CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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

ANALYSIS OF RELIEF EFFECT OF CERVUS ELAPHUS BLOOD WINE ON PHYSICAL ACTIVITY INDUCED FATIGUE

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Article history:
Received:
15 November 2016
Accepted:
24 August 2016

Keywords:
Cervus elaphus blood wine;
Physical activity induced fatigue;
Mice;
Fatigue resistance;
Quality analysis.

ABSTRACT
This study detected quality indexes of Cervus elaphus blood wine and analyzed its effect on physical activity induced fatigue of mice, providing a theoretical basis and data support for the further development and value improvement of Cervus elaphus blood wine. Several major quality indexes of Cervus elaphus blood wines from three batches were detected and analyzed. Besides, the effect of Cervus elaphus blood wine in relieving physical activity induced fatigue was analyzed by comparing the effects of Cervus elaphus blood wines in three different doses and wine base on the resistance of physical activity induced fatigue of mice with a wine base control group. It was found that, effects of Cervus elaphus blood wines in different doses on the weight of mice had no significant difference, but Cervus elaphus blood wine could effectively prolong the loaded swimming time of mice, significantly increase the level of hepatic glycogen of mice, reduce the content of serum urea and blood lactic acid of mice, and relieve physical activity induced fatigue of mice. Cervus elaphus blood wine containing many nutritional components such as amino acid, vitamin and lipid can enhance immunity and produce fatigue resistance effect. Cervus elaphus blood wines in different doses can relieve fatigue of mice to various degrees; hence Cervus elaphus blood wine has good edible value and broad market.

1. Introduction

Cervus elaphus blood refers to the blood of Cervus elaphus deer or red deer. A large number of animal experiments and clinical researches in recent years have suggested that, Cervus elaphus blood, in indeed, has many healthcare functions including maintaining beauty, keeping young, treating anemia, regulating immunity, delaying aging, relieving fatigue and improving memory, which provide a new development space and opportunity for Cervus elaphus industry and relevant medical and health care product industries (Gombotz, 2012; Liu et al., 2013; Cook et al., 2010). Wine made from Cervus elaphus blood, commonly known as healthcare wine, is a kind of valuable medicinal wine which integrates the edible value and medical value of Cervus elaphus blood; it has significant healthcare and medicinal efficacy and functions of enhancing immune functions, improving working ability, relieving fatigue, and improving sleep (Wiebrecht et al., 2014; Hou et al., 2011).

Physical activity induced fatigue refers to temporary decline of the maximum contraction or the maximum output power of muscle induced by physical activities. The occurrence of physical activity induced fatigue and the degree of fatigue are determined by considering the functional level and motor activity of

tissues and organs in human body. Causes for physical activity induced fatigue mainly include energy exhaust, metabolite accumulation and disordered endocrine regulation (Oliveira et al., 2013; Ishii et al., 2013). As the physical structure of mice is similar to that of human body, this study selected mice as experimental subjects for the analysis of anti-fatigue function of Cervus elaphus blood wine.

In recent years, many experts and scholars in China and abroad have explored the functions of Cervus elaphus blood. In 2015, Esbaugh A J et al. (Esbaugh et al., 2015) pointed out that, Cervus elaphus blood could promote the formation and maturity of T4 cells, which suggested it might have the function of enhancing the immune function of human body. Chang CW et al. (Chang et al., 2016] found that, oral administration of Cervus elaphus blood could accelerate the healing of ulcer and wound, strengthen the regeneration process of skin, further promote the healing of bone fracture as well as the metabolism of carbohydrate and nitrogen matters, improve memory, gastrointestinal function, sleep and vision, regulate blood pressure, resist radiation, reduce toxic and side effects of chemotherapy, and clear and nourish throat. It is of great significance to carry out studies on healthcare foods which are mainly made of Cervus elaphus blood and discuss over its processing techniques and specific healthcare functions, because those actions have important economic significance and scientific values to the improvement of deep processing level of Cervus elaphus blood and the expansion of its application approaches (Kanter et al., 2014). This work provides theoretical and data support for the further development and utilization of Cervus elaphus blood wine through analyzing the quality of Cervus elaphus blood wine and studying the effect of Cervus elaphus blood wine on the resistance of physical activity induced fatigue of mice.

2. Materials and methods

2.1. Experimental subjects and materials

(1) Cervus elaphus Linnaeus, Chinese date, arillus longan, Chinese wolfberry fruit and ginseng (Beijing Chinese traditional medicine wholesale market, China).

(2) Cervus elaphus blood wine (Yanghe Wine Co., Ltd., China).

(3) One hundred and sixty male Kunming mice, weighed 20 g, were randomly divided into four groups, i.e., wine base control group (wine base 15% v/v), Cervus elaphus blood gavage groups (gavage using Cervus elaphus blood wine in doses of 4.6 mL/kg.Bw/d, 9.2 mL/kg.Bw/d and 18.4 mL/kg.BW/d), 40 in each group. All mice could take food and water freely. All animal experiment operations followed the requirements of experimental animal management committee, reviewed and approved by the animal ethics committee and verified by pathologists.

2.2. Experimental instruments and reagents

2.2.1 Experimental instruments

Instruments included microbiological incubator (Shanghai Heheng Instrument and Equipment Co., Ltd., China), spectrophotometer (Hangzhou Qianjiang Instrument and Equipment Co., Ltd., China), alcoholmeter (Shanghai Shangbi Experimental Instrument Co., Ltd, China), atomic absorption spectrophotometer (AAS) (Beijing Dafengrui Instrument Co., Ltd., China), swimming box (50 cm × 50 cm × 40 cm), thermostat water bath (Beijing Xinbiao Tengda Instrument and Equipment Co., Ltd., China), second chronograph, electronic scale, etc.

2.2.2. Experimental reagents

Reagents included serum urea detection kit, hepatic glycogen detection kit, whole blood lactate detection kit, sodium sulfite, sodium hydroxide, absolute ether, sulfuric acid, acetonitrile, methyl alcohol, etc.

2.3. Preparation technique of Cervus elaphus blood wine

Firstly, serum was separated from Cervus elaphus blood and then the PH value of Cervus elaphus blood was decreased to 7. After one-day enzymolysis using protease, the Cervus elaphus blood was mixed with white wine (65% v/v) and immersed in a closed container. Fifteen days later, it was filtered after the removal of supernate.

Secondly, Chinese date and arillus longan selected were denucleated and beaten to pieces; ginseng, Chinese wolfberry fruit and cervus elaphus linnaeus selected were dried and grinded. All the processed raw materials were mixed up, mixed with white wine (65% v/v), and immersed in a closed container for 15 days. The mixture was stirred for 15 min every day. Prefiltration was performed fifteen days later.

The Cervus elaphus blood and raw materials which were processed by prefiltration were mixed up and blended with honey for the second filtration.

The liquid obtained after the second filtration was tested and encapsulated. Finally, Cervus elaphus blood wine was obtained.

2.4. Properties of Cervus elaphus blood wine finished products

Cervus elaphus blood wine obtained was red brown, clear, mellow, warm and tasty. Besides, it had functions of nourishing blood, boosting essence, promoting circulation of blood, removing stasis, eliminating swelling, resisting aging, maintaining beauty and extending life. The Cervus elaphus blood wine contained 80 % of water and 16% ~ 17% of organic matters; protein was the major component. The alcohol degree of the Cervus elaphus blood wine was 35% (v/v) and 1 ml of Cervus elaphus blood wine contained 0.97 g of herbal component. The wine had a high medical value and edible value.

2.5. Experimental method

2.5.1. Mouse loaded swimming experiment

Each mouse was gavaged with 0.4 ml of Cervus elaphus blood wine, once each day, totally for one month. Half an hour after the last time of gavage, lead sheath whose weight was 5% that of a mouse was bounded on the tail of mouse. Then the mice were put into the swimming box. The swimming time of each mouse from the beginning of swimming to death was recorded.

2.5.2. Detection of serum urea of mice

Half an hour after the last time of gavage, mice were put into the swimming box for 90 min-swimming.

At the end of swimming, the mice were taken out of the swimming box. Blood was collected from eyeballs of the mice after one hour of rest. After the blood froze, it was centrifuged at 14000 rpm. Ten minutes later, the upper-layer serum was taken for detection. Serum urea was detected using urea detection reagents.

2.5.3. Detection of hepatic glycogen of mice

Half an hour after the last time of gavage, the liver was removed and put into a liquid nitrogen container for detection.

Then liver specimens were rinsed with normal saline. After the removal of water using filter paper, the weight of tissue was weighed precisely.

The specimen and alkaline liquor (weight of specimen: volume of alkaline liquor: 1:3) were added into a tube and cooked with boiling water. Twenty five minutes later, it was cooled using flowing water.

Finally, hepatic glycogen of mice was detected using glycogen detection liquid made of glycogen hydrolysate.

2.5.4. Detection of blood lactic acid of mice

Half an hour after the last time of gavage, blood was collected from eyeballs of mice through angular vein using capillary glass tubes.

The mice were put into the swimming box for unloaded swimming after blood collection. Ten minutes later, blood was collected from eyeballs of the mice for the detection of the content of blood lactic acid.

After half an hour of rest, 20 μl of blood was collected from eyeballs of each mouse for the detection of blood lactic acid.

2.6. Data statistics

SPSS ver. 19.0 was used to statistically analyze experimental data. The difference between the experimental groups and the control group was analyzed; the relevant data were processed by one-way analysis of variance using SPSS ver. 19.0. The measured data were expressed as mean ± standard deviation (SD). The comparison between groups was statistically processed by analysis of variance. $p < 0.05$ indicated the difference had statistical significance.

3. Results and discussions

3.1. Detection of physicochemical indexes of Cervus elaphus blood wine (Table 1)

Table 1. Statistics of the measured indexes of Cervus elaphus blood wine

Test item	Unit	Batch No. 20080310	Batch No. 20080517	Batch No. 20080819
Cervus elaphus blood wine identification test	-	Positive	Positive	Positive
Panaxoside Re	Mg/L	110	109	105
The degree of alcohol (v/v)	%	35.76	35.84	35.97
Acetic acid	g/L	1.35	1.43	1.39
Methyl alcohol	g/mL	0.6	0.6	0.5
Lead	Mg/L	<0.1	<0.1	<0.1
Manganese	Mg/L	<1	<1	<1
Arsenic	Mg/L	<0.1	<0.1	<0.1
Total number of bacterial colony	cfu/mL	<1	<1	<1
Yeast	cfu/mL	<1	<1	<1

The above statistical data suggested that, all values of the indexes were up to specifications.

3.2. Detection results of microbacterial indexes (Table 2)

Table 2. Statistical table of detects results of microbiological indexes of Cervus elaphus blood wine

Test item	Unit	Batch No. 20080310	Batch No. 20080517	Batch No. 20080819
Total number of bacterial colony	cfu/mL	<1	<1	<1
Coli group	MPN/100mL	<3	<3	<3
Mycete	cfu/mL	<1	<1	<1
Saccharomycetes	cfu/mL	<1	<1	<1

It could be known from Table 2 that, the total bacterial count was smaller than 1 cfu/ml, the total count of coli group was smaller than 3 MPN/100 ml, and the total count of mould and saccharomycetes was smaller than 1 cfu/ml, conforming to food microbiological detection standard.

3.3. Effects of Cervus elaphus blood wine on swimming time of mice

Mice were put into swimming box for loaded swimming.

Compared to the control group, mice in the experimental groups swam for a longer time, suggesting Cervus elaphus blood wine could slow down the appearance time of fatigue and strengthen the exercise tolerance. The time of loaded swimming was a key index for evaluating physical activity induced fatigue. The results of effects of Cervus elaphus blood wine on the swimming time of mice are shown in Table 3.

Table 3. Effects of Cervus elaphus blood wine on the swimming time of mice

Group	Wine base control group	Low dose group	Medium dose group	High dose group
The number of animals	10			
Time of loaded swimming	4.3±1.4	5.0±1.2	5.7±1.4	6.4±1.3

Through the results of the one-way analysis of variance, as shown in Table 3, we found that, the loaded swimming time of mice in the experimental groups had remarkable difference ($p < 0.05$) and the loaded swimming time of three experimental groups were longer than that of the wine base control group, suggesting the intake of Cervus elaphus blood wine could effectively improve the exercise tolerance of mice, prolong loaded swimming time and relieve physical activity induced fatigue. Besides, 18.4 mL/kg.BW/d was considered as the best intake amount.

3.4. Effects of Cervus elaphus blood wine on the weight of mice

Table 4 demonstrates the weight change of mice in the initial stage and later stage of test.

Table 4. Statistical table of weight of mice in groups in the initial stage and later stage of test

Group	Swimming group			Serum urea group			Hepatic glycogen group			Blood lactic acid group		
	The number of mice (n)	Weight (g)		The number of mice (n)	Weight (g)		The number of mice (n)	Weight (g)		The number of mice (n)	Weight (g)	
		Initial stage	Later stage		Initial stage	Later stage		Initial stage	Later stage		Initial stage	Later stage
Wine base control group	10	21.1	35.0	10	21.1	35.5	10	21.0	35.6	10	21.5	35.5
Low dose group	10	21.3	35.0	10	20.9	35.4	10	21.1	35.4	10	21.6	35.4
Medium dose group	10	20.8	35.6	10	21.5	35.3	10	21.3	35.2	10	21.3	35.2
High dose group	10	21.0	34.8	10	21.3	35.0	10	21.1	35.2	10	21.3	35.7

Table 4 demonstrates that, one-way analysis of variance found that, the weight of the swimming group, serum urea group, hepatic glycogen group and blood lactic acid group was not significantly different with three dose groups, so did the three dose groups and the wine base control group ($p > 0.05$). These data indicated that, Cervus elaphus blood wine had no remarkable effect on the weight of mice.

3.5. Effects of Cervus elaphus blood wine on serum urea and hepatic glycogen of mice (Table 5)

One-way factor analysis of variance found that, the content of serum urea and hepatic

glycogen of mice between groups had significant difference; the content of serum urea in the high dose group was much lower than that of the medium dose group and the medium dose group. the content of serum urea and hepatic glycogen of the medium dose group was much lower compared to that of the low dose group after physical activity; the content of serum urea of the three dose groups was much lower than that of the wine base control group after physical activity; the level of hepatic glycogen of the three dose group was much higher than that of the wine base control group after physical activity; results indicated that, Cervus elaphus blood wine could effectively improve the adaptability of mice to exercise load, increase the level of hepatic glycogen, accelerate the removal of serum urea, and relieve physical activity induced fatigue.

3.6. Effects of Cervus elaphus blood wine on blood lactic acid of mice

One-way analysis of variance found that, the average value of area under the curve of blood lactic acid of mice in different experimental groups had remarkable difference; the area under the curve of blood lactic acid of the low dose group was much higher than that of the medium group before swimming, at the end of swimming and after rest and the area under the curve of blood lactic acid of the medium dose group was much higher than that of the high dose group in the three stages; the content of blood lactic acid of the three dose groups was significantly lower than that of the wine base control group. The findings suggested that, Cervus elaphus blood wine could effectively reduce the content of blood lactic acid after physical activity and relieve fatigue.

Table 5. Effects of Cervus elaphus blood wine on serum urea and hepatic glycogen of mice

Group	Wine base control group	Low dose group	Medium dose group	High dose group
The number of mice (n)	10			
Hepatic glycogen (mg/g liver tissue)	18.5±1.4	20.0±1.6	21.9±1.8	23.0±1.2
Serum urea (mmol/L)	9.0±1.1	8.1±0.9	7.2±0.3	6.8±0.8

Cervus elaphus blood wine made by novel preparation technique looks red-brown and clear, smells mellow and tastes soft, sweet and clear (Cui et al., 2013; Vilgis, 2013; Xiong et al., 2012). This study explored the effect of Cervus elaphus blood wine in relieving exercise induced fatigue by detecting and analyzing several major quality indexes of Cervus elaphus blood wine from three different batches and comparing the effects of Cervus elaphus blood wine in different doses and wine base on exercise induced fatigue (Peng et al., 2012). Each milliliter of the prepared Cervus elaphus blood wine contained 0.97 g of herbal components; there was 55 ml in each bottle and the degree of alcohol was 35% (v/v). Loaded swimming experiment was performed on mice and the content of serum urea, hepatic glycogen and blood lactic acid of mice gavaged with Cervus elaphus blood wine was detected. Results demonstrated that, the intake of Cervus elaphus blood wine could effectively strengthen the exercise tolerance of mice, prolong the loaded swimming time of mice, relieve exercise induced fatigue, improve the adaptability of mice to exercise load, accelerate the elimination of urea, reduce serum urea of mice, increase the level of hepatic glycogen, and effectively reduce the generation of blood lactic acid of mice. These results all suggest that, Cervus elaphus blood wine can relieve exercise induced fatigue, strengthen resistance, reduce the occurrence of fatigue phenomenon and improve athletic ability.

4. References

Chang, C.W., Huang, TZ., Chang, W.H., et al. (2016). Acute Garcinia mangostana, (mangosteen) supplementation does not alleviate physical fatigue during exercise: a randomized, double-blind, placebo-controlled, crossover trial. *Journal of the International Society of Sports Nutrition,* 13(1), 1-8.

Cook, L., Cooper, N. (2010). Eltrombopag – a novel approach for the treatment of chronic immune thrombocytopenic purpura: review and safety considerations. *Drug Design Development & Therapy,* 4(5), 139–145.

Cui, Y., Lv, W., Fu, R.X. (2013). Development and Analysis of Aroma Compounds of Low Alcohol Blue Berry Wine. *Advanced Materials Research,* 791-793, 244-247.

Esbaugh, A.J., Ern, R., Nordi, W.M., et al. (2015). Respiratory plasticity is insufficient to alleviate blood acid–base disturbances after acclimation to ocean acidification in the estuarine red drum, Sciaenops ocellatus. *Journal of Comparative Physiology B,* 186(1), 1-13.

Gombotz, H. (2012). Patient Blood Management: A patient-orientated approach to blood replacement with the goal of reducing anemia, blood loss and the need for blood transfusion in elective surgery. *Transfusion Medicine & Hemotherapy,* 39(2), 67-72.

Hou, H., Bao, Y., Li, Q. et al. (2011). Preparation of blood-deficient model and research of angelica polysaccharide on enriching blood in chickens. *Evidence-based Complementary and Alternative Medicine,* 2012(3), 440-448.

Ishii, H., Nishida, Y. (2013). Effect of Lactate Accumulation during Exercise-induced Muscle Fatigue on the Sensorimotor Cortex. *Journal of Physical Therapy Science,* 25(12), 1637-42.

Kanter, R., Augusto, G.F., Walls, H.L., et al. (2014). 4th Annual Conference of the Leverhulme Centre for Integrative Research on Agriculture and Health (LCIRAH), Agri-food policy and governance for nutrition and health, London, 3–4 June 2014. *Food Security,* 6(5), 747-753.

Liu, H., Luiten, P.G.M., Eisel, U.L.M, et al. (2013). Depression after myocardial infarction: TNF-α-induced alterations of the blood–brain barrier and its putative therapeutic implications. *Neuroscience & Biobehavioral Reviews,* 37(4), 561-72.

Oliveira, A.R.D., Vanin, A.A., Marchi, T.D. et al. (2013). What is the ideal dose and power output of low-level laser therapy (810 nm) on muscle performance and post-exercise recovery? Study protocol for a double-blind, randomized, placebo-controlled trial. *Trials,* 15(1), 1-7.

Peng, L., Han, Y.B., Li, H.E. et al. (2012). Experimental study on effect of coenzyme Q(10) on the ability of anti-fatigue and anoxia endurance in mice. *Chinese Journal of Health Laboratory Technology,* 37(1), 483-496.

Vilgis, T.A. (2013). Texture, taste and aroma: multi-scale materials and the gastrophysics of food. *Flavour,* 2(1), 1-5.

Wiebrecht, A., Gaus, W., Becker, S. et al. (2014). Safety aspects of Chinese herbal medicine in pregnancy—Re-evaluation of experimental data of two animal studies and the clinical experience. *Complementary Therapies in Medicine,* 22(5), 954-964.

Xiong, L., Yang, Y.H. (2012). Research on fermentation and blending techniques of honey-longan seed wine. *Journal of Sichuan Agricultural University,* 30(2), 201-204.