CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

EFFECTS OF ANTI-FATIGUE FUNCTION OF SPINE GRAPE AND CERASUS HUMILIS NORMAL JUICE ON PHYSICAL FITNESS OF STUDENTS AND DEVELOPMENT OF SPORTS

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Article history:
Received:
4 January 2016
Accepted:
20 June 2016

Keywords:
Grape and cerasus humilis
Normal juice;
Sports beverage;
Anti-fatigue activity;
Physical fitness;
Development of sports.

ABSTRACT
Adolescent students can gain energy and nutritional substances and recover from fatigue by drinking sports beverage after sports. Taking spine grape and cerasus humilis as raw materials, we developed a kind of anti-fatigue sports beverage with special flavor according to national formulation requirements for sports beverage. Besides, anti-fatigue activity function of the sports beverage was evaluated by carrying out animal experiment. The beverage made from spine grape and cerasus humilis is proved to be a natural sports beverage with good anti-fatigue function and can effectively strengthen physical performance, relieve fatigue, help remove free radicals brought by high-strength or exhaustive exercise, prevent early generation of fatigue, increase human tolerance, improve overall athletic ability and promote the development of sports field.

1. Introduction

Comprehensive development of adolescent students has long been a hot topic concerned worldwide (Kokko et al., 2006; Hu et al., 2015; Akiyama et al., 2013). Having a healthy body is the basic requirement put forward by Central Committee of the Chinese Communist Party and the State Council for adolescent students from the perspectives of Chinese talent cultivation and sustainable development strategy, which is not only the premise for serving the country and people but also the sign of vigorous vitality of Chinese nation (Sun, 2013; P-Mar-Apr, 2006). Physical fitness is an important measuring standard for physique conditions. Good physical fitness is an important condition for physical education and sports. Athletic ability of teenagers which can be reflected directly by physical fitness is an important sign of physique conditions. Overall, good physical fitness during adolescence is an important condition for healthy life in future (Wang et al., 2015; Zheng et al., 2013; Feng et al., 2014). Due to the demand of sportsmen and body builders, functional sports beverage develops (Dimitrovski et al., 2016). Internal environment will change, i.e., human body will be tired, lose water and have excessive free radicals when sports load of human body exceeds certain value. Therefore, it is of great significance to supplement exogenous substrates to relieve fatigue and help rapid recovery of body (Spierer et al., 2013; Galemore, 2011; Kinnear and Kock, 2011); moreover, it provides a good environment for expansion of sports beverage market. This study focuses on two natural fruits, i.e., spine grape (Lai et al., 2014) and cerasus humilis (Chang et al., 2011), which contain rich vitamins, amino acid, anthocyanin, resveratrol and microelement. Spine grape and cerasus humilis normal juice can relieve fatigue, remove residual free radicals and accelerate recovery. Functional sports beverage made

from spine grape and cerasus humilis is useful for improving athletic ability of adolescent students and promote the development of sports field.

2. Materials and methods

2.1. Experimental materials

Sports beverage made from spine grape and cerasus humilis, Gatorade sports beverage and normal saline were taken as research objects. One hundred healthy Kunming mice (50 males and 50 females), weighed (18 ± 2) g, were selected. Experimental instruments included superoxide dismutase (SOD) detection kit, malondialdehyde (MDA) detection kit, blood urea nitrogen (BUN) detection kit, blood lactic acid (LAC) detection kit, liver glycogen detection kit, visible spectrophotometer, TDL-40B low-temperature high-speed centrifuge, FA/JA electron balance, tank for loaded swimming (70 × 50 × 40 cm), lead sheath, etc.

2.2. Experimental method

Raising and grouping of experimental animals

Before exhaust experiment, the experimental mice were raised for a period of time; and in that period, the mice were quarantined. During quarantine, the mice ate and drank freely at temperature of 20 ~ 30 °C. One week later, all qualified mice experienced two-day adaptive swimming in a tank with water deep of 30 cm and temperature of 25 ± 2 °C. After swimming training, mice which grew normally and were able to swim were selected and divided into three groups, i.e., group one, group two and group three (control group), 10 in each group. Mice in group one received gavage of sports beverage made from spine grape and cerasus humilis; mice in group two received gavage of Gatorade sports beverage; mice in group three received gavage of normal saline. The gavage was performed for 20 days, once a day, with a daily dose of 0.1ml/10g (beverage quantity/weight).

Safety inspection

During 20 days, food and water intake, defecation, growth, behavior and toxic signs of mice in three groups were observed. After experiment, whether liver, heart, lung, kidney, stomach, jejunum, spleen, thymus gland and ovary/testis of test mice had injury or pathological changes were checked.

Determination of growth speed of mice

Weight of experimental mice would change before and after experiment; hence tracking and record were necessary in order to calculate the average net weight gain.

Loaded swimming experiment

Gavage lasted for 20 days. Thirty minutes after the last gavage, the mouse was loaded with lead sheath whose weight was 5% that of mouse on the tail and put in a 30 cm-deep swimming tank with water temperature of 25 ± 2 °C. The experiment ended when mice ran out of energy, i.e., mice failed to float or show righting reflex after sinking into water for 10 s. That period of time was regarded as duration of loaded swimming.

Determination of viscera index of mice

Weight of the mice was measured after sports. Then the mice were killed and the organs were taken out for weighing. Viscera index was calculated (viscera index was expressed by the ratio of weight of organs (mg) and weight (g)). The experimental operation strictly followed the instruction on blood lactic acid detection kit.

Determination of LAC content

The mice were taken out of water after running out of energy; then blood was immediately extracted from eye socket for preparation of serum. Then the content of blood lactic acid was detected using blood lactic acid detection kit and blood lactic acid instrument. The experimental operation strictly followed the instruction on the blood lactic acid kit.

Determination of hepatic glicogen content

Organs were taken out after exhaustive mice were killed. The organs were washed by normal saline and dried by filter paper. 100 mg of liver was weighed precisely using electronic analytical balance. The content of hepatic glucogen was detected using hepatic glucogen kit. The experimental operation strictly followed the instruction on the hepatic glucogen kit.

Determination of superoxide dismutase (SOD) content

Mice were taken out from water after running out of energy; then blood was immediately extracted from eye socket. Serum was prepared and then the content of SOD in serum was detected. The experimental operation strictly followed the instruction on the SOD detection kit.

Determination of MDA content

Mice were taken out from water after running out of energy; then blood was immediately extracted from eye socket. After preparation of serum, the content of MDA in serum was detected using visible spectrophotometer. The experimental operation strictly followed the instruction on the MDA detection kit.

Determination of BUN content

Mice were taken out from water after running out of energy; then blood was immediately extracted from eye socket. After 3 hour-standing, the blood was centrifuged at 3000 r/min at low temperature for 15 min. Finally 1μl of serum was obtained. BUN detection kit was used to detect the content of serum BUN. The experimental operation strictly followed the instruction on the BUN detection kit.

2.3. Statistical analysis

Statistical Package for Social Sciences (SPSS) was used for statistical analysis. Data were expressed as mean ± SD and processed by independent sample t test. Difference was considered statistically significant if $p < 0.05$.

3. Results and discussions

3.1. Analysis of safety of sports beverage made from spine grape and cerasus humilis

During 20 days, mice in the three groups behaved normal in aspects of eating, water drinking and defecation and grew well; besides, no obvious changes or intoxicating phenomenon were observed. At the end of the experiment, organs of the experimental mice were examined; and no significant pathological changes were observed, suggesting no damage was induced by the test sample. Therefore, we consider sports beverage made from spine grape and cerasus humilis is safe and nontoxic.

3.2. Changes of growing speed of mice under the effect of sports beverage made from spine grape and cerasus humilis

Results of weight and net weight increase of mice in the three groups before and after experiment are shown in Table 1. Average net weight increase of mice that were gavaged with sports beverage made from spine grape and cerasus humilis for 20 days was 4.823 g and average net weight increase of mice that were gavaged with Gatorade sports beverage was 4.941 g, higher than that of the mice in the control group; but there was no significant difference. Therefore, sports beverage made from spine grape and cerasus humilis was considered to have no effects on growth of body.

3.3.Changes of duration of exhaustive swimming under the effects of sports beverage made from spine grape and cerasus humilis

The effect of anti-fatigue drugs can be evaluated through loaded swimming experiment; exercise tolerance directly affects duration of swimming and thus reflects fatigue degree after sports (Ting-Jun and Yi-Qing, 2012).

Table 1. Changes of growing speed of mice under the effect of sports beverage made from spine grape and cerasus humilis (mean ± SD, n = 10)

Group	Weight before experiment (g)	Weight after experiment (g)	Average net weight increase (g)
Group one	18.781±2.649	22.997±1.008	4.982±1.718
Group two	17.963±2.481	22.756±1.067	4.818±1.766
Group three (control group)	19.276±1.957	23.845±0.738	4.988±2.001

Table 2 shows duration of loaded swimming of mice gavaged with different things. We found 20-day gavaging of sports beverage made from spine grape and cerasus humilis and Gatorade sports beverage can significantly extend duration of loaded swimming; compared to the control group, there were significant differences. But duration of loaded swimming of two sports beverage groups had no significant difference. Thus sports beverage made from spine grape and cerasus humilis has certain anti-fatigue effect.

Table 2. Changes of duration of loaded swimming under the effect of sports beverage made from spine grape and cerasus humilis (mean ± SD, n = 10)

Group	Duration of loaded swimming (min)
Group one	26.458±2.741
Group two	29.836±3.391
Group three (control group)	17.569±2.756

3.4. Changes of visceral index of mice under the effects of sports beverage made from spine grape and cerasus humilis

Ratio of visceral index to weight of mice in each group at the end of experiment is shown in Table 3. The ratio of liver, kidney and spleen to weight of mice gavaged with sports beverage made from spine grape and cerasus humilis and mice gavaged with Gatorade sports beverage had no significant difference. However, the ratio of thymus gland to weight of mice gavaged with sports beverage was significantly different from that of mice in the control group.

3.5. Changes of biochemical indexes of mice after exhaustive swimming under the effect of sports beverage made from spine grape and cerasus humilis

Content of LAC

In the beginning of sports, glycolysis reaction provides energy for muscle and moreover a large amount of LAC generates. When sports end, glycolysis stops and LAC reduces, which leads to the decrease of LAC content and recovery of body. It can be seen that, lowering output of LAC and improving removal speed of LAC is effective in resisting fatigue. The content of LAC becomes higher as LAC produced during sports will rapidly penetrate into blood; the phenomenon continues until LAC in muscle and blood reaches a balance. Content of LAC of mice after exhaustive swimming is shown in Table 4. It can be seen from Table 4 that, the content of LAC of mice gavaged with sports beverage made from spine grape and cerasus humilis and Gatorade sports beverage was 7.147 ± 0.502 mmol/ml and 6.566 ± 0.418 mmol/ml, much lower than the control group; there were significant differences. However, there was no significant difference of content of LAC between mice gavaged with sports beverage made from spine grape and cerasus humilis and mice gavaged with Gatorade sports beverage. Therefore, it can be concluded that, sports beverage made from spine grape and cerasus humilis has strong effect in removing LAC and relieving fatigue.

Table 3. Changes of visceral index of mice under the effect of sports beverage made from spine grape and cerasus humilis (mean ± SD, n = 10)

Group	Liver (mg/10g)	Spleen (mg/10g)	Kidney (mg/10g)	Thymus gland (mg/10g)
Group one	6.522±0.398	0.739±0.071	6.852±0.398	0.354±0.006
Group two	6.587±0.408	0.728±0.047	7.065±0.189	0.352±0.008
Group three (normal group)	6.392±0.465	0.757±0.052	6.557±0.485	0.313±0.001

Content of hepatic glycogen

Hepatic glycogen can provide energy for contraction of muscle fiber and keep normal level of glucose in blood. The availability of hepatic glycogen is the key factor influencing the beginning of muscle fatigue (Eduardo et al., 2009). The content of hepatic glycogen of mice after exhaustive swimming is shown in Table 4. Both of the content of hepatic glycogen of mice gavaged with sports beverage made from spine grape and cerasus humilis and Gatorade sports beverage had significant difference with that of the control group; but the difference between the two groups was not remarkable. Thus it can be concluded that, the two kinds of sports beverage can provide more hepatic glycogens and lower consumption of hepatic glycogen, thus to improve physical fitness, tolerance and fatigue resistance.

Content of SOD

SOD is the only enzyme whose substrate is oxygen free radical and an important enzyme which is effective in removing hydrogen peroxide. As shown in Table 4, the content of SOD of mice gavaged with sports beverage made from spine grape and cerasus humilis and Gatorade sports beverage significantly increased, both of which showed a significant difference from the control group; but the content of SOD of mice gavaged with sports beverage made from spine grape and cerasus humilis and Gatorade sports beverage had no remarkable difference. The action mechanism may be that, functional sports beverage contains substances that can activate activity of antioxidant enzyme or induce synthesis of antioxidant enzyme.

Content of MDA

MDA can be used for measuring metabolism of free radicals. MDA as the representative product of lipid peroxide can reflect the level of free radicals objectively. The change direction of activity of SOD is opposite to change direction of MDA. Research results are shown in Table 4. Compared to the control group, the content of MDA of mice gavaged with sports beverage made from spine grape and cerasus humilis and mice gavaged with Gatorade sports beverage had remarkable decline; however, the content of MDA of mice gavaged with sports beverage had no significant difference. Therefore, it can be concluded that, sports beverage made from spine grape and cerasus humilis is effective in improving antioxidant ability and inhibiting lipid peroxidation.

Content of BUN

Catabolism of nitrogen substance can be directly reflected by the content of BUN and it is also the evaluation index for sports load that can be tolerated in special conditions. Table 4 shows the changes of content of BUN of mice after loaded swimming. It can be seen from Table 4 that, BUN content of mice gavaged with sports beverage and mice gavaged with normal saline had no significant difference ($p > 0.05$).

When sports time was lower than 30 min, there was no involvement of protein during energy supply; as BUN is the product of protein metabolism, there are no remarkable changes of BUN. That might also be the reason why the regulatory effects of the sports beverages on BUN are not remarkable.

Table 4. Effects of sports beverage made from spine grape and cerasus humilis on biochemical indexes of mice after exhaustive swimming (mean ± SD, n = 10)

Group	LAC (mmol/ml)	Hepatic glycogen (mg/g)	SOD (u/ml)	MDA (nmol/ml)	BUN (mmol/ml)
Group one	7.147±0.502	2.321±0.276	129.685±11.321	2.681±0.316	22.985±2.256
Group two	6.566±0.418	2.348±0.296	137.521±6.758	2.411±0.359	25.174±1.285
Group three (control group)	10.078±0.832	1.859±0.187	114.556±5.875	4.215±0.348	24.295±1.927

In this study, we gavaged mice that weighed 18 g with normal saline, sports beverage made from spine grape and cerasus humilis and Gatorade sports beverage for 20 days, then observed the growth condition of mice and tested the effects of sports beverage on weight and visceral index of mice. Research results showed that, food intake and water drinking conditions of mice were normal; no mice died; there was no significant difference of weight before and after experiment; no pathological changes and abnormality were observed in various organs after anatomy; visceral index of liver, spleen and kidney of mice gavaged fluctuated within normal scope, and the difference between mice gavaged with sports beverage and normal saline had no statistical significance. Therefore, it can be concluded that, sports beverage made from spine grape and cerasus humilis has no adverse effects on growth and development of animals and it is safe and non-toxic.

Wild spine grape which contains natural antioxidative substance such as procyanidine and resveratrol is the main component of sports beverage made from spine grape and cerasus humilis. It has been found that, resveratrol has good immunomodulatory effect (Basini et al., 2010; Delmas et al., 2013). Besides, a study (Liu and Huang, 2006) suggested that long-term intensive training could result in apoptosis of lymphocyte through consuming nutritional substances, changing neuro-endocrine function and attacking membrane of immune cells and thus lead to decline of immune function. In the test, thymus index of the mice gavaged with sports beverage was much higher than that of the control mice, which was because of polyphenols contained in the sports containing spine grape.

SOD, the defense system of human body, can eliminate free radicals to keep its dynamic balance (Tina et al., 2009; Cai and Wang, 2014). In the experiment, two kinds of sports beverage both led to significant increase of SOD, suggesting functional sports beverage was able to eliminate free radicals and strengthen antioxidant capacity. MDA as the final product of metabolism of lipid peroxidation cam indirectly reflect the severity of injury of cells induced by oxygen free radical (Valente et al., 2011). Besides, the functional sports beverages resulted in significant decline of content of MDA, which further suggested the ability of eliminating free radicals of functional sports beverage. Hence it can be concluded that, the sports beverage can improve antioxidant capacity and thus enhance athletic ability of mice.

Besides, it has been pointed out that, procyanidine in red grape wine can eliminate free radicals, resist oxidation and prevent cardiovascular disease and atherosclerotic plaque (Gutha et al., 2010). Therefore, it can be concluded that, sports beverage made from spine grape and cerasus humilis has functions of eliminating free radicals and resisting oxidation, which is because of the high content of procyanidine in the sports beverage made from spine grape and cerasus humilis.

Sports beverage made from spine grape and cerasus humilis as a natural sports beverage with effective anti-fatigue function can effectively enhance body functions, relieve fatigue, help eliminate free radicals induced by high-strength or exhaustive sports, improve

tolerance and enhance overall athletic ability; hence it can fully satisfy the need of teenagers during sports. The development of sports beverage made from spine grape and cerasus humilis extends the market of sports beverage.

4. Conclusions

Wild spine grape and cerasus humilis are the raw materials of the developed sports beverage. The application of two fruits in development of functional sports beverage can not only stimulate appetite of people with full flavor, but also can promote the supplement of body fluid of teenagers during sports and satisfy their need of nutrition. We break the conventional idea of supplementing athletes with sugar and electrolyte only and make use of the high content of resveratrol and procyanidine to delay sense of fatigue and enhance sports performance. Besides, in view of the high safety and functionality, the sports beverage is expected to be promising in the market of functional sports beverage.

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