Scientific Research and Essay Vol. 5 (2), pp. 148-153, 18 January, 2010
Available online at http://www.academicjournals.org/SRE
ISSN 1992-2248 © 2010 Academic Journals

Full Length Research Paper

Effect of the grape seed proanthocyanidin extract on the free radical and energy metabolism indicators during the movement

Ying Shan[1]*, Xiao-hang Ye[1] and Hui Xin[2]

[1]Department of Physical Education and Military Training, Zhejiang University of Technology, Hangzhou, Zhejiang Province 310014, China.
[2]Institute of Physical Education, Minzu University of China, Beijing 100081, China.

Accepted 2 December, 2009

In order to explore the mechanism of grape seed proanthocyanidin extract (GSPE) against exercise-induced fatigue, the mice movement model was used, and the free radical and energy metabolism indicators during movement were measured. The results show that grape seed proanthocyanidin extract can improve the activity of antioxidant enzymes such as SOD and GSH-Px in the liver in exercised mice, and lower the MDA content, which may produce the effect of anti-oxidation by increasing the activity of antioxidant enzyme in the body; GSPE can keep the blood glucose in exercised mice on a stable level, increase the liver glycogen and muscle glycogen reserves, and prevent the blood glucose content, as well as the liver glycogen and muscle glycogen content, reducing after long time exercise; Meanwhile, GSPE can improve the level of TC, TG and FFA in plasma in exercised - mice, influence the fat metabolism under different conditions and promote the fat utilization. All the results above may be the anti-fatigue mechanism of GSPE.

Key words: Grape seed proanthocyanidin extract, free radicals, energy metabolism.

INTRODUCTION

Proanthocyanidins is a compound extracted from grape seed and its basic structure unit is the catechins (Figure 1). Proanthocyanidins contains catechin monomer, dimer and trimer, all of which are water-soluble molecules and contain a number of phenolic hydroxyls (Bagchi et al., 2002). Polyphenolic compounds having a very important function of antioxidant, they can clean off the free radicals, and reduce the membrane lipid peroxidation, so they can reduce the occurrence of free radical-related diseases and delay aging (Morillas-Ruiz et al., 2006; Silva et al., 2007; Iacopini et al., 2008). Current researches have shown that grape seed proantho-cyanidin extract can clear off free radicals, protect the over-oxidative damage caused by free radicals (Feng et al., 2005; Spranger et al., 2008), and prevent a range of diseases caused by free radicals, such as myocardial infarction, atherosclerosis, drug-induced liver and kidney injury; what's more, it has functions of anti-thrombotic, anti-tumor, anti-mutagenic, anti-radiation, and anti-fatigue (Yamakoshi et al., 1999; Bagchi et al., 2000; Sano et al., 2005; Qin et al., 2006; Engelbrecht et al., 2007).

Exercise - induced fatigue means that the body can not maintain its function at a certain level or can not maintain a predetermined exercise intensity. It is closely related to the athlete's athletic ability and the improvement of athletic performance (Chi et al., 2008; Wu et al., 2008). This experiment is a preliminary study of the effect of Grape seed proanthocyanidin extract on the free radical and energy metabolism indicators during movement. We explored the anti-fatigue mechanism of GSPE from the anti-oxidation system, energy metabolism, etc, trying to provide theoretical guidance and experimental evidence for using GSPE in sports practice.

*Corresponding author. E-mail: yingshankm@qq.com, yingshankm@163.com. Tel:/Fax: +86 13336064842.

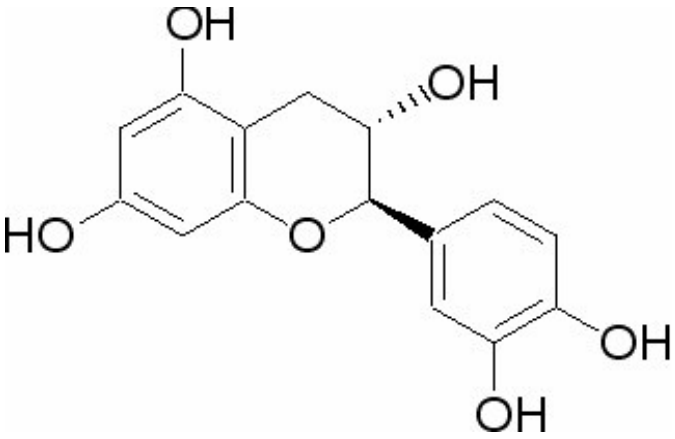

Figure 1. Structure of catechin.

MATERIALS AND METHODS

Materials and reagents

Grape Seeds (purchased in the local wine factory, and identified by Professor Lee, a biologist of Minzu University of China); SOD assay kit, MDA assay kit, GSH-Px assay kit, liver and muscle glycogen detection kit, TG, TC detection kit, free fatty acid detection kit (purchased from Nanjing Jiancheng Bioengineering Institute).

Laboratory instruments

Blood glucose meters (Kyoto Corporation, Japan); Automatic biochemical analyzer (Olympus Corporation, Japan); MK3-based microplate reader (Thermo Corporation, USA); desktop Eppendorf (Heraeus Company, Germany); FZ102-type plants grinder (Shanghai shengqi Instrument Corporation); RE52CS rotary evaporator (Shanghai Rongsheng Instrument Factory); SHB-□ recycled water Multi-purpose vacuum pump (Zhengzhou Great Wall Industry and Trade Branch Corporation, Ltd.). AL104 Electronic Analytical Balance (Mettler - Toledo Instruments Co., Ltd.)

Preparation of grape seed proanthocyanidin extract

Grape seeds were crushed with muller, and filtered with sieve, ≤ 1mm. Then the powder was degreased with petroleum ether (30-60℃) and dried. Weigh accurately 10 g powder was Weighed accurately and put it into 250 ml Round-bottom flask, then 60% ethanol extract was put into the flask according to the solid - liquid ratio 1:12. Next, the mixture was extracted for 2 h at 60℃ and filtered after that. The residues needed to be extracted for another two times and filtered too, then all the filtrate we obtained were mixed and concentrated. Finally dried via vacuum drying and ready for use. The extract rate of proanthocyanidins was 6.17% as determined by the method mentioned in Reference (Feng and Chen, 2003).

EXPERIMENTAL ANIMALS AND GROUPING

Kunming mice (male), 4 weeks old, weighing 18 – 20 g and provided by Hangzhou animal husbandry center. The mice were fed with basal foods, the room temperature being 23 ± 5℃, relative humidity being 40 - 70% and lighting being along with natural changes. After being adaptively fed for a week, 40 of the mice were chosen randomly and divided into four groups: GSPE sport group (GSG), GSPE quiet group (GQG),sport control group (SCG) and quiet control group (QCG). Each group had 10 mice. GSPE groups (GSG and GQG) were force-fed with GSPE by gastrogavage and the dosage was 200 mg / kg·d, while the control groups (SCG, QCG) were force-fed with distilled water of the same volume (0.02 ml / g·d), and all mice were fed continuously for 2 weeks. 30 min after the last gavage, the quiet group (GQG, QCG) did no exercise, while the exercise group (GSG, SCG) swam with free load for 120 min. The mice were dried after swimming. Then all the mice's eyes were picked so as to obtain blood, and the needed materials were obtained by dislocating the mice to death. The obtained blood was placed in anticoagulant tube and centrifuged to obtain plasma; took the liver and quadriceps were taken quickly and frozen them by liquid nitrogen. All the materials were ready to be tested.

Statistical analysis

Data was expressed as $\bar{x} \pm s$. All data was statistically analyzed on microcomputer by SPASS13.0 statistical package. Measurement data was homogeneity of variance as determined by the homogeneity of variance test, differences between the two groups were determined by t test; multiple sets of data were analyzed by single-factor analysis of variance (One-way ANOVA). $P < 0.05$ was considered statistically significant.

RESULTS

Effects of GSPE in SOD, GSH-Px, MDA levels in the mice's liver

The effects of GSPE in SOD, GSH-Px, MDA levels in the mice's liver were shown in Table 1. The following results can be seen from Table 1. In a quiet state, the SOD and GSH-Px levels in livers in the mice which were forced-fed GSPE increased, while the MDA level declined; as compared with the quiet control group, the level of SOD significantly increased ($P < 0.05$); the level of GSH-Px did not change significantly ($P > 0.05$); and the level of MDA decreased significantly ($P < 0.05$). After swimming with free load for 120 min, the levels of SOD and GSH-Px in the liver in control group decreased significantly ($P < 0.05$) as compared with the quiet control group, while the MDA level significantly increased ($P < 0.05$). After load - free swimming, the SOD and GSH-Px levels in liver were increased significantly ($P < 0.05$) as compared with the sport control group, while the level of MDA decreased significantly ($P < 0.05$). These results suggest that GSPE

Table 1. The effects of GSPE in SOD, GSH-Px and MDA level in the mice's liver ($\bar{x} \pm s$) .

Group	SOD (U/mg.pro)	GSH-Px (U/mg.pro)	MDA (nmol/mg.pro)
Quiet control group (QCG)	746.58 ± 41.31	76.47 ± 13.24	1.41 ± 0.38
GSPE quiet group (GQG)	816.41 ± 34.27*	81.26 ± 10.46	1.23 ± 0.27*
Sport control group (SCG)	652.86 ± 31.82*	65.79 ± 12.93*	1.69 ± 0.34*
GSPE sport group (GSG)	706.53 ± 23.45*#	70.42 ± 11.58*#	1.48 ± 0.19*#

*P < 0.05 as compared with quiet control group ; #P < 0.05 as compared with sport control group.

Table 2. The effects of GSPE in mice in the level of blood glucose and liver and muscle glycogen ($\bar{x} \pm s$) .

Group	Blood glucose (mmol/L)	Liver glycogen (mg/g)	Muscle glycogen (mg/g)
Quiet control group (QCG)	5.26 ± 0. 23	7.23 ± 0.37	3.21 ± 0.15
GSPE quiet group (GQG)	5.19 ± 0. 17	11.93 ± 0.16	4.43 ± 0.24
Sport control group (SCG)	3.52 ± 0. 13*	4.26 ± 0.28*	1.31 ± 0.18*
GSPE sport group (GSG)	4.88 ± 0. 21*#	6.01 ± 0.31*#	2.46 ± 0.27*#

*P < 0.05 as compared with quiet control group; #P < 0.05 as compared with sport control group.

Table 3 The effects of GSPE in the level of TC, TG and FFA in mice's plasma ($\bar{x} \pm s$) .

Group	TC (mmol/L)	TG (mmol/L)	FFA (umol/l)
Quiet control group (QCG)	2.51 ± 0.12	1.57 ± 0.15	125 ± 6.21
GSPE quiet group (GQG)	2.63 ± 0.19	1.42 ± 0.19*	114 ± 6.67
Sport control group (SCG)	2.27 ± 0.13*	1.37 ± 0.06*	189 ± 4.25*
GSPE sport group (GSG)	2.11 ± 0.08*#	1.28 ± 0.25*	109 ± 4.38*#

*P < 0.05 as compared with quiet control group; #P < 0.05 as compared with sport control group.

can enhance the activity of antioxidant enzymes in liver, and inhibit the production of lipid peroxide.

Effects of GSPE in mice in the levels of blood glucose and liver and muscle glycogen

The effects of GSPE in mice in the level of blood glucose and liver and muscle glycogen were shown in Table 2. As can be seen from Table 2, as compared with the quiet control group, in a quiet state, the levels of liver and muscle glycogen in the mice which were force-fed GSPE significantly increased (P < 0.05), while the blood glucose level had no significant change (P > 0.05). After swimming with free load for 120 min, the blood glucose and liver and muscle glycogen levels in mice in the control group decreased significantly (P < 0.05) as compared with the quiet control group. After the mice being force-fed GSPE and driven to swim with free load, the blood glucose and liver and muscle glycogen levels in mice were significantly increased as compared with the sport control group (P < 0.05). These results suggest that exercise can decrease the level of blood glucose, liver glycogen and muscle glycogen, meanwhile, GSPE has functions such as keeping the stable level of blood glucose, increasing the reserves of liver and muscle glycogen, and preventing the level of blood glucose and the liver and muscle glycogen from decreasing after long time exercise.

Effects of GSPE in the level of TC, TG, FFA in mice's plasma

The effects of GSPE in the level of TC, TG, FFA in mice's plasma were shown in Table 3. As can be seen from Table 3, in a quiet state, as compared with the quiet control group, the TC and FFA levels in plasma in mice

which were force-fed GSPE had no significant change ($P > 0.05$), while the TG level decreased significantly ($P < 0.05$). After free - load swimming for 120 min, the levels of TC and TG in plasma in control group decreased significantly as compared with the quiet control group ($P < 0.05$), while the FFA level significantly increased ($P < 0.05$). After the mice being force-fed GSPE and driven to swim with free load, the level of TC, TG and FFA in plasma significantly decreased as compared with the sport control group ($P < 0.05$).

These results suggest that GSPE has functions of impacting fat metabolism in different conditions and promoting the utilization of fat.

DISCUSSION

The process of free radicals' generation and removal maintains homeostasis in normal physiological conditions. When doing prolonged and strenuous exercise, the body's ability of removing oxygen free radicals can not balance the oxygen free radicals generated during the exercise, and this will result in the imbalance between antioxidant and oxidation, then the body cells is in a state of oxidative stress (Martínez-Cayuela, 1995; Yu, 2003; Reid, 2008). There is a linear relationship between the increase of oxygen free radicals during exercise and the reduction of exercise capacity, and the main performance is that the oxygen free radicals results in the lipid peroxidation in the biofilm in various organs of the body (Coyle et al., 1997; Wan and Li, 2007). Malondialdehyde (MDA) is a lipid peroxide which is produced when the free radicals attack on the polyunsaturated fatty acids in biofilm, it is a sensitive indicator which can be used to measure the free radicals' damage and the free radical metabolism, and the damage situation as well as the metabolism of free radicals can be reflected by determining the content of MDA. GSH-Px and SOD are key enzymes in the body to eliminate free radicals. SOD can change the highly toxic superoxide anions (O_2^-) to O_2 and H_2O_2, then H_2O_2 and O_2^- react while the iron-chelating compounds exist, and produce ·OH which has strong activity; meanwhile, GSH-Px can further catalyzed the reduction of GSH and H_2O_2, oxidize H_2O_2 to H_2O and prevent the production of highly toxic OH. This study shows that, after swimming for 120 min, the activity of the antioxidant enzymes SOD and GSH-Px in liver significantly decreased, while MDA content significantly increased. It indicates that when doing vigorous exercise, the activity of SOD and GSH-Px in the body's organizations significantly decreased, and oxygen free radicals can not be clear away in time then the radicals will attack the membrane and produce MDA. The results are same as the existing conclusions (Xiong and Zhang, 2005; Bai XX. 2006; Yang et al., 2007). On the other hand, GSPE can improve the activity of antioxidant enzymes SOD and GSH-Px in liver and decrease the content of MDA. It indicates that the antioxidant function of GSPE maybe work by increasing the activity of body's antioxidant enzymes.

Glucose is the main energy material when body is doing anaerobic exercise, it is also the only fuel cells in the three nutrients that can synthesize ATP when doing anaerobic exercise. Glucose oxidation is the major energy source of body's high-intensity exercise (Wan and Li, 2007). Even on the condition of prolonged and low intensity exercise, glucose oxidation is also the first energy to be used, fat and protein are used only when the available sugar are used up (Coyle et al., 1997). During long time and high-intensity exercise, the glycogen reserves before exercise will impact the endurance training and the competition of athletic ability directly. For some sports which energy are supplied mainly by glycolysis, enough muscle glycogen reserves before the match are necessary, the reason is that too low muscle glycogen reserves will inhibit lactate production and reduce anaerobic metabolism. The importance of Liver glycogen in the exercise capacity is reflected in the endurance sports. Liver glycogen decomposes quickly during exercise and release glucose into the blood, in order to maintain the balance of blood glucose (Quintanilha, 1984).

The body absorbs glucose from the blood and consume muscle glycogen at the same time, and blood glucose can be supplemented to maintain balance by the decomposition of liver glycogen, when a large number of muscle glycogen and liver glycogen is consumed, and the content of blood glucose is dropped, the central nervous system can not be supplied with enough energy, the systemic fatigue and physical decline will occur (Zhao et al., 2007). In addition, the depletion of glycogen reserves can also lead to the shortage of oxaloacetate generation, then the acetyl CoA produced by fatty acid oxidation can not synthesize with oxaloacetate and produce the citric acid, which will participate in the citric acid cycle oxidation, then fatty acids are limited to supply energy by oxidation in the mitochondria (Chen et al., 2002; Ma et al., 2008).

Therefore, though the storage of fat in the human body is rich, sufficient glycogen reserves are still needed. The experimental results show that GSPE can significantly enhance the liver and muscle glycogen contents in mice in a quiet state, postpone the reduction of liver and muscle glycogen reduced by long time exercise and prevent the reduction of blood glucose after long time exercise. This shows that GSPE has effects on increasing the liver and muscle glycogen reserves, reducing the consumption of sugar and keeping the level of blood sugar stable. These effects of GSPE can guarantee the energy supply in central nervous system, sports organizations such as muscle and red blood cells, delay the occurrence of fatigue, thereby improve the exercise capacity. This may be one of the mechanisms of its anti-fatigue effect.

Prolonged and endurance exercise can cause depletion of glycogen reserves, accelerate the fat mobilization, then increase the free fatty acids (FFA) released into the plasma. If the generation rate of plasma free fatty acid in plasma is more than the oxidation utilization, the plasma FFA will significantly increase (Ma et al., 2008). The concentration of plasma FFA, especially unsaturated fatty acids is too high will inhibit the functions of Na^+-ATP enzyme in muscle cell membrane and Ca^{2+}-ATP enzyme in sarcoplasmic reticulum, then the capacity of these two kinds of enzymes hydrolysis adenosine triphosphate (ATP) enzyme is diminished, and this will impact the formation of muscle membrane action potentials, as well as the absorption of Ca^{2+} in sarcoplasmic reticulum, then the processes of muscle contraction and relaxation are all affected, and this is an important reason for the formation of exercise-induced fatigue (Clausen, 2003; Qu, 2008). In addition, free fatty acids in plasma can competing with tryptophan to combine with albumin and increase the concentration of free tryptophan in plasma; tryptophan is the precursor of 5 - HT, and the increase of 5 - HT can also result in the central nervous system fatigue (Xu et al., 2008; Ma et al., 2008). The experimental results show that in a quiet state, after force-feeding GSPE, the levels of TC and FFA in plasma in mice have no significant changes as compared with the quiet control group, while the TG level significantly decreased, indicating that GSPE can affect the fat metabolism in normal mice and lower blood lipids; this effects of GSPE may enhance the body to oxidize and use fat. After prolonged exercise, TC and TG in plasma in mice - decreased significantly as compared with the quiet control group, while the level of FFA significantly increased; the following factors are related to the decrease of TG in plasma: triglycerides are consumed in sports, endogenous synthesis of triglycerides is reduced and the activity of lipid protein lipase increased promotes the clearance of triglycerides (Clausen, 2003). The level of FFA- increased significantly because that the generation of free fatty acid in plasma is more than the body's fat oxidation utilization. After prolonged exercise and force-feeding GSPE, the concentrations of TC and FFA in plasma were lower than the sport control group, and TG also has the trend of reducing; it indicates that GSPE has a strong ability of fat removal, and its effects on FFA in plasma may be that it can increase the quantity of mitochondria in muscle and improvethe mitochondrial's ability of oxidizing and utilizing fatty acid, then it can speed up the clearance of plasma FFA. Thus, GSPE can effect the fat metabolism under different conditions and promote the fat utilization. This may be another mechanism of its anti-fatigue effect.

Conclusion

The results above show that GSPE can significantly increase the activity of antioxidant enzymes in mice and clear the free radicals in body, so it can protect the body against free radical damage. At the same time, GSPE can affect glucose metabolism in mice, increase the liver and muscle glycogen reserves, reduce the consumption of glucose and keep the level of blood glucose stable. In addition, GSPE can affect the fat metabolism in mice and promote the utilization of fat.

ACKNOWLEDGEMENTS

This research was supported by education department of China's Zhejiang province scientific research grant (Project No. 20080543).

REFERENCES

Bagchi D, Bagchi M, Stohs SJ, Das DK, Ray SD, Kuszynski CA, Joshi SS, Pruess HG (2000). Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention. Toxicol. 148: 187-197.

Bagchi D, Bagchi M, Stohs SJ, Ray SD, Sen CK, Pruess HG (2002). Cellular protection with proanthocyanidins derived from grape seeds. Ann. New York Acad. Sci. 957: 260-270.

Bai XX (2006). The effect of Arctium lappal on the exhausting exercising capability of rats. J. Phys. Edu. 13: 53-56.

Chen KT, SuCH, Hsin LH, Su YC, Su YP, Lin JG (2002). Reducing fatigue of athletes following oral administration of Huangqi Jianzhong Tang. Acta Pharmacologica Sinica 23: 757-761.

Chi AP, Chen JP, Wang ZZ, Xiong ZY, Li QX (2008). Morphological and structural characterization of a polysaccharide from Gynostemma pentaphyllum Makino and its anti-exercise fatigue activity. Carbohydr. Poly. 74: 868-874.

Clausen T (2003). Na^+-K^+ pump regulation and skeletal muscle contractility. Physiol. Rev. 83: 1269-1273.

Coyle EF, Jeukendrup AE, Wagenmakers AJ, Saris WH (1997). Fatty acid oxidation is directly regulated by carbohydrate metabolism during exercise. Am. J. Physiol.- Endocrinol. Metab. 273: E268-E275.

Engelbrecht AM, Mattheyse M, Ellis B, Loos B, Thomas M, Smith R, Peters S, Smith C, Myburgh K (2007). Proanthocyanidin from grape seeds inactivates the PI3-kinase/PKB pathway and induces apoptosis in a colon cancer cell line. Cancer Lett. 258: 144-153.

Feng JG, Chen LQ (2003). Determination of Procyanidin in Grape Seed Extracts. China Food Additives 6: 103-105.

Feng YZ, LiuYM, Fratkins JD, LeBlan MH (2005). Grape seed extract suppresses lipid peroxidation and reduces hypoxic ischemic brain injury in neonatal rats. Brain Res. Bull. 66: 120-127.

Iacopini P, Baldi M, Storchi P, Sebastiani L (2008). Catechin, epicatechin, quercetin, rutin and resveratrol in red grape: Content, in vitro antioxidant activity and interactions. J. Food Comp. Anal. 21: 589-598.

Ma L, Cai DL,Li HX, Tong B, Song LH, Wang Y (2008). Anti-fatigue effects of salidroside in mice. J. Med. Colleges of PLA 23: 88-93.

Martínez-Cayuela M (1995). Oxygen free radicals and human disease. Biochimie 77: 147-161.

Morillas-Ruiz JM, Villegas Garcia JA, Lo´pez FJ, Vidal-Guevara ML, Zafrilla P (2006). Effects of polyphenolic antioxidants on exercise-induced oxidative stress. Clin. Nutr. 25: 444-453.

Qin MQ, Zhang GQ, Qin ZL (2006). Effects of OPC on CK,MDA,SOD,GSH-PX for Taekwondo athletes after heavy load training. J. Shandong Inst. Phys. Edu. Sports 22: 72-73.

Qu Y (2008). On the Cause and Removing Method of Motion Fatigue. J. Baotou Vocat. Techn. Coll. 9: 32-34.

Quintanilha AT (1984). Effects of physical exercise and/or vitamin E on tissue oxidative metabolism. Biochem. Soc. Transac. 12: 403-404.

Reid MB (2008). Free radicals and muscle fatigue: Of ROS, canaries, and the IOC. Free Radical Biol. Med. 44: 169-179.

Sano T, Oda E, Yamashita T, Naemura A, Ijiri Y, Yamakoshi J, Yamamoto J (2005). Anti-thrombotic effect of proanthocyanidin, a

purified ingredient of grape seed. Thrombosis Research 115: 115-121.
Silva EM, Souza JNS, Rogez H, Rees JF, Larondelle Y (2007). Antioxidant activities and polyphenolic contents of fifteen selected plant species from the Amazonian region. Food Chem. 101: 1012-1018.
Spranger I, Sun B, Mateus AM, Freitas V, Ricardo-da-Silva JM (2008). Chemical characterization and antioxidant activities of oligomeric and polymeric procyanidin fractions from grape seeds. Food Chem. 108: 519-532.
Wan BJ, Li YR (2007). Research On Anti - fatigue Effect of Herba Eclipta. J. Anhui Sports Sci. 28: 51-53.
Wu LL, Miao AQ, Ji SM, Liu G (2008). Study the Function of Antifatigue after Exercise with the Prescription of Bei Li. J. Sports Sci. 29: 40-42.
Xiong ZY, Zhang QJ (2005). Efects of Melatonin and Exhaustive Exercise on Anti-oxidation System in Endurance-trained Mice's liver, Skeletal M us cle and Kidney. Chinese J. Sports Med. 24: 210-213.
Xu CX, Liu HT, Wang J (2008). Changes of 5-HT and TPH in the Ventral Horn of Spinal Cord after Exercise-induced Fatigue. Chinese J. Sports Med. 27: 560-562.
Yamakoshi J, Kataok S, Koga T, Ariga T (1999). Proanthocyanidin-rich extract from grape seeds attenuates the development of aortic atherosclerosis in cholesterol-fed rabbits. Atherosclerosis 142: 139-149.
Yang XY, Zhang Y, Liu HL, Lin JS, Cheng GY (2007). Research on the Taurine Complex Liquid Effecting on the White Rats Ability of Anti Sports Fatigue. Sport Sci. Technol. 28: 43-45.
Yu F (2003). The present study and application of the scavenger of the oxygen free radical to sports. J. Northwest Normal University (Natural Science Edition) 3: 113-116.
Zhao WS, Eerdun, Ma YY, Wang J, Menghebaoligao, Zhang YT (2007). The Influence of Lüjian Sports Drink on Some Physiological Indexes of Boxing Athletes and Kickboxing Athletes. Med. J. Nat. Defending Forces in North China 19: 43-46.