supplementation after an exhaustive running (Con: 1.2 ±0.2 U/ml; ExSCP 0.5: 1.1 ±0.3 U/ml; ExSCP 1.0: 1.2±0.4 U/ml,$p>0.05$) despite higher in the former than the latter two groups. However, the SOD levels in the Ex group (0.8±0.4 U/ml) were significantly lower than those in the Con and ExSCP 1.0 groups ($p<0.05$) at post-exhaustive

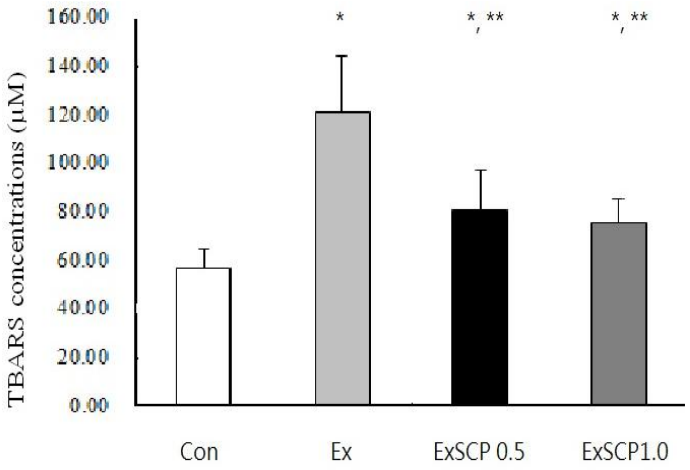

Figure 1: The TABRS concentrations in the Con, Ex, ExSCP 0.5 and ExSCP 1.0 groups at post-exhaustive running.
*significantly different from the Con, ** significantly different from the Ex.

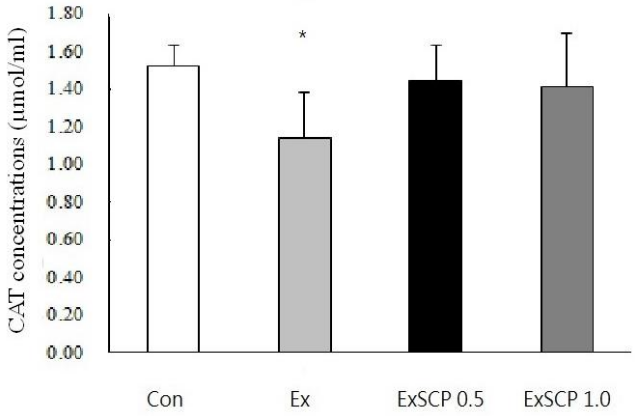

Figure 2: The CAT concentrations in the Con, Ex, ExSCP 0.5 and ExSCP 1.0 groups at post-exhaustive running.
* significantly different from the Con.

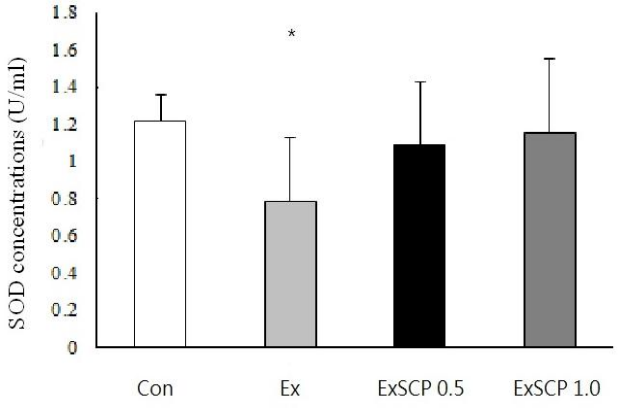

Figure 3: The SOD concentrations in the Con, Ex, ExSCP 0.5and ExSCP 1.0 groups at post-exhaustive running.
* significantly different from the Con and ExSCP 1.0.

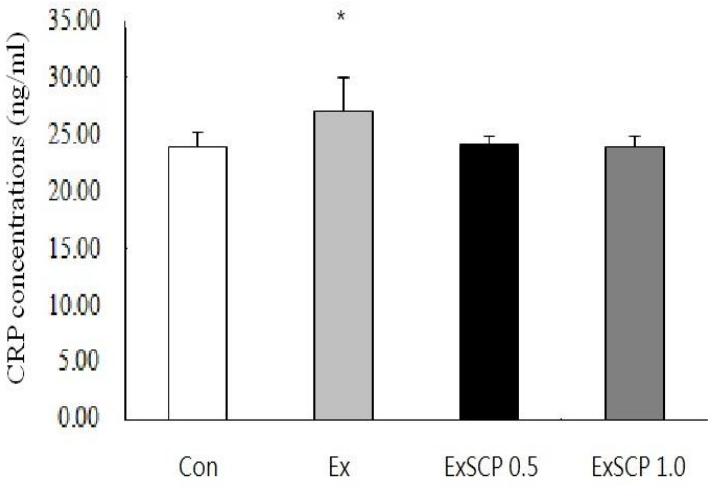

Figure 4: The CRP concentrations in the Con, Ex, ExSCP 0.5 and ExSCP 1.0 groups at post-exhaustive running.
* significantly different from the Con.

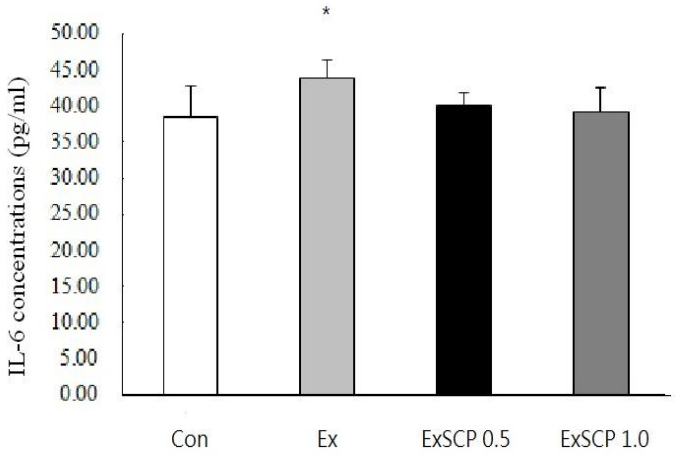

Figure 5: The IL-6 concentrations in the Con, Ex, ExSCP 0.5 and ExSCP 1.0 groups at post-exhaustive running.
* significantly different from the Con.

running. For the GPx levels after an exhaustive running, there was no significant difference between the four groups, although the Ex, ExSCP 0.5 and ExSCP 1.0 groups (7.5 ± 1.7,7.6 ± 1.5, 7.6 ± 1.4 nmol/ml) were lower than the Con (9.6 ± 2.4 nmol/ml) after an exhaustive running.

For the inflammatory markers, the CRP and IL-6 concentrations in the three groups with an exhaustive running were higher as compared with those in the Con group after an exhaustive running; however, only the Ex group was significantly higher than the Con group ($p<0.05$). The CRP and IL-6 concentrations tended to be lower in the ExSCP 0.5 (p =0.075 and p = 0.053) and ExSCP 1.0 groups (p =0.057 and p = 0.068) than in the Ex group (Figures 4 and 5)

DISCUSSION

Similar to previous studies, an acute bout of exhaustive running led to obvious increases in TBARS levels and inflammatory markers in the present study. However, a four-week SCP supplementation attenuated increased TBARS levels in the two groups with SCP supplementation as compared with the Ex group at post-exhaustive running. On the other hand, the tendency to be lower for CRP and IL-6 concentrations in the two groups with SCP supplementation as compared with the Ex group suggest further studies on the effect of SCP supplementation on anti-inflammation.

In the current study, the TBARS concentrations in the Ex,

EXSCP 0.5 and ExSCP 1.0 groups were significantly higher as compared with the Con group after an exhaustive running. The result of one bout of acute exhaustive running leading to an increase in oxidative stress was the same in previous studies (Huang et al., 2008; Viña et al., 2000). On the other hand, studies (Powers and Jackson, 2008; Urso and Clarkson, 2003) reported that antioxidant enzymes, superoxide dismutase, catalase and glutathione peroxidase attenuated an increase in oxidative stress because these enzymes transformed free radicals and intermediate products into water and oxygen. In this study, the TABRS levels in the ExSCP 0.5 and 1.0 groups were significantly lower as compared with those in the Ex group after an exhaustive running. Secondly, the SOD levels in the ExSCP 1.0 group were significantly higher than those in the Ex group at post-exhaustive running besides comparable SOD levels between the two groups with SCP supplementation and Con groups. Based on these two main outcomes, this study suggests that SCP supplementation might reduce oxidative stress by enhancing the antioxidant enzyme system. Several studies (Liu and Finley, 2005; Gabrielska and Oszmiański, 2005) have shown that the chain reactions of free radicals and lipid peroxidation formation could be inhibited and terminated by galactosides and glucosides, which were identified in the composition of SCP (Charles et al., 2008). Furthermore, Charles and Huang (2009) verified that the SCP benefited the mRNA transcription of SOD and decreased superoxide radicals. Therefore, this study suggested that the composition of SCP might be the factor for mitigating TBARS levels in the two groups with SCP supplementation as compared with the Ex group by elevating antioxidant enzymes, especially for SOD.

On the other hand, despite slight difference in TBARS and SOD levels between the two groups with different SCP dosages, this study could not draw the conclusion for the dose-dependent effect of SCP supplementation because there was no obvious difference between those two SCP groups in other antioxidant enzymes or the time to exhaustion.

With regard to the inflammatory markers in the present study, the IL-6 and CRP levels showing a significant increase in the Ex group and slightly higher in the ExSCP 0.5 and 1.0 groups as compared with the Con group indicated an increase in inflammation after one bout of acute exhaustive running. This result is in line with previous studies (Comassi et al., 2015; Suzuki et al., 2003) that also reported an obvious increase in the markers of inflammation after an acute bout of strenuous exercise. Similarly, a study (Joseph et al., 2011) reported that increased inflammatory factors induced by an acute bout of exercise could be reduced by the intake of polysaccharides extracted from *Ganoderma lucidum*. In this regard, Ramberg et al. (2010) found that the glucan component of polysaccharides could attenuate inflammatory response by strengthening the immunological system. The same component (glucan) was identified in the SCP (Charles et al., 2008). In this study, the CRP and IL-6 concentrations tended to be lower in the two groups with SCP supplementation than in the Ex. Hence, further studies are necessary for the effect of SCP on attenuating inflammation or the response of immunity system.

This study is not without limitations. Eliciting an increase in oxidative stress and inflammation via an exhaustive running was requisite in this study. Regarding the effect of SCP on endurance performance, an increase in the content of muscle glycogen by SCP supplementation during six days of experimental model probably played an important role in endurance performance (Yen et al., 2013). Because the dosage (the ExSCP 1.0 group) and the supplementation period (28 vs. 6 days) in this study were higher and longer than those of Yen et al. (2013), respectively in addition to one group with the same SCP dosage as Yen et al's study, the present study inferred that increased muscle glycogen by SCP supplementation might be partly responsible for longer running time. However, the lack of data regarding the content of muscle glycogen, blood glucose concentrations or carbohydrate oxidation (Jeukendrup, 2004; Stellingwerff et al., 2007) was the limitation of this study. Although studies (Bloomer, 2008; Goto et al., 2003) earlier reported that antioxidant capacity was enhanced by regular exercise or training, this part could not be examined because the control group did not perform the same exhaustive running. On the other hand, the longer running time might result in higher oxidative stress and inflammatory markers and more antioxidant enzymes consumption. To complete a given distance at the same exercise intensity might be considered for the dose-response effect in further studies.

Conclusion

The SCP supplementation ameliorated exercise-induced oxidative stress by enhancing some of antioxidant enzymes in this study. With respect to inflammatory markers, the CRP and IL-6 levels after an exhaustive running tended to be lower via SCP supplementation as compared with the Ex group without SCP supplementation. Further studies regarding which mechanism might be responsible for the effect of sweet cassava polysaccharides (SCP) on attenuating oxidative stress and inflammation synchronously due to strenuous exercise are required.

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