Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Anti-Fatigue Activities and Phytochemical Compositions of Turnip (*Brassica rapa* L.) Extracts

Yi Zhou, Zhanqiang Li[1]

Department of Eco-environmental Engineering, Qinghai University, 251 Ningda Road, Xining 810016, [1]Research Center for High Altitude Medicine, Qinghai University, Xining, Qinghai, China

Received: 03-Nov-2020 Revised: 28-May-2021 Accepted: 04-Aug-2021 Published: 24-Jan-2022

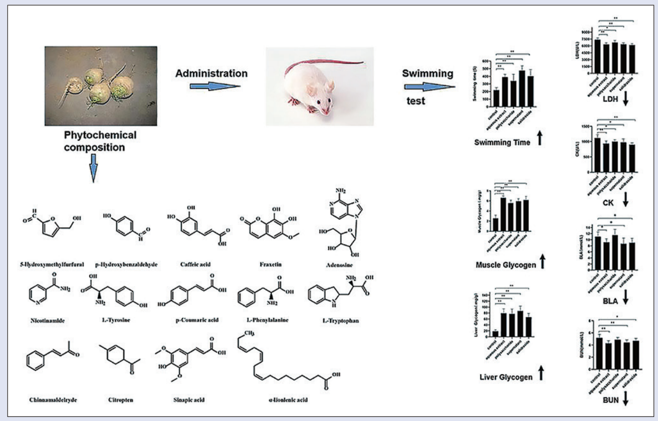
ABSTRACT

Background: Turnip (*Brassica rapa* L.) is a biennial plant, which belongs to *Cruciferae Brassica*, and is widely distributed at an altitude of 3000-4000 m in the Qinghai-Tibetan Plateau in western China. Turnip has been shown to have anti-hypoxia activities; however, there is limited scientific evidence of its anti-fatigue effects. **Objectives:** The objective is to evaluate the anti-fatigue properties and characterize the phytochemical compositions of turnip. **Materials and Methods:** Different proportions of turnip extract, including the total aqueous extract (50 mg/kg, per oral, [p. o.]), supernatant (50 mg/kg, p. o.), and polysaccharide (50 mg/kg, p. o.), were administered to mice, once a day for 20 days; following which, the exhaustive swimming time, liver and muscle glycogen, lactate dehydrogenase (LDH), creatine kinase (CK), blood lactic acid (BLA), and blood urea nitrogen (BUN) levels were tested. Ultra-high-performance liquid chromatography and Q-Exactive Orbitrap high-resolution mass spectrometry were used to analyze the turnip compounds. **Results:** Turnip, especially the supernatant portion, increased the exhaustive swimming time (476.01 ± 59.63 vs. 217.89 ± 32.70 s, $P < 0.01$), levels of liver glycogen (87.22 ± 16.28 vs. 18.53 ± 4.82 mg/kg, $P < 0.01$), and muscle glycogen (5.93 ± 0.51 vs. 2.56 ± 0.62 mg/kg, $P < 0.01$); decreased the activities of LDH (6,351.84 ± 344.81 vs. 7,359.54 ± 382.48 U/L, $P < 0.01$) and CK (974.66 ± 112.89 vs. 1,115.69 ± 113.52 U/L, $P < 0.05$); and decreased the levels of BLA (8.68 ± 1.86 vs. 10.99 ± 1.52 mmol/L, $P < 0.05$) and BUN (4.40 ± 0.44 vs. 5.21 ± 0.52 mmol/L, $P < 0.01$) when compared to the control group, and exhibited the best performance in relieving fatigue. Fourteen low-molecular-weight chemicals were detected in the supernatant portion, some of which had anti-fatigue effects. **Conclusion:** Our findings suggest that turnip prevents fatigue in mice and has the potential to be developed into health products to reduce fatigue.

Key words: Anti-fatigue activities, exhaustive swimming test, mice, phytochemical compositions, turnip

SUMMARY

- Turnip has anti-fatigue effects as it can increase liver and muscle glycogen levels, and decrease LDH, CK BLA, and BUN levels. Phytochemical analysis demonstrated that turnip contains several energy source substances. Further studies on the mechanism and exploitation of turnip should be performed in the future.



Abbreviations used: LDH: Lactate dehydrogenase; CK: Creatine kinase; BLA: Blood lactic acid; BUN: Blood urea nitrogen; AMS: Acute mountain sickness; UHPLC-Q-Exactive Orbitrap MS/MS: Ultra-high-performance liquid chromatography and Q-Exactive Orbitrap high-resolution mass spectrometry; NAD+: Nicotinamide adenine-reduction; GPCR: G protein-coupled receptors; 5-HMF: 5-hydroxymethylfurfural.

Correspondence:

Dr. Yi Zhou,
College of Eco-environmental Engineering, Qinghai University, 251 Ningda Road, Xining 810016, Qinghai, China.
E-mail: 2013990028@qhu.edu.cn.

DOI: 10.4103/pm.pm_470_20

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

The Qinghai-Tibetan Plateau is known as the Roof of the World, with an average altitude of more than 4,000 m. It covers approximately one-fourth of China's total area. Every year, millions of newcomers arrive from a low altitude to Qinghai-Tibetan Plateau; therefore, altitude-related medical problems in both Qinghai and Tibet are important considerations for further study. Rapid ascent to a high-altitude environment causes symptoms such as fatigue, headache, chest distress, and short breath. These symptoms are known as acute mountain sickness (AMS), which usually occurs 6-12 h after ascending to a high-altitude environment; if the ascent is increased, AMS can last for >3 days.[1] Among these symptoms, fatigue is a particularly obvious and challenging problem. With the onset of fatigue, labor efficiency decreases by 12.61% and 18.78% at the altitude of 3500 m and 4500 m compared to that in low altitude, respectively.[2] Moreover, the incidence of fatigue after acute exposure to high-altitude ranges from 10% to 50%.[3] Easing fatigue is key to reducing AMS, and fatigue is a general phenomenon of poor acclimatization to hypoxia at high altitude. Hypoxia can lead to functional impairment and physiological abnormalities of the body because the available oxygen reaching the body tissues is

Cite this article as: Zhou Y, Li Z. Anti-fatigue activities and phytochemical compositions of turnip (*Brassica rapa* L.) extracts. Phcog Mag 2021;17:857-64.

reduced.[4] Hypoxia increases respiratory work, activates the sympathetic nervous system, reduces peripheral oxygen content, and aggravates fatigue.[5] Hypoxia also decreases mitochondrial oxidative flux and ATP production;[6] in which case, the change in energy metabolism may also impact high altitude fatigue.[7] Thus, one way to overcome these problems is to develop a targeted medicine to strengthen anti-hypoxia and anti-fatigue capability. Currently, several drugs are available for the treatment of high-altitude illnesses, including acetazolamide,[8,9] dexamethasone,[10] nifedipine,[11] and aminophylline.[12] However, all of these drugs have obvious side effects if taken long-term. For this reason, plants native to the plateau have been considered attractive alternatives given that the unique growth environment imparts distinct biological activities.

Turnip (*Brassica rapa* L.) is a biennial plant belonging to *Cruciferae brassica*, which is widely distributed in the Qinghai-Tibetan Plateau in western China at an altitude of 3000-4000 m. Turnip is one of the oldest cultivated vegetables and is recorded in Tibetan medical books as "SiBuYiDian," with anti-hypoxic effects and the ability to alleviate fatigue. Indeed, turnip root is used as a folk medicine by Tibetan people to relieve hypoxia over an extended period.[13] Turnip root also contributes to promoting hypoxia tolerance in healthy humans.[14] As hypoxia can aggravate fatigue,[5] we hypothesized that if a plant had anti-hypoxic effects, it is conceivable that it will also have anti-fatigue effects. To date, there have been limited systematic studies on the anti-fatigue activities of turnip; therefore, for the first time, we aim to evaluate the anti-fatigue effects and characterize the phytochemical compositions in turnip in order to attenuate fatigue in people who ascend to a high-altitude environment. This work has significant benefits in terms of development and usage of plateau plants.

MATERIALS AND METHODS

Materials and reagents

Turnip roots were collected from the Yushu in Qinghai, China, on October 15, 2019, and verified by Prof. Dr. Zhangqiang Li, Qinghai University, Xining. The sample was deposited in Qinghai University, College of Eco-environmental Engineering, with the herbarium plant number BB20191015. The content of liver glycogen, muscle glycogen, lactate dehydrogenase (LDH), creatine kinase (CK), blood lactic acid (BLA), and blood urea nitrogen (BUN) was detected according to the procedures used by Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All other chemicals and reagents used in the experiments were analytical grade.

Turnip portion extractions

The air-dried turnip roots were crushed and extracted three times (1 h and each time) with distilled water at 100°C. All of the extracts were combined, filtered with a circulating water vacuum pump (SHZ-D III, Zhengzhou Yarong Instrument Co., Ltd., China), and concentrated under reduced pressure at 60°C using a rotary evaporator (EYELA, N-1210, Tokyo, Japan) to obtain the total extract portion (portion I: Aqueous extract). One moiety was removed and four volumes of ethanol were added, and the mixture was kept overnight at 4°C. The sample was then centrifuged at 4000 rpm for 20 min to obtain the supernatant and precipitation. The supernatant was concentrated at 60°C using a rotary evaporator (EYELA, N-1210, Tokyo, Japan) (portion II: Supernatant, the percentage yield of supernatant in extract is 64%), and the precipitation was washed with ethanol (portion III: Polysaccharide). All three portions were recovered by freeze-drying (EYELA, FOU-2110, Tokyo, Japan) before evaluating the anti-fatigue activities.

Evaluation of anti-fatigue effects of turnip in mice

Animal grouping and treatment

Male Kunming mice (18-22 g) were purchased from the Experimental Animal Center of Xi'an Jiaotong University, China (permit number: SCXK 2017-003). All animals were placed in a well-ventilated animal room at 25°C ± 5°C for 12 h. The care and use of animals were conducted in accordance with the standards established by the Guide for the Care and Use of Experimental Animals of Qinghai Province and approved by the local Ethics Committee. After a week of adaptive feeding, the mice were randomly divided into five groups (n = 20 per group), and each group was randomly divided into two subgroups; 10 mice were subjected to the exhaustive swimming test and 10 mice were tested for biochemical parameters and orally administered different treatments for 20 days. The groups were as follows: The aqueous extract group (50 mg/kg), the supernatant group (50 mg/kg), the polysaccharide group (50 mg/kg), the control group (distilled water, 10 ml/kg), and the positive control group (salidroside, 50 mg/kg).

Exhaustive swimming test

After 20 days, 10 mice in each group were selected for the exhaustive swimming test. Briefly, 1 h after the last treatment, the mice were with a lead weight, that is, ~5% of the body weight, attached at the base of the tail. The mice were placed in the swimming tank (90 cm in length, 60 cm in width and 60 cm in depth) with 50-cm deep water at 25 ± 1°C. The mice continued to move their limbs until they were exhausted and failed to rise above the surface after 10 s.[15]

Analysis of the biochemical parameters related to fatigue

After 20 days, the remaining 10 mice in each group were selected for the determination of blood biochemical parameters and glycogen. One hour after the last treatment, the mice were placed in the same pool to swim without loads for 60 min and then rested for 10 min. Mice were anesthetized with an intraperitoneal injection of chloral hydrate (10%, 0.06 ml/20 g), and blood was collected in 1.5 mL Eppendorf tubes (NEST, Guangzhou Saiguo Biotech Co., Ltd) by removing the left eyeball. Plasma was prepared at 3500 rpm and centrifuged at 4°C for 15 min to determine the levels of LDH, CK, BLA, and BUN. The liver and hind leg muscles were collected, frozen in liquid nitrogen, and maintained at−80°C until the glycogen concentration was analyzed.[16] All parameters were measured in accordance with the recommended procedures provided by the kits purchased from Nanjing Jiancheng Bioengineering Institute as mentioned above.

Ultra-high-performance liquid chromatography and Q-exactive orbitrap high-resolution mass spectrometry analysis of the phytochemical composition of turnip supernatants

The supernatant portion was evaporated and then freeze-dried into powder. The powder was weighed to 0.01 g and dissolved in 10 mL of 80% methanol/distilled water for 10 min at room temperature by ultrasonication, and then centrifuged at 12,000 rpm for 10 min. The supernatant was filtered using a 0.22-μm filter membrane, and 1 μL of the filtrate was loaded into the ultra-high-performance liquid chromatography and Q-Exactive Orbitrap high-resolution mass spectrometry (UHPLC-Q-Exactive Orbitrap MS/MS) system. Chromatographic separation was conducted on a Dionex Ultimate 3000 UHPLC system (Thermo Fisher Scientific, Waltham, MA, USA). The separation was achieved with a Thermo Scientific Hypersil GOLD aQ C_{18} Column (2.1 mm × 100 mm, 1.9 μm) at 40°C at a flow rate of 0.4 mL/min. The mobile phase consisted of acetonitrile (A) and water (B), with a gradient of 2% A at 0-2 min, 2%–98% A at 2-15 min, and 98% A at 15-19 min. A Q-Exactive hybrid quadrupole-orbitrap mass spectrometer (Thermo Scientific, Waltham, MA, USA) using heat electrospray ionization was operated under positive- and negative-ion modes to achieve MS. The capillary temperature was 320°C, the flow rate of sheath gas was 40 arbitrary units, and the auxiliary gas was set to 10 arbitrary units at 350°C. The capillary voltage was set to + 4.0 or – 2.8 kV in the positive and negative mode, respectively. The resolution of the full MS scan was 70,000, and the range was 100-1500 *m/z*. Samples were analyzed at 20, 30, and 40 normalized collision energies in the MS2 mode, with a resolution of 17,500. Data were collected and analyzed using Thermo Xcalibur 3.0 software (Thermo Scientific, Waltham, MA, USA).[17]

Statistical analysis

All data are expressed as mean ± standard deviation. The data were analyzed by SPSS18.0 (SPSS Inc., Chicago, IL, USA) software. Differences among groups were analyzed by one-way analysis of variance followed by paired Student's *t*-test. $P < 0.05$ was considered statistically significant.

RESULTS

Effects of turnip on exhaustive swimming test

The exhaustive swimming test can evaluate the endurance of mice, and the improvement of exercise endurance is a direct index of anti-fatigue effects.[15] The exhaustive swimming time is shown in Figure 1. Compared to the control group, the mice that were administered aqueous extract, polysaccharide, supernatant, and salidroside had a significantly prolonged survival time in the exhaustive swimming test. In particular, among all experimental groups, the supernatant had the most obvious effect of prolonging swimming time compared with the control group (476.01 ± 59.63 vs. 217.89 ± 32.70 s, $P < 0.01$), and it had better effects than polysaccharide and positive control. The results indicated that turnip could elevate the exercise tolerance of mice. As the supernatant portion exhibited the best performance in prolonging swimming time, it is likely that this portion contains low molecular weight bioactive compounds.

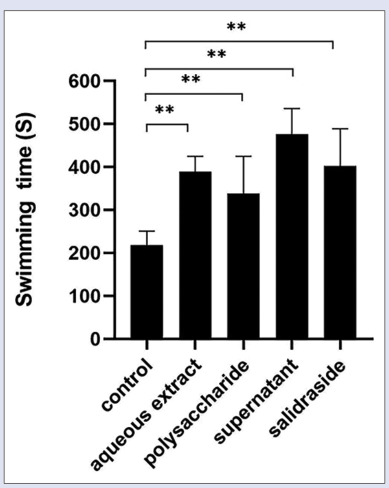


Figure 1: Effects of turnip on the exhaustive swimming time of mice. The X-axis denotes different groups as follows: The control group (distilled water, 10 ml/kg), aqueous extract group (50 mg/kg), polysaccharide group (50 mg/kg), supernatant group (50 mg/kg), and salidroside-positive control group (50 mg/kg). Data are presented as mean ± standard deviation ($n = 10$). Compared to the control group, $^{**}P < 0.01$

Liver and muscle glycogen test

The amount of accumulated glycogen in the liver and muscle is shown in Figure 2. After administering the different portions of turnip, the liver glycogen contents of each treatment group increased significantly ($P < 0.01$) compared to the control group, and the most obvious increase was observed in the supernatant group compared with the control group (87.22 ± 16.28 vs. 18.53 ± 4.82 mg/kg, $P < 0.01$). Muscle glycogen also increased significantly in the other four groups compared to the control group. Under the same dosage, the muscle glycogen levels in the supernatant group were higher than those in polysaccharide and positive control groups.

Blood biochemical parameters related to the fatigue test

After forced swimming for 60 min without loads, blood was collected and plasma was prepared to determine LDH, CK, BLA, and BUN. All measured blood biochemical parameters are shown in Figure 3. When given different portions of turnip, there was a marked reduction in changes of LDH compared to the control group; particularly, the supernatant group (6351.84 ± 344.81 vs. 7359.54 ± 382.48 U/L, $P < 0.01$) was more significant than the polysaccharide group (6750.65 ± 435.83 vs. 7359.54 ± 382.48 U/L, $P < 0.01$). The activity of CK decreased in the other four groups compared to the control group ($P < 0.05$, $P < 0.01$), and the most obvious decrease was observed in the aqueous extract group (938.33 ± 84.51 vs. 1115.69 ± 113.52 U/L, $P < 0.01$).

Compared to the control group, the content of BLA decreased in the aqueous extract group (9.18 ± 1.12 vs. 10.99 ± 1.52 mmol/L, $P < 0.05$) and supernatant group (8.68 ± 1.86 vs. 10.99 ± 1.52 mmol/L, $P < 0.05$), while it was increased in the polysaccharide group (11.48 ± 1.89 vs. 10.99 ± 1.52 mmol/L). The level of BUN also decreased after the administration of different portions of turnip, while only the aqueous extract and supernatant groups presented a statistically significant difference (4.28 ± 0.45 and 4.40 ± 0.44 vs. 5.21 ± 0.52 mmol/L, $P < 0.01$, respectively). There was no significant difference in BUN in the

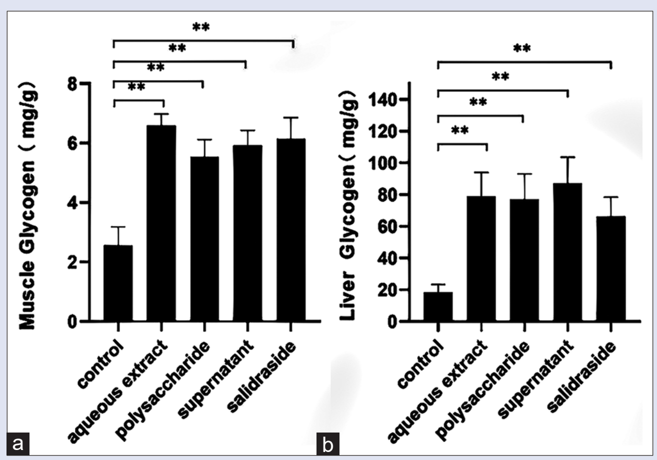


Figure 2: Effects of turnip on the muscle and liver glycogen content in mice. The X-axis denotes different groups as follows: The control group (distilled water, 10 ml/kg), aqueous extract group (50 mg/kg), polysaccharide group (50 mg/kg), supernatant group (50 mg/kg), and salidroside-positive control group (50 mg/kg). (a) Statistical analysis of muscle glycogen and (b) liver glycogen content. Data are presented as mean ± standard deviation ($n = 10$). Compared to the control group, $^{**}P < 0.01$

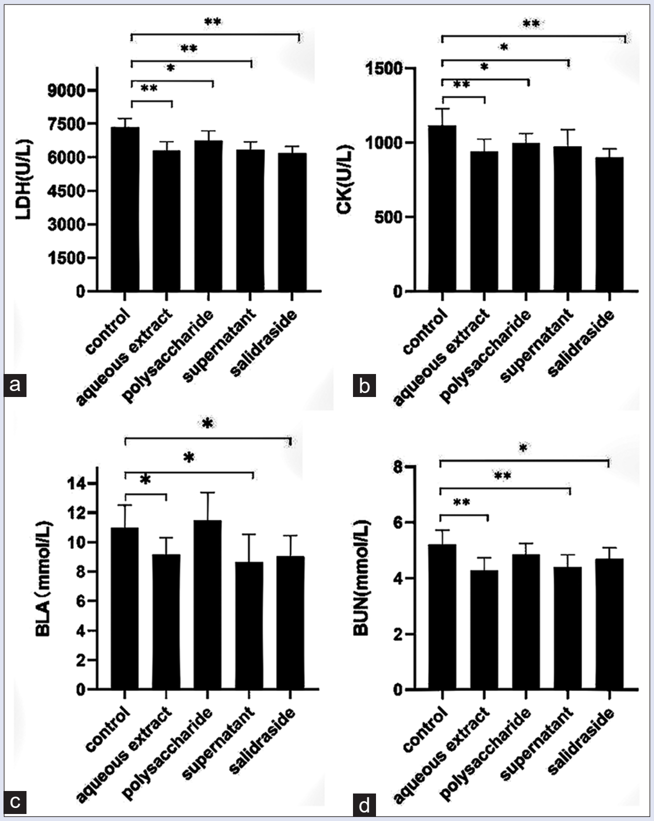


Figure 3: Effects of turnip on the content of blood biochemical parameters related to fatigue in mice. The X-axis denotes different groups as follows: The control group (distilled water, 10 ml/kg), aqueous extract group (50 mg/kg), polysaccharide group (50 mg/kg), supernatant group (50 mg/kg), and salidroside-positive control group (50 mg/kg). (a) Statistical analysis of LDH: Lactate dehydrogenase; (b) CK: Creatine kinase; (c) BLA: Blood lactic acid; and (d) BUN: Blood urea nitrogen. Data are presented as mean ± standard deviation (n = 10). Compared to the control group, $^{*}P < 0.05$, $^{**}P < 0.01$

polysaccharide group, which suggested that the supernatant had better anti-fatigue effects than polysaccharide.

UHPLC-Q-Exactive Orbitrap MS/MS analysis of turnip components

The supernatant portion not only significantly prolonged the exhaustive swimming time but also reduced blood biochemical parameters compared to the other portions. Ultra-high-performance liquid chromatography and the Q-Exactive Orbitrap high-resolution mass spectrometry (UHPLC-Q-Exactive Orbitrap MS/MS) method were used to analyze the components in the supernatant; the chromatogram of positive and negative ion modes is shown in Figure 4.

Fourteen low-molecular-weight compounds were detected in the supernatant portion. The chromatographic retention times, accurate molecular mass, and MS/MS data are listed in Table 1, and included 5-hydroxymethylfurfural, p-hydroxybenzaldehyde, caffeic acid, fraxetin, nicotinamide, adenosine, L-tyrosine, p-coumaric acid, L-phenylalanine, L-tryptophan, cinnamaldehyde, citropten, sinapic acid, and α-linolenic acid. The structure of these compounds is shown in Figure 5. All the compounds were identified as follows: 5-Hydroxymethylfurfural with protonated m/z 127.0390 ([M + H] +, $C_6H_6O_3$),[18] p-hydroxybenzaldehyde with protonated m/z 123.0439 ([M + H] +, $C_7H_6O_2$),[19] caffeic acid

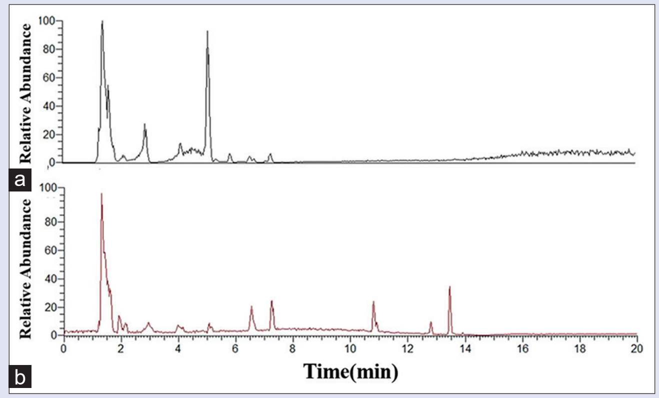


Figure 4: UHPLC-Q-Exactive hybrid quadrupole-orbitrap mass analysis chromatogram of the supernatant portion. (a) Total ion chromatogram (TIC) in positive electrospray ionization (ESI) mode. (b) TIC chromatogram in negative ESI mode

with protonated m/z 181.0493 ([M + H] +, $C_9H_8O_4$),[20] fraxetin with a protonated m/z 209.0442 ([M + H] +, $C_{10}H_8O_5$),[21] p-coumaric acid with a protonated m/z 165.0540 ([M + H] +, $C_9H_8O_3$),[22] cinnamaldehyde with a protonated m/z 133.0648 ([M + H] +, C_9H_8O),[23] sinapic acid with a protonated m/z 223.0612 ([M + H] +, $C_{11}H_{12}O_5$),[24] and α-linolenic acid with a protonated m/z 279.2319 ([M + H] +, $C_{18}H_{30}O_2$).[25] Furthermore, nicotinamide, adenosine, L-tyrosine, L-phenylalanine, and L-tryptophan were based on MS/MS data and phytochemical compositions, as well as the previously published studies.[26,27]

DISCUSSION

In this study, we examined the antifatigue effects and bioactive ingredients of turnip. We found that, besides improved endurance performance after the forced swimming test, turnip increased liver and muscle glycogen deposition, decreased tissue damage makers, including LDH and CK, and decreased the accumulation of metabolites, including BLA and BUN, thereby predicting the function of turnip on antifatigue. Meanwhile, as the supernatant portion exhibited the greatest antifatigue effect, the UHPLC-Q-Exactive Orbitrap MS/MS method was used to analyze the components of the supernatant. As a result, 14 compounds were detected, some of which were related to anti-fatigue effects based on the previous literature.

Turnip, a plateau plant, has long been used as a folk medicine and food. As turnip has been previously shown to relieve hypoxia,[13,14] we sought to examine its antifatigue effect when ascending to a high altitude given that hypoxia can aggravate fatigue.[5-7] Our study explored the adaptability of mice during exercise using biochemical indices of swimming assessment.

First, we explored the sports endurance in mice. The exhaustive swimming test was used as the experimental exercise model and is extensively used for the evaluation of animal sports endurance.[28-30] In our study, the different portions of turnip had prolonged the swimming time of mice, indicating that turnip could induce resistance to fatigue. The supernatant portion had the most significant effect in prolonging the swimming time, indicating that the supernatant had the greatest anti-fatigue effects and could effectively enhance the stamina of mice; thus, we consider that this portion possesses bioactive ingredients.

The biochemical parameters were evaluated to further clarify the effect of each portion. Liver and muscle glycogen are sensitive parameters related to fatigue.[31] Because carbohydrates are the most important energy source and are deposited in the body as blood glucose and glycogen. Glycogen deposition in the liver and muscle tissue can be converted into glucose

Table 1: Results of ultra-high-performance liquid chromatography - Q-exactive orbitrap mass spectrometry /mass spectrometry analysis of the supernatant portion

Retention time/min	Ion	Theoretical value	Actual value	Secondary mass	Molecular formula	Name
1.47	$[M+H]^+$	127.0390	127.0389	109.0286, 81.0341,69.0342	$C_6H_6O_3$	5-Hydroxymethylfurfural
1.47	$[M+H]^+$	123.0s441	123.0439	100.0247, 105.0036, 82.0143	$C_7H_6O_2$	p-Hydroxybenzaldehyde
1.88	$[M+H]^+$	181.0495	181.0493	163.0386, 135.0439	$C_9H_8O_4$	Caffeic acid
1.93	$[M+H]^+$	209.0445	209.0442	194.0208, 149.0230	$C_{10}H_8O_5$	Fraxetin
1.99	$[M+H]^+$	123.0553	123.0553	80.0500, 96.0447, 106.0289	$C_6H_6N_2O$	Nicotinamide
3.25	$[M+H]^+$	268.1040	268.1030	136.0616	$C_{10}H_{13}N_5O_4$	Adenosine
3.40	$[M+H]^+$	182.0812	182.0808	136.0216, 123.0263	$C_9H_{11}NO_3$	L-Tyrosine
3.40	$[M+H]^+$	165.0546	165.0543	95.0495, 119.0492	$C_9H_8O_3$	p-Coumaric acid
4.26	$[M+H]^+$	166.0863	166.0859	120.0808	$C_9H_{11}NO_2$	L-Phenylalanine
5.21	$[M+H]^+$	205.0972	205.0970	118.0651, 146.0597, 188.0701	$C_{11}H_{12}N_2O_2$	L-Tryptophan
5.42	$[M+H]^+$	133.0648	133.0646	105.0701, 95.0493	C_9H_8O	Cinnamaldehyde
6.76	$[M+H]^+$	207.0652	207.0648	179.0698, 151.0754	$C_{11}H_{10}O_4$	Citropten
6.77	$[M-H]^-$	223.0612	223.0610	119.0854	$C_{11}H_{12}O_5$	Sinapic acid
12.30	$[M+H]^+$	279.2319	279.2315	-	$C_{18}H_{30}O_2$	α-Linolenic

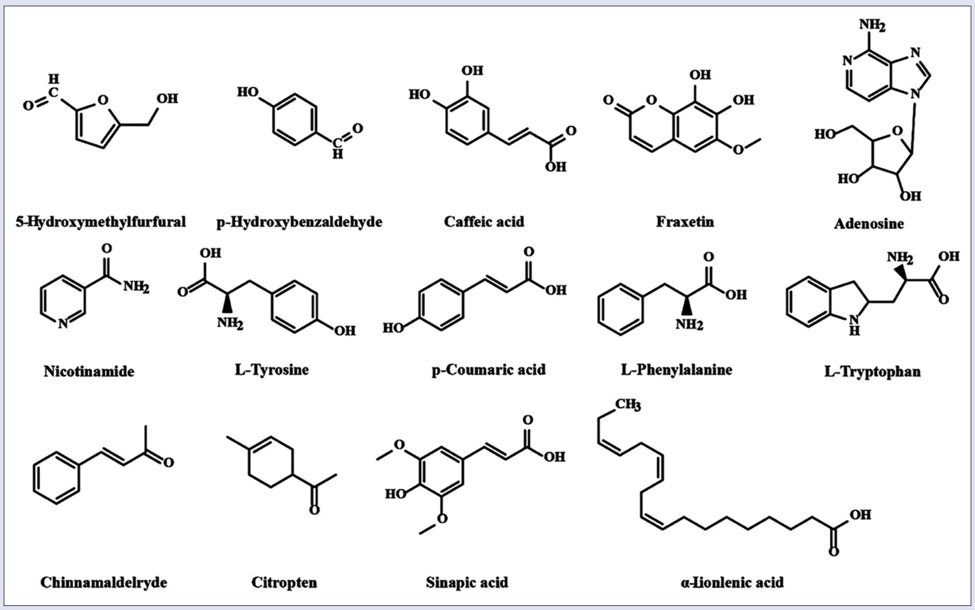


Figure 5: Structure of the 14 compounds detected with UHPLC-Q-Exactive Orbitrap MS/MS

at any time according to human needs to satisfy the energy demands and also plays an important role in prolonging exercise time.[32,33] Muscle glycogen provides sufficient energy for muscle contraction.[34] The increase in liver glycogen is conducive to the enhancement of exercise endurance.[35,36] Depletion of liver glycogen makes it difficult to maintain blood glucose, and the resulting hypoglycemia damages the nervous function.[37] When the glycogen is largely consumed, fatigue will occur. Liver and muscle glycogen should always be determined when studying the anti-fatigue effect.[38,39] The results of our study demonstrated that treatment with turnip increased the levels of liver and muscle glycogen; therefore, one possible explanation for the anti-fatigue effects of turnip is that it could reduce glycogen consumption and improve energy metabolism. Taken together, these factors improve the endurance of mice in the exhaustive swimming test.

LDH and CK in plasma are important indicators for fatigue evaluation. LDH catalyzes the conversion of pyruvate into lactic acid in glycolysis, and its activity indicates the degree of lactate metabolism. LDH can quench BLA, which is the product of anaerobic metabolism of glucose during high-intensity exercise.[29,40] The CK level is a critical indicator of fatigue[41] and supports the production of energy needed during exercise. Under normal conditions, the muscle cell structure is intact, and LDH and CK rarely permeate the cell membrane. High-intensity weight bearing exercise can cause tissue damage, which is closely related to exercise-induced fatigue; thus, LDH and CK found abundantly in plasma are necessary to be tested.[42] The results of our study demonstrated that the activities of LDH and CK in different portions of turnip were significantly lower than those in the control group; therefore, another possible explanation of the anti-fatigue effects of turnip is that it could ease muscle damage, and maintain and protect the integrity of the cell membrane after swimming exercise.

BLA is one of the products generated as a result of energy metabolism during muscle activity, and its accumulation is associated with fatigue.[43] According to some studies, lactic acid itself is not a direct cause of fatigue, but the H^+ dissociated from lactic acid reduces the pH value, decreases phosphofructokinase activity, affects Ca^{2+} release, impairs the contraction ability of muscle fibers, and accelerates fatigue.[44] In long-term high intensive exercise, the human body cannot obtain sufficient energy by metabolizing sugar and fat, so protein and amino acid may participate in energy supply causing an increase in urea nitrogen. BUN is the product of this energy metabolism process, and it is a sensitive index to evaluate the bearing capability in fatigue.[45,46] When the body is poorly adapted for exercise endurance, the level of BUN increases.[47] More importantly, if an animal cannot be adapted to exercise, the BLA and BUN levels will increase.[48] If a plant inhibits the accumulation of BLA and BUN, the plant can be said to possess anti-fatigue effects.[40] In this study, turnip could reduce the levels of BLA and BUN in fatigued mice; thus, turnip contributes to the aerobic metabolism to product ATP and slows down the metabolism rate of protein, ultimately, improving metabolic exercise, activating the energy metabolism, and accelerating the restoration of energy. Through the exhaustive swimming test and evaluation of biochemical parameters, we verified the anti-fatigue effect of turnip.

We next evaluated the chemical components of turnip. Many studies have focused on the extraction and anti-fatigue effects of macromolecular substances, such as polysaccharides in plants.[49-53] However, in our study, we separated extraction portions of turnip, including the total aqueous extract, supernatant, and polysaccharide; although all portions resisted fatigue, the supernatant portion had the greatest anti-fatigue effects. Therefore, the importance of low molecular weight compounds cannot be ignored. We focused on the compounds in supernatant, except polysaccharide, using the UHPLC-Q-Exactive Orbitrap MS/MS method. Fourteen compounds were detected as follows: 5-hydroxymethylfurfural, p-hydroxybenzaldehyde, caffeic acid, fraxetin, nicotinamide, adenosine, L-tyrosine, p-coumaric acid, L-phenylalanine, L-tryptophan, cinnamaldehyde, citropten, sinapic acid, and α-linolenic acid. Based on a literature review, we found that these compounds have been shown to have anti-fatigue effects. Caffeic acid is a phenolic compound present in many plants. Caffeic acid extracted from Taraxacum officinal can lower glucose in the blood and decrease the BLA concentrations to exert anti-fatigue effects.[54] Nicotinamide belongs to nicotinamide adenine-reduction (NAD^+), which is essential to counteract oxidative damage.[55] NAD+ is converted into $NADH_2$ and ATP through oxidative phosphorylation, and it is the direct energy source for exercise. A nicotinamide-rich diet can improve physical endurance.[56] The triple-combination of nicotinamide, casein, and guanosine can significantly enhance the exercise performance and exhibit anti-fatigue effects in mice.[57] Adenosine is present in almost all cells of the human body under physiological conditions and is further produced under conditions of hypoxia or energy consumption. Adenosine is a critical intermediate metabolite of nucleic acids and is an important signaling molecule. It regulates the cellular response to hypoxia, energy depletion, and tissue damage by activating G protein-coupled receptors on multiple cell types.[58] Dietary nucleotides containing adenosine exert anti-fatigue effects, which may be related to inhibiting oxidative stress and improving mitochondrial function.[35] Amino acids are one of the main building blocks of life. Twenty proteinogenic L-amino acids form the materials used for protein synthesis. Nonessential amino acid can be synthesized by many cells from metabolic intermediates, but essential amino acid must be acquired from nutrients.[59] Tryptophan and phenylalanine are essential amino acids, and turnip contains both, suggesting that turnip has high nutritional value. The supernatant portion of turnip includes caffeic acid, nicotinamide, adenosine, and amino acids, all of which function as energy sources to improve physical endurance; this is a powerful explanation for the significant anti-fatigue effects of the supernatant portion.

Other compounds in the supernatant portion of turnip, such as 5-hydroxymethylfurfural (5-HMF), are commonly found in many daily foods and have anti-inflammatory effects.[60] p-Coumaric acid is a hydroxycinnamic acid found in many plants[61] and is a well-known antioxidant agent.[62-64] Cinnamaldehyde is a bioactive substance, which has anti-fungal, anti-bacterial, anti-inflammatory, anti-mutagenic, and antioxidant effects.[65,66] Citropten (5,7-dimethoxycoumarin) is a plant secondary metabolite, which can prevent depression induced by chronic mild stress in rats.[67] α-Linolenic acid is an essential fatty acid that cannot be synthesized by human and must be supplied through the diet.[68] α-Linolenic acid shows diverse protective effects such as anti-cardiovascular disease,[69] anti-cancer,[70] anti-neuropathy,[71] anti-inflammatory, and antioxidative effects.[72] All of these compounds can be considered bioactive ingredients of turnip. It will be meaningful to systematically explore the anti-fatigue effects of these compounds in future studies.

Our study confirmed the antifatigue effect of turnip and identified its low molecular-weight compounds. Further studies should focus on clarifying the detailed signaling pathways underlying these effects.

CONCLUSION

In this study, turnip had prominent anti-fatigue properties, especially the supernatant portion. Turnip enhanced the metabolic exercise and activated energy metabolism by increasing liver and muscle glycogen levels. In addition, turnip could efficiently alleviate muscle damage by decreasing the LDH and CK activities in plasma and could accelerate the restoration of energy by decreasing the BLA and BUN levels. Phytochemical analysis demonstrated that turnip contains some energy source substances, which is a powerful explanation for the anti-fatigue effects of turnip.

In summary, the current study demonstrated that turnip exhibited good anti-fatigue effects, which may be due to the improvement of energy metabolism, which is a new finding for screening anti-fatigue medicine. More details about signaling pathways underlying these effects and exploitation of turnip need to be illuminated in the future.

Acknowledgements

The authors would like to thank the Department of Research Center for High Altitude Medicine, Qinghai University, for providing the facilities for this work.

Financial support and sponsorship

This research was funded by the National Natural Science Foundation of China (Nos 82060731) and Natural Science Foundation of Qinghai Province (Nos 2019-ZJ-981Q).

Conflicts of interest

There are no conflicts of interest.

REGERENCES

1. Jin J. Acute mountain sickness. JAMA 2017;318:1840.
2. Cheng FY, Jeng MJ, Lin YC, Wang SH, Wu SH, Li WC, *et al.* Incidence and severity of acute mountain sickness and associated symptoms in children trekking on Xue Mountain, Taiwan. PLoS One 2017;12:e0183207.
3. Koirala P, Wolpin SE, Peterson JT. High altitude illness: Knowledge, practice, and attitudes of porters in Nepal. Wilderness Environ Med 2018;29:431-6.
4. Chen CJ, Wang WY, Wang XL, Dong LW, Yue YT, Xin HL, *et al.* Anti-hypoxic activity of the ethanol extract from *Portulaca oleracea* in mice. J Ethnopharmacol 2009;124:246-50.
5. Vogiatzis I, Athanasopoulos D, Boushel R, Guenette JA, Koskolou M, Vasilopoulou M, *et al.* Contribution of respiratory muscle blood flow to exercise-induced diaphragmatic fatigue in trained cyclists. J Physiol 2008;586:5575-87.
6. Nanadikar MS, Vergel Leon AM, Borowik S, Hillemann A, Zieseniss A, Belousov VV, *et al.* O_2 affects mitochondrial functionality *ex vivo*. Redox Biol 2019;22:101152.
7. Horscroft JA, Kotwica AO, Laner V, West JA, Hennis PJ, Levett DZ, *et al.* Metabolic basis to Sherpa altitude adaptation. Proc Natl Acad Sci U S A 2017;114:6382-7.
8. Ke T, Wang J, Swenson ER, Zhang X, Hu Y, Chen Y, *et al.* Effect of acetazolamide and gingko biloba on the human pulmonary vascular response to an acute altitude ascent. High Alt Med Biol 2013;14:162-7.

9. Penninga L, Wetterslev J, Penninga EI, Gluud C. Acetazolamide for the prevention of acute mountain sickness: Time to move on. High Alt Med Biol 2013;14:85-6.
10. Stream JO, Grissom CK. Update on high-altitude pulmonary edema: Pathogenesis, prevention, and treatment. Wilderness Environ Med 2008;19:293-303.
11. Deshwal R, Iqbal M, Basnet S. Nifedipine for the treatment of high altitude pulmonary edema. Wilderness Environ Med 2012;23:7-10.
12. Yang B, Wang GY, Chen B, Qin RB, Xi SL, Chen L. Anti-hypoxia and anti-oxidation effects of aminophylline on human with acute high-altitude exposure. Chin Med Sci J 2007;22:62-5.
13. Xie Y, Jiang S, Su D, Pi N, Ma C, Gao P. Composition analysis and anti-hypoxia activity of polysaccharide from Turnip. Int J Biol Macromol 2010;47:528-33.
14. Chu B, Chen C, Li J, Chen X, Li Y, Tang W, *et al.* Effects of Tibetan turnip (*Brassica rapa* L.) on promoting hypoxia-tolerance in healthy humans. J Ethnopharmacol 2017;195:246-54.
15. Yong-Xin X, Jian-Jun Z. Evaluation of anti-fatigue activity of total Saponins of Radix notoginseng. Indian J Med Res 2013;137:151-5.
16. Yu Y, Wu G, Jiang Y, Li B, Feng C, Ge Y, *et al.* Sea cucumber peptides improved the mitochondrial capacity of mice: A potential mechanism to enhance gluconeogenesis and fat catabolism during exercise for improved antifatigue property. Oxid Med Cell Longev 2020;2020:4604387.
17. Jing L, Su S, Zhang D, Li Z, Lu D, Ge R. Srolo bzhtang, a traditional tibetan medicine formula, inhibits cigarette smoke induced airway inflammation and muc5ac hypersecretion via suppressing IL-13/STAT6 signaling pathway in rats. J Ethnopharmacol 2019;235:424-34.
18. Qin L, Zhang YY, Xu XB, Wang XS, Liu HW, Zhou DY, *et al.* Isotope dilution HPLC-MS/MS for simultaneous quantification of acrylamide and 5-hydroxymethylfurfural (HMF) in thermally processed seafood. Food Chem 2017;232:633-8.
19. Zheng M, Gu S, Chen J, Luo Y, Li W, Ni J, *et al.* Development and validation of a sensitive UPLC-MS/MS instrumentation and alkaline nitrobenzene oxidation method for the determination of lignin monomers in wheat straw. J Chromatogr B Analyt Technol Biomed Life Sci 2017;1055-1056:178-84.
20. Kadam D, Palamthodi S, Lele SS. LC-ESI-Q-TOF-MS/MS profiling and antioxidant activity of phenolics from *L. Sativum* seedcake. J Food Sci Technol 2018;55:1154-63.
21. Wang Z, Zhu W, Liu H, Wu G, Song M, Yang B, *et al.* Simultaneous determination of aesculin, aesculetin, fraxetin, fraxin and polydatin in beagle dog plasma by UPLC-ESI-MS/MS and its application in a pharmacokinetic study after oral administration extracts of *Ledum palustre* L. Molecules 2018;23:2285.
22. Kumar A, Kumar P, Koundal R, Agnihotri VK. Antioxidant properties and UPLC-MS/MS profiling of phenolics in Jacquemont's Hazelnut kernels (*Corylus jacquemontii*) and its byproducts from western Himalaya. J Food Sci Technol 2016;53:3522-31.
23. Ji B, Zhao Y, Zhang Q, Wang P, Guan J, Rong R, *et al.* Simultaneous determination of cinnamaldehyde, cinnamic acid, and 2-methoxy cinnamic acid in rat whole blood after oral administration of volatile oil of Cinnamoni ramulus by UHPLC-MS/MS: An application for a pharmacokinetic study. J Chromatogr B Analyt Technol Biomed Life Sci 2015;1001:107-13.
24. Lin L, Yang Q, Zhao K, Zhao M. Identification of the free phenolic profile of Adlay bran by UPLC-QTOF-MS/MS and inhibitory mechanisms of phenolic acids against xanthine oxidase. Food Chem 2018;253:108-18.
25. Zhou B, Lin C, Xie S, Zhou X, Zhang F, Ye X, *et al.* Determination of four omega-3 polyunsaturated fatty acids by UPLC-MS/MS in plasma of hyperlipidemic and normolipidemic subjects. J Chromatogr B Analyt Technol Biomed Life Sci 2019;1126-1127:121762.
26. He M, Wu H, Nie J, Yan P, Yang TB, Yang ZY, *et al.* Accurate recognition and feature qualify for flavonoid extracts from Liang-Wai Gan Cao by liquid chromatography-high resolution-mass spectrometry and computational MS/MS fragmentation. J Pharm Biomed Anal 2017;146:37-47.
27. Pandey R, Kumar B, Meena B, Srivastava M, Mishra T, Tiwari V, *et al.* Major bioactive phenolics in Bergenia species from the Indian Himalayan region: Method development, validation and quantitative estimation using UHPLC-QqQLIT-MS/MS. PLoS One 2017;12:e0180950.
28. Wang X, Xing R, Chen Z, Yu H, Li R, Li P. Effect and mechanism of mackerel (*Pneumatophorus japonicus*) peptides for anti-fatigue. Food Funct 2014;5:2113-9.
29. You L, Zhao M, Regenstein JM, Ren J. *In vitro* antioxidant activity and *in vivo* anti-fatigue effect of loach (*Misgurnus anguillicaudatus*) peptides prepared by papain digestion. Food Chem 2011;124:188-94.
30. Narkhede AN, Jagtap SD, Nirmal PS, Giramkar SA, Nagarkar BE, Kulkarni OP, *et al.* Anti-fatigue effect of Amarkand on endurance exercise capacity in rats. BMC Complement Altern Med 2016;16:23.
31. Suh SH, Paik IY, Jacobs K. Regulation of blood glucose homeostasis during prolonged exercise. Mol Cells 2007;23:272-9.
32. Jung KA, Han D, Kwon EK, Lee CH, Kim YE. Antifatigue effffect of *Rubus coreanus* Miquel extract in mice. J Med Food 2007;10:689.
33. Jin HM, Wei P. Anti-fatigue properties of Tartary buckwheat extracts in mice. Int J Mol Sci 2011;12:4770-80.
34. Jung K, Kim IH, Han D. Effect of medicinal plant extracts on forced swimming capacity in mice. J Ethnopharmacol 2004;93:75-81.
35. Xu M, Liang R, Li Y, Wang J. Anti-fatigue effects of dietary nucleotides in mice. Food Nutr Res 2017;61:1334485.
36. Bo Y, Lu ZX, Bie XM, Lu FX, Huang XQ. Scavenging and anti-fatigue activity of fermented defatted soybean peptides. Eur Food Res Technol 2008;226:415-21.
37. Dohm GL, Tapscott EB, Barakat HA, Kasperek GJ. Influence of fasting on glycogen depletion in rats during exercise. J Appl Physiol Respir Environ Exerc Physiol 1983;55:830-3.
38. Ma L, Cai DL, Li HX, Tong BD, Song, LH, Wang Y. Anti-fatigue effects of salidroside in mice. J Med Coll PLA 2008;23:88-93.
39. Zhong L, Zhao L, Yang F, Yang W, Sun Y, Hu Q. Evaluation of anti-fatigue property of the extruded product of cereal grains mixed with *Cordyceps militaris* on mice. J Int Soc Sports Nutr 2017;14:15.
40. Wang L, Zhang HL, Lu R, Zhou YJ, Ma R, Lv JQ, *et al.* The decapeptide CMS001 enhances swimming endurance in mice. Peptides 2008;29:1176-82.
41. Brancaccio P, Maffulli N, Limongelli FM. Creatine kinase monitoring in sport medicine. Br Med Bull 2007;81-82:209-30.
42. Ebbeling CB, Clarkson PM. Exercise-induced muscle damage and adaptation. Sports Med 1989;7:207-34.
43. Darques JL, Decherchi P, Jammes Y. Mechanisms of fatigue-induced activation of group IV muscle afferents: The roles played by lactic acid and inflammatory mediators. Neurosci Lett 1998;257:109-12.
44. Chin ER, Allen DG. The contribution of pH-dependent mechanisms to fatigue at different intensities in mammalian single muscle fibres. J Physiol 1998;512:831-40.
45. Jia JM, Wu CF. Anti-fatgue activity of tissue culture extracts of *Saussurea* involucrata. Pharm Biol 2008;46:433-6.
46. Liu JY, Feng CP, Li X, Chang MC, Meng JL, Xu LJ. Immunomodulatory and antioxidative activity of *Cordyceps militaris* polysaccharides in mice. Int J Biol Macromol 2016;86:594-8.
47. Huang WC, Chiu WC, Chuang HL, Tang DW, Lee ZM, Wei L, *et al.* Effect of curcumin supplementation on physiological fatigue and physical performance in mice. Nutrients 2015;7:905-21.
48. Zhang Y, Yao X, Bao B, Zhang Y. Anti-fatigue activity of a triterpenoid-rich extract from Chinese bamboo shavings (*Caulis bamfusae* in Taeniam). Phytother Res 2006;20:872-6.
49. Gao H, Zhang W, Wang B, Hui A, Du B, Wang T, *et al.* Purification, characterization and anti-fatigue activity of polysaccharide fractions from okra (*Abelmoschus esculentus* (L.) Moench). Food Funct 2018;9:1088-101.
50. Surhio MM, Wang Y, Fang S, Li J, Ye M. Anti-fatigue activity of a *Lachnum polysaccharide* and its carboxymethylated derivative in mice. Bioorg Med Chem Lett 2017;27:4777-80.
51. Zhao HP, Zhang Y, Liu Z, Chen JY, Zhang SY, Yang XD, *et al.* Acute toxicity and anti-fatigue activity of polysaccharide-rich extract from corn silk. Biomed Pharmacother 2017;90:686-93.
52. Wei W, Li ZP, Zhu T, Fung HY, Wong TL, Wen X, *et al.* Anti-fatigue effects of the unique polysaccharide marker of *Dendrobium officinale* on BALB/c mice. Molecules 2017;22:E155.
53. Liu J, Du C, Wang Y, Yu Z. Anti-fatigue activities of polysaccharides extracted from *Hericium erinaceus.* Exp Ther Med 2015;9:483-7.
54. Zhang JC, Chen J. The effects of *Taraxacum officinale* extracts (TOE) supple mentation on physical fatigue in mice. Afr J Tradit Complement Altern Med 2011;8:128-33.
55. Belenky P, Bogan KL, Brenner C. NAD+ metabolism in health and disease. Trends Biochem Sci 2007;32:12-9.
56. Sukhodub A, Sudhir R, Du Q, Jovanovis S, Reyes S, Jovanovic A. Nicotinamide-rich diet improves physical endurance by up-regulating SUR2A in the heart. J Cell Mol Med 2011;15:1703-12.
57. Harwanto D, Ardiansyah A, Jin HJ, Choi JS, Jin DH, Hong YK. The hot water extract and active components nicotinamide and guanosine of the leather carp *Cyprinus carpio* nudis improve exercise performance in mice. J Food Biochem 2019;43:e13004.
58. Idzko M, Ferrari D, Eltzschig HK. Nucleotide signalling during inflammation. Nature 2014;509:310-7.
59. Bröer S, Bröer A. Amino acid homeostasis and signalling in mammalian cells and organisms. Biochem J 2017;474:1935-63.

60. Kong F, Lee BH, Wei K. 5-Hydroxymethylfurfural mitigates lipopolysaccharide-stimulated inflammation via suppression of MAPK, NF-κB and mTOR activation in RAW 264.7 cells. Molecules 2019;24:E275.
61. El-Seedi HR, El-Said AM, Khalifa SA, Göransson U, Bohlin L, Borg-Karlson AK, *et al*. Biosynthesis, natural sources, dietary intake, pharmacokinetic properties, and biological activities of hydroxycinnamic acids. J Agric Food Chem 2012;60:10877-95.
62. Kiliç I, Yeşiloğlu Y. Spectroscopic studies on the antioxidant activity of p-coumaric acid. Spectrochim Acta A Mol Biomol Spectrosc 2013;115:719-24.
63. Kadoma Y, Fujisawa S. A comparative study of the radical-scavenging activity of the phenolcarboxylic acids caffeic acid, p-coumaric acid, chlorogenic acid and ferulic acid, with or without 2-mercaptoethanol, a thiol, using the induction period method. Molecules 2008;13:2488-99.
64. Zang LY, Cosma G, Gardner H, Shi X, Castranova V, Vallyathan V. Effect of antioxidant protection by p-coumaric acid on low-density lipoprotein cholesterol oxidation. Am J Physiol Cell Physiol 2000;279:C954-60.
65. Jaganathan SK, Mazumdar A, Mondhe D, Mandal M. Apoptotic effect of eugenol in human colon cancer cell lines. Cell Biol Int 2011;35:607-15.
66. Roth-Walter F, Moskovskich A, Gomez-Casado C, Diaz-Perales A, Oida K, Singer J, *et al*. Immune suppressive effect of cinnamaldehyde due to inhibition of proliferation and induction of apoptosis in immune cells: Implications in cancer. PLoS One 2014;9:e108402.
67. Yang W, Wang H. 5,7-dimethoxycoumarin prevents chronic mild stress induced depression in rats through increase in the expression of heat shock protein-70 and inhibition of monoamine oxidase-A levels. Saudi J Biol Sci 2018;25:253-8.
68. Burdge GC. Metabolism of alpha-linolenic acid in humans. Prostag Leukotr Ess 2006;75:161-8.
69. Chiang YL, Haddad E, Rajaram S, Shavlik D, Sabaté J. The effect of dietary walnuts compared to fatty fish on eicosanoids, cytokines, soluble endothelial adhesion molecules and lymphocyte subsets: A randomized, controlled crossover trial. Prostaglandins Leukot Essent Fatty Acids 2012;87:111-7.
70. Deshpande R, Mansara P, Suryavanshi S, Kaul-Ghanekar R. Alphalinolenic acid regulates the growth of breast and cervical cancer cell lines through regulation of NO release and induction of lipid peroxidation. J Biochem Mol Biol 2013;2:6-17.
71. Delplanque B, Du Q, Agnani G, Le Ruyet P, Martin JC. A dairy fat matrix providing alpha-linolenic acid (ALA) is better than a vegetable fat mixture to increase brain DHA accretion in young rats. Prostaglandins Leukot Essent Fatty Acids 2013;88:115-20.
72. Erdinest N, Shmueli O, Grossman Y, Ovadia H, Solomon A. Antiinflammatory effects of alpha linolenic acid on human corneal epithelial cells. Invest Ophth Vis Sci 2012;53:4396-406.