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# Qianhu (*Peucedanum praeruptorum* Dunn) Improves exercise capacity in mice by regulating Nrf2/HO-1 oxidative stress signaling pathway

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## Abstract

This study assessed the effect of Qianhu (*Peucedanum praeruptorum* Dunn) on the recovery of movement in mice with D-galactose-induced dyskinesia. The evaluation of the ability of mice to exercise revealed that Qianhu increased the running and swimming time to exhaustion in mice with dyskinesia. In addition, measurement of biochemical indices in mice showed that Qianhu altered the serum levels of blood urea nitrogen (BUN), blood lactic acid (BLA), malonaldehyde (MDA), liver glycogen (HG), muscle glycogen (MG), while the levels of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) remained normal. Additionally, Qianhu regulated the mRNA expression of copper/zinc-superoxide dismutase (Cu/Zn-SOD), manganese-superoxide dismutase (Mn-SOD), catalase (CAT), heme oxygenase 1 (HO-1), nuclear factor erythroid2-related factor (Nrf2) and syncytin-1 in mice and also protected mice against D-galactose-induced oxidative stress. The analysis of the chemical composition of Qianhu revealed that it mainly contains isochlorogenic acid B, myricetin, baicalin, luteolin, and kaempferol, which are known excellent antioxidants that protect against tissue damage due to oxidative stress and have anti-aging properties. Thus, these compounds may be the active components in Qianhu that improve the ability of mice to exercise, and may also represent the key compounds for its use as natural medicine or health food.

**Keywords** Qianhu, Exercise capacity, Oxidative stress, Nrf2/HO-1 signaling pathway, mRNA

## Introduction

As people age, their organs and tissues lose their ability to function properly, including their muscles. As a result of this deterioration in organ and muscle function, particularly liver function, the body's ability to move also deteriorates. Long-distance runners have

higher telomerase activity in their leukocytes than non-runners, suggesting that exercise may slow the aging process. Long-distance runners also have slower heart rates and lower blood pressure and cholesterol levels, indicating a relationship between physical endurance and aging [1]. Maintaining consistent and an adequate exercise program of mobility, stability and strength helps improve the performance of the cardiovascular system. The benefits of regular exercise include a healthy metabolism, a delayed onset of arteriosclerosis, and a reduction in diseases associated with liver dysfunction [2]. In addition, other factors can contribute to the poor health associated with aging, such as abnormal blood circulation, the buildup of harmful metabolic chemicals, and the imbalance between

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the consumption and absorption of energy-generating nutrients and other substances in the body. Aging of the body and exercise are intertwined, and the ability to exercise can promote regular exercise, which can delay aging. Liver function impairment due to aging, metabolic imbalance, and increased fatigue lead to decreased exercise capacity. Maintaining a reasonable exercise regimen can improve the liver function, relieve fatigue, and maintain the vitality of the body [3].

The liver is a key regulator of systemic energy homeostasis through the production of energy by oxidative phosphorylation, as a result it is highly vulnerable to oxidative stress. Insufficient production of antioxidant enzymes will result in dysfunction of liver mitochondrial respiratory complexes and generation of reactive oxygen species (ROS) as a by-product of mitochondrial oxidative phosphorylation, leading to mitochondrial DNA damage, hepatocyte aging, and hepatocellular carcinoma [4]. The continuous accumulation of free radicals in the body due to liver aging impacts normal metabolism, increases body fatigue, and decreases exercise capacity, thereby lowering the quality of life for the elderly. Proper functioning and health of organs and tissues, especially the liver, enable the body to continually counteract oxidative stress and decrease ROS-induced aging [5]. Therefore, liver function, aging, and motor abilities are all intertwined. Accordingly, improving motor function is an effective strategy to delay aging by protecting liver function and improving motor function.

Qianhu (*Peucedanum praeruptorum* Dunn) is a traditional Chinese medicine and functional food in China, with various beneficial pharmacological properties, including expectorant, antitussive, anti-inflammatory, analgesic, anti-myocardial ischemia, anti-cardiac arrhythmia, platelet aggregation inhibitory, and microcirculation-improving effects [6–10]. In addition, Qianhu is used as an ingredient in food preparation. The botanical functional food products have been confirmed to have antioxidant and anti-aging effects, and some have also been shown to improve exercise capacity [11, 12]. The results of in vitro studies showed that Qianhu scavenged hydroxyl free radicals and superoxide anion free radicals, and also had a good inhibitory effect on lipid peroxidation, which was dose-dependent. The antioxidant effect of Qianhu has been found to be related to its various active ingredients, including flavonoids, such as baicalin and kaempferol [13]. This study investigated the effects of Qianhu on exercise capacity, analyzed its active components to provide a theoretical basis for its application as an antioxidant, and contributed to the understanding of the function and efficacy mechanism of natural functional food resources.

## Materials and methods

### Qianhu extraction

Two hundred grams of freeze-dried Qianhu (Hebei Anguo Ruiqi Traditional Chinese Medicine Co., Ltd., Anguo City, Hebei, China) were ground into a fine powder and dissolved in 4 L of 70% (v/v) ethanol. Then, the mixture was extracted at 60 °C for 3 h to obtain the Qianhu extract. The solvent was then removed using a rotary evaporator.

### Animal model

The experimental study lasted 10 weeks. The 50 ICR 6-week old mice (half male and half female) used in this study were purchased from Shaanxi University of Chinese Medicine (Xi'an, Shaanxi, China, Institutional Animal Care and Use Committee (IACUC) number: SYXX (Shaanxi) 2022–008) and kept for 7 days in an environment with temperature and humidity that promoted adaptive feeding. Subsequently, the mice were divided into 5 groups (with 10 mice in each group): normal, model, vitamin C (VC), low-dose Qianhu (L-Qianhu), and gastric high-dose Qianhu (H-Qianhu) groups. For six weeks, mice in all groups, received intraperitoneal injections of normal saline (10 mL/kg of body weight (b.w.)) after being administered a 5% (w/w) D-galactose solution at a daily dose of 100 mg/kg b.w. [14]. From week 7, mice in the normal and model groups were gavaged with distilled water (10 mL/kg b.w.). Mice in the VC group were administered a daily dose of 100 mg/kg b.w. of vitamin C, while mice in the L-Qianhu and H-Qianhu groups were administered a daily dose of 50 and 100 mg/kg b.w. of Qianhu, respectively. At 10 weeks, after completing time-to-fatigue running and swimming tests, blood was collected by retro-orbital venous plexus sampling, and all mice were euthanized. The liver and skeletal muscle were subsequently dissected for subsequent use.

### Mouse running to exhaustion test

After administering the corresponding treatment by oral gavage, the mice were forced to run to exhaustion on a running wheel (YH-CS, Wuhan Yihong Technology Co., Ltd, Wuhan, Hubei, China) set to 20 rpm, by giving 5 consecutive shocks, and the running time to exhaustion of the mice was recorded.

### Mouse swimming to exhaustion test

After administering the corresponding treatment by intragastric administration, the mice were placed in a homemade thermostatic water tank with water at a temperature of 30 °C and a depth of 20 cm. Then, the mice were forced to swim to exhaustion, which was considered

to be after sinking and being unable to resurface for 10 s, and the swimming time to exhaustion of the mice was recorded.

#### Determination of the serum indexes in mice

The serum levels of blood urea nitrogen (BUN), blood lactic acid (BLA), malonaldehyde (MDA), liver glycogen (HG), muscle glycogen (MG), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) were determined in retro-orbital blood samples. The blood was centrifuged at 4 °C for 10 min at 1500 rpm, and the upper serum layer was collected. All assays were performed according to the recommendations of the kits' manufacturer (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China) [15].

#### Preparation of mouse tissue sections

The dissected mouse liver and skeletal muscle tissue were washed 3 times with saline and preserved in 10% (v/v) formalin. The fixed tissue was dehydrated at 4 °C for 48 h, paraffin-embedded, sliced into 5–10 µm slices, and stained with hematoxylin and eosin (H&E). Eventually, tissue pathology was examined under an Olympus BX53 light microscope (Olympus Corporation, Tokyo, Japan) [16].

#### Detection of mRNA expression in mouse tissues

After weighing 0.2 g of mouse liver or skeletal muscle tissue, and washing with saline, total RNA was extracted by homogenizing the tissue samples in 1.0 mL RNAzol (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) according to the manufacturer's instructions. After measuring the absorbance values of the extracted RNA samples at 260 and 280 nm, the RNA concentration was calculated and its quality was assessed by determining the OD<sub>260</sub>/OD<sub>280</sub> ratio, and the final concentration was adjusted to 1 µg/L and used for cDNA synthesis. After cDNA synthesis, each sample was subjected to gene expression analysis by quantitative real-time polymerase chain reaction (qPCR). The qPCR was performed

in a 20 µL reaction containing 1 µL cDNA, 10 µL SYBR Green PCR Master Mix (Thermo Fisher Scientific Inc., Waltham, MA, USA), 1 µL each of upstream and downstream primers (Table 1), and 7 µL of sterile distilled water, using the StepOnePlus™ real-time PCR System (Thermo Fisher Scientific Inc.). The mRNA amplification conditions were as follows: denaturation at 95 °C for 60 s, followed by 40 cycles at 95 °C for 15 s, 55 °C, and 72 °C for 35 s). GAPDH was used as the internal parameter, and the relative expression intensity of each gene was calculated using the 2<sup>-ΔΔCt</sup> method [17].

#### High-performance liquid chromatography

Standards and Qianhu extracts were dissolved in methanol to obtain the test solutions. Then, the chemical composition of the Qianhu extract samples was determined by high-performance liquid chromatography (HPLC) on a Thermo DIONEX UltiMate 3000 HPLC system (Thermo Fisher Scientific Inc.), equipped with an Acclaim™ 120 C18 column (4.6 mm × 150 mm, 5 µm), using a mobile phase of methanol-0.5% glacial acetic acid, detection wavelength of 328 nm, column temperature of 35 °C, flow rate of 0.6 mL/min, and sample intake of 20 µL.

#### Statistical analysis

Data were collected for each mouse, and the final experimental results are presented as the mean ± standard deviation. The SPSS software (IBM Corporation, Armonk, NY, USA) was used to determine whether the differences determined by the one-way analysis of variance were statistically significant at the *P* < 0.05 level.

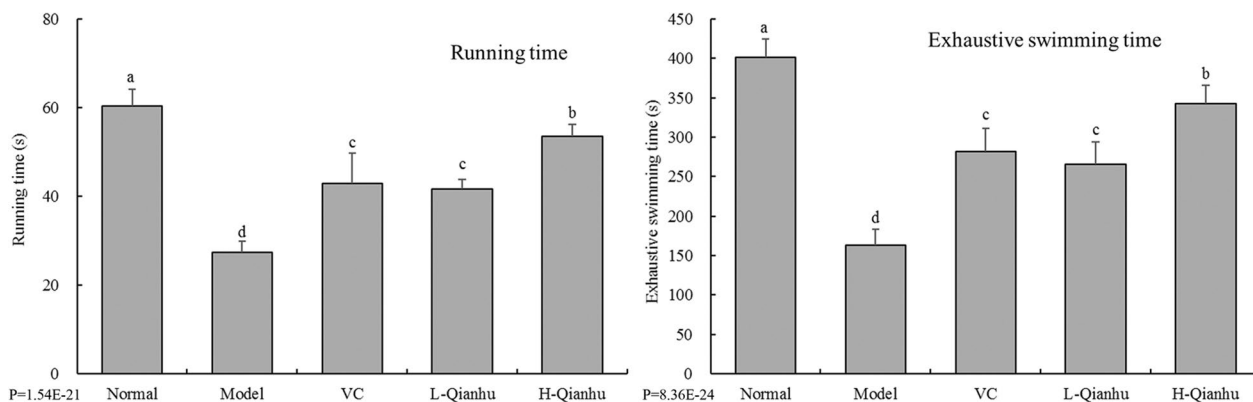
## Results

#### Effects of Qianhu on the exercise capacity of mice

The results shown in Fig. 1 reveal that the normal group mice were able to run and swim for a longer period of time before exhaustion than the model group mice. Supplementation with Qianhu and VC significantly (*P* < 0.05) increased the running and swimming time to exhaustion

**Table 1** The primer sequences used in this experiment

Gene name	Forward sequence	Reverse sequence
Cu/Zn-SOD	5'-AACCAGTTGTGTTGTGAGGAC-3'	5'-CCACCATGTTTCTTAGAGTGAGG-3'
Mn-SOD	5'-CAGACCTGCCTTACGACTATGG-3'	5'-CTCGGTGGCGTTGAGATTGTT-3'
CAT	5'-GGAGGCGGGAACCCAATAG-3'	5'-GTGTGCCATCTCGTCAGTGAA-3'
HO-1	5'-GCTGGTGATGGCTTCTTGTA-3'	5'-ACCTCGTGGAGACGCTTTACAT-3'
Nrf2	5'-AGTGACTCGGAAATGGATGAG-3'	5'-TGTGCTGGCTGTGCGTTAGG-3'
Syncytin-1	5'-GTTAACTTTGTCTCTCCAGAATCGA-3'	5'-CATCAGTACGTGGGCTAGCA-3'
GAPDH	5'-TGACCTCAACTACATGGTCTACA-3'	5'-CTTCCCATTCTCGGCCTTG-3'



**Fig. 1** The running and exhaustive swimming times in aged mice with impaired exercise ability. **a–d** The same lowercase letters indicate no significant difference between the two groups at the level of  $P < 0.05$ , and different lowercase letters indicate a significant difference between the two groups,  $n = 10$

of physically impaired mice compared to the model group, with H-Qianhu having the most marked effect on the running and swimming endurance to exhaustion, significantly ( $P < 0.05$ ) better than that with L-Qianhu and VC.

#### Serum levels of BUN, BLA, MDA, HG, MG, SOD, and GSH-Px in mice

The serum levels of BUN, BLA, and MDA in the normal group mice were significantly ( $P < 0.05$ ) lower than those in the other groups. In contrast, the serum levels of HG, MG, SOD and GSH-Px were significantly ( $P < 0.05$ ) greater, as shown in Fig. 2. Moreover, the serum levels of biochemical indices in mice of the model group showed the opposite trend as those in mice of the normal group. In the relative model group, dandelion and VC lowered the serum levels of BUN, BLA, and MDA in mice with impaired liver function. However, H-dandelion could reduce the increased levels of HG, MG, SOD activity and GSH-Px activity closer to normal.

#### Pathological changes in the mouse liver

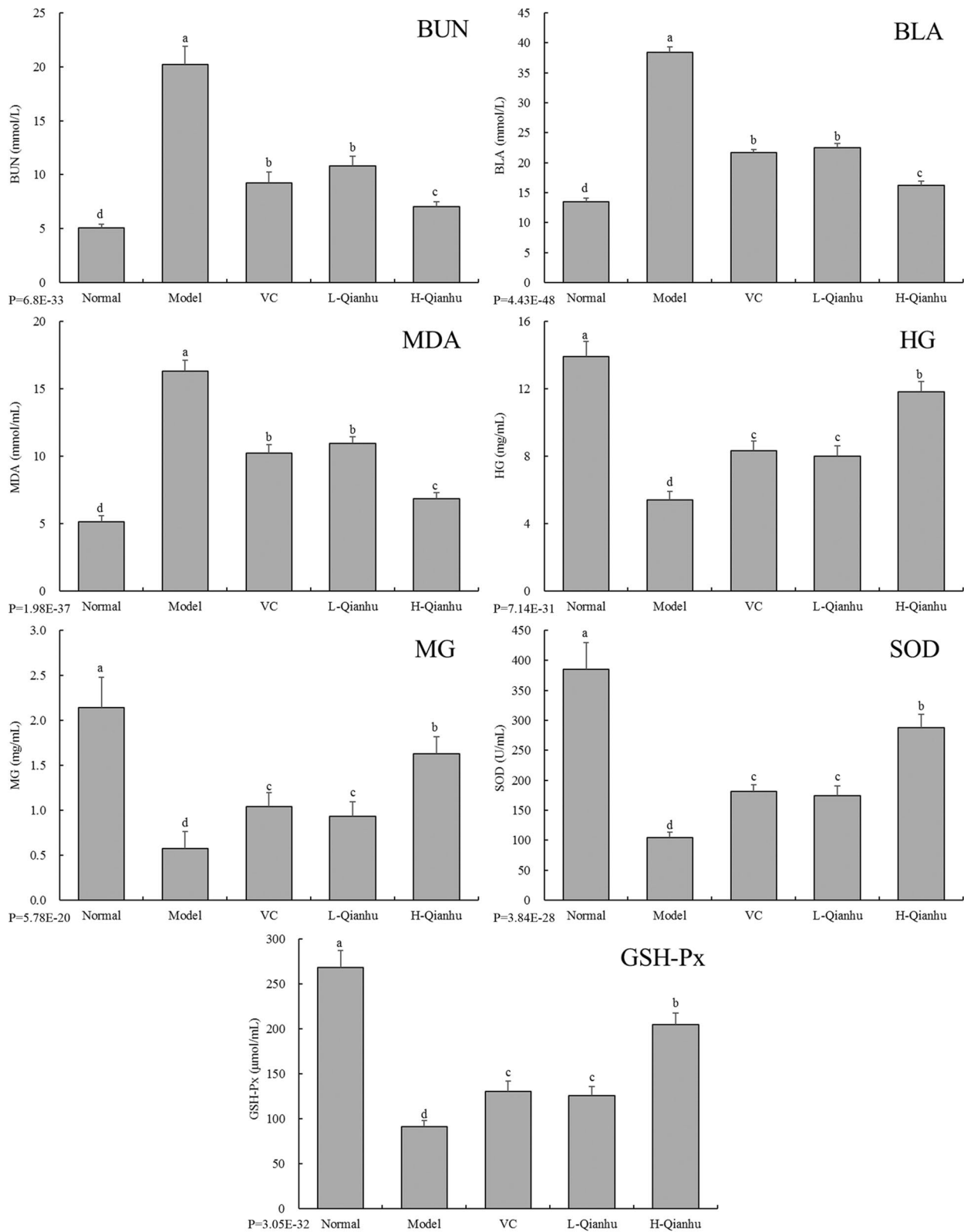
The microscopic tissue morphology of mouse liver is shown in Fig. 3. The normal liver is transparent and intact, and its hepatocytes are radially centered on the central vein. In the model group, the hepatic leaflet structure is disrupted, the hepatocyte organization is disordered, cell membrane and nuclear envelope are ruptured, and apoptotic bodies are also formed. Both Qianhu and VC were able to minimize hepatocyte damage in physically impaired mice. The liver lobular structure was mostly intact after H-Qianhu treatment, although some cells in the L-Qianhu and VC groups were destroyed.

#### Gene expression analysis in mouse liver and skeletal muscle tissues

As shown in Fig. 4, the normal group mice had higher expression levels of Cu/Zn-SOD, Mn-SOD, CAT, HO-1, and Nrf2 than the model group mice. Meanwhile, in the liver tissues of physically impaired mice, both Qianhu and H-Qianhu upregulated the expression of nNOS, Cu/Zn-SOD, Mn-SOD, CAT, HO-1, and Nrf2, but H-Qianhu upregulated their expression to a greater extent, bringing their expression levels closer to those of the normal group mice. The results of the mRNA expression study in skeletal muscle tissues were consistent with those of the expression analysis of Cu/Zn-SOD, Mn-SOD, CAT, HO-1, and Nrf2 in liver tissues (Fig. 5). Additionally, the skeletal muscle of the model group mice showed the highest expression level of syncytin-1. Also, treatment with H-Qianhu, L-Qianhu, and VC upregulated the expression of syncytin-1 in skeletal muscle of physically impaired mice, but the treatment with H-Qianhu upregulated its expression to a greater extent, bringing its expression level closer to that of the normal group.

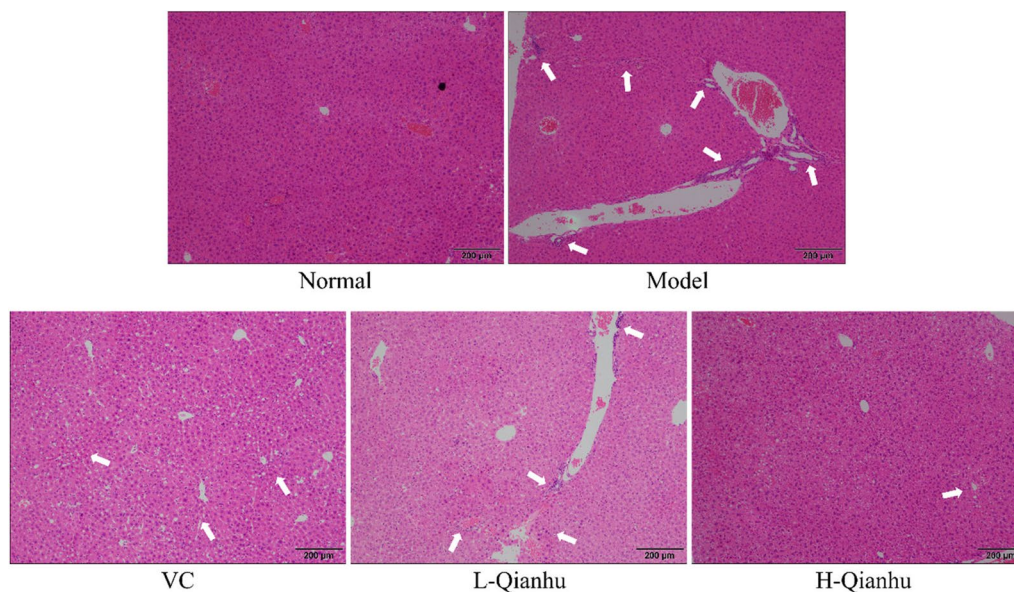
#### Chemical composition of Qianhu

As shown in Fig. 6, Qianhu is mainly composed of isochlorogenic acid B (15.42 mg/g), myricetin (76.47 mg/g), baicalin (33.82 mg/g), luteolin (239.68 mg/g), and kaempferol (223.08 mg/g). The levels of luteolin and kaempferol are relatively high, suggesting that these two substances might be the most important active ingredients.



**Fig. 2** The serum BUN, BLA, MDA, HG, MG, SOD, and GSH-Px levels in aged mice with impaired exercise ability. **a–d** The same lowercase letters indicate no significant difference between the two groups at the level of  $P < 0.05$ , and different lowercase letters indicate a significant difference between the two groups,  $n = 10$





**Fig. 3** The observation of liver pathological H&E stained sections in aged mice with impaired exercise ability. The arrows indicate inflammatory infiltration of liver tissue and hepatocyte damage

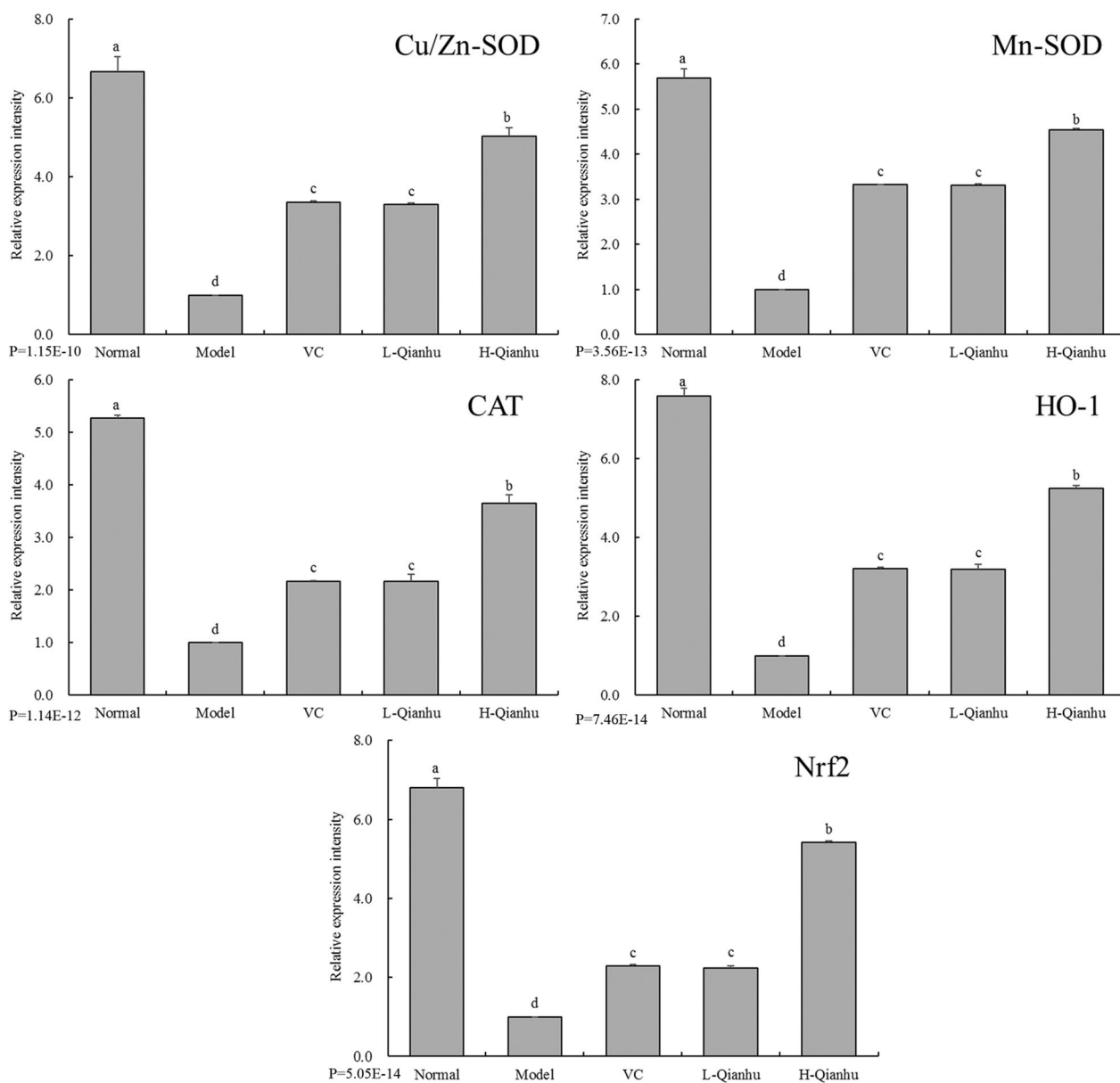
## Discussion

In humans, aging is associated with liver dysfunction, liver metabolic disorders, and increases free radicals in the body. The excessive accumulation of free radicals causes organ damage and function impairment, including movement-related organs and tissues, eventually resulting in decreased physical exercise ability and frequent fatigue [18]. An appropriate exercise regimen can reduce free radical damage to human cell membranes, allow cells to maintain the normal oxidative phosphorylation, maintain mitochondrial structure and functional integrity, and promote liver health. Moreover, being in a good physical condition can also help maintain the ability to exercise [19]. Animal studies on running and swimming endurance to exhaustion often validate exercise capacity, which is the most direct evidence of fatigue resistance and a crucial indicator of the body's resistance to metabolic imbalance [20]. The effect of Qianhu in mice with low exercise capacity was also investigated in this study to determine the effect of Qianhu on exercise ability. The findings revealed that Qianhu might extend running and swimming endurance to exhaustion in mice, implying that Qianhu improves exercise ability.

With aging, when the human body is active for a long period of time, abnormal glucose metabolism and lipid metabolism will ensue, and a large amount of protein and amino acids will be consumed, thus excessively increasing the BUN level in the body. During strenuous activity, the human body will momentarily experience hypoxia,

causing a shift in the normal energy metabolism process and the consequent accumulation of a high amount of lactic acid in the muscle in a short period of time, leading to fatigue. At the same time, lactic acid deposited in the muscle will progressively enter the bloodstream to produce BLA. Therefore, BLA may be used as a key index to measure the anti-fatigue response in the body [21]. Moreover, the magnesium levels in body tissues is directly connected to exercise endurance and resistance to fatigue. When the levels of HG and MG decrease in muscle tissues, the level of energy source compounds decreases, leading to the use of MG to produce the necessary energy via glycolysis. During glycolysis, a considerable amount of lactic acid accumulates in the muscle tissues and is released into the bloodstream, reducing the activity of the muscle, thereby decreasing the capacity of the body to exercise [22]. Strenuous and excessive exercise increases free radicals in the body due to higher metabolism.

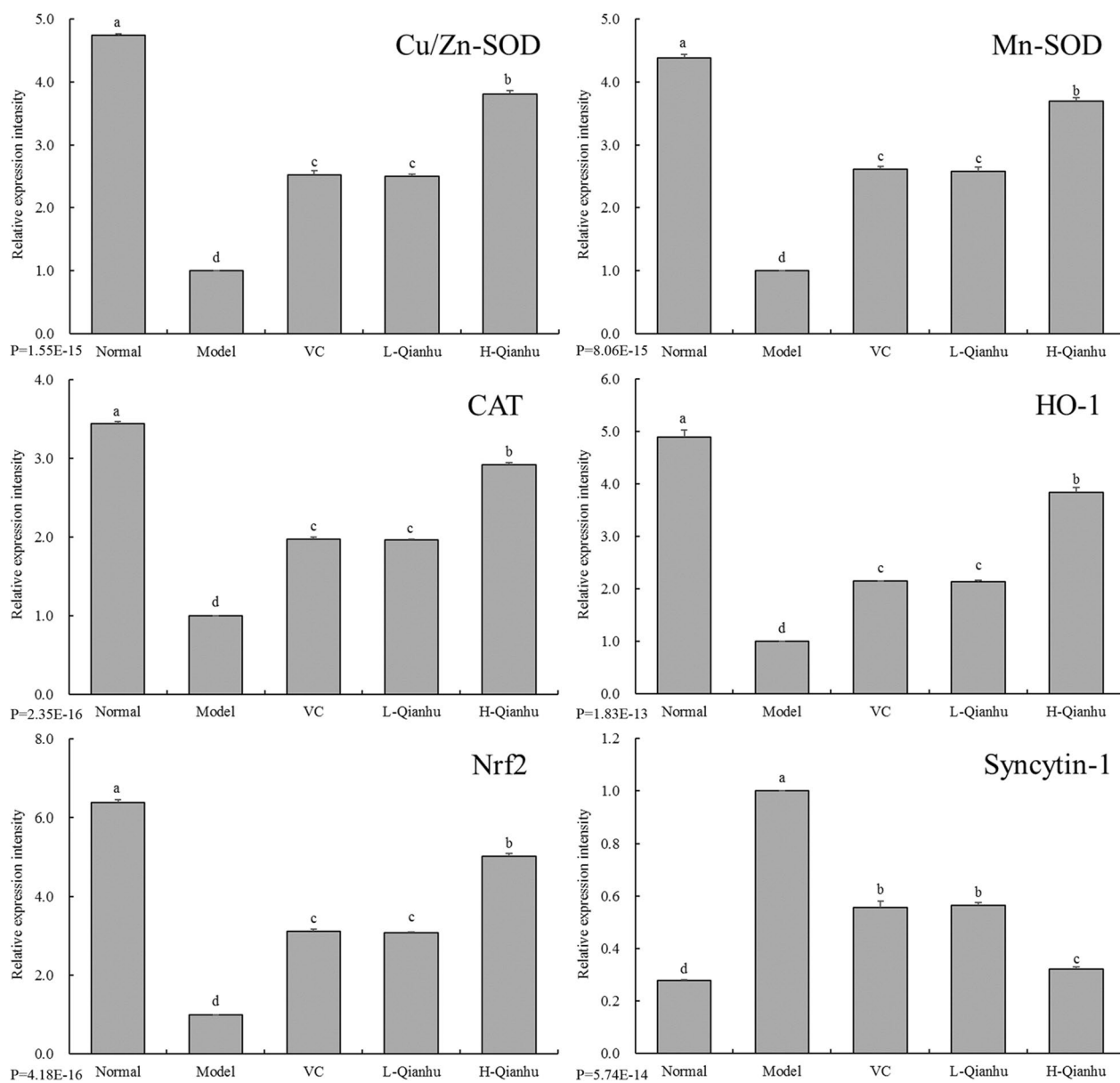
The major antioxidant enzymes CAT, GSH-Px, and SODs maintain normal oxidative stress level and eliminate excess free radicals in the body. GSH-Px is involved in preserving the structural and functional integrity of the cell membrane by catalyzing the inactivation of hydrogen peroxide and lipid peroxides to protect cells from peroxidative damage [23]. SODs are key antioxidant enzymes involved in maintaining the oxidation-antioxidant balance in the body. SODs catalyze the dismutation of superoxide radicals into molecular oxygen and hydrogen peroxide and effectively eliminate superoxide anion



**Fig. 4** The mRNA expression of Cu/Zn-SOD, Mn-SOD, CAT, HO-1 and Nrf2 of liver tissue in aged mice with impaired exercise ability. **a–d** The same lowercase letters indicate no significant difference between the two groups at the level of  $P < 0.05$ , and different lowercase letters indicate a significant difference between the two groups,  $n = 10$

radicals, limiting the generation of dangerous hydroxyl radicals and protecting cells and tissues. When the accumulated free-base forms in the body significantly exceed the antioxidant enzyme defense capacity, free radicals will destroy tissue, by peroxidation of unsaturated lipids in the cell membrane, reducing membrane fluidity, and impairing cell function, resulting in production of a large amount of MDA. MDA will further destroy the structure of the cell membrane, leading to cell swelling and necrosis, thus the level of MDA in the body can also

indirectly reflect the levels of free radicals in the body [24]. The level of lipid peroxides in the body increases with age, and the level of activity of antioxidant enzymes in the body determines the extent of excessive free radical damage to tissue cells. Detecting antioxidant enzyme activity in the body can reflect the strength of the body's resistance to oxidative stress, degree of fatigue, and exercise capacity [25]. The experimental results of this study showed that Qianhu can have a marked upregulatory effect on the expression of SODs and GSH-Px in serum



**Fig. 5** The mRNA expression of Cu/Zn-SOD, Mn-SOD, CAT, HO-1, Nrf2 and syncytin-1 of skeletal muscle tissue in aged mice with impaired exercise ability. **a–d** The same lowercase letters indicate no significant difference between the two groups at the level of  $P < 0.05$ , and different lowercase letters indicate a significant difference between the two groups,  $n = 10$

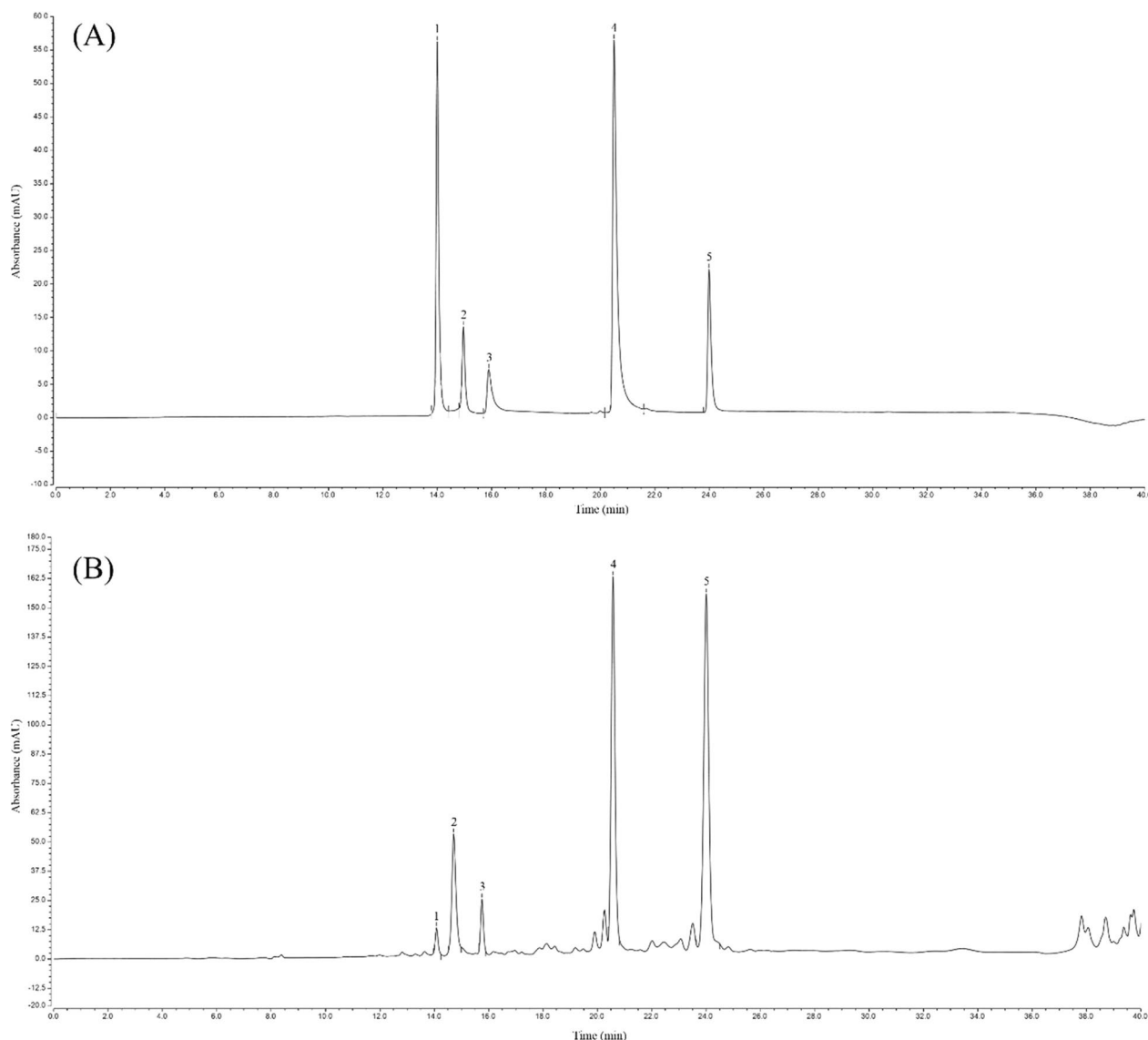
of physically impaired mice, thereby delaying aging and improving exercise endurance and ability to exercise.

The liver is the main organ of the body's oxidative stress response, which may well reflect the degree of oxidation in the body. It is also an organ where free radicals and lipid peroxides are produced, thus increased free radicals can reflect actual liver tissue damage. Furthermore, liver dysfunction leads to lower frequency of vasoconstriction in the liver and reduces blood flow to many organs, decreasing exercise capacity [26]. In this

study, microscopic examination of pathological sections revealed obvious liver tissue degeneration and lesions, which were effectively alleviated by treatment with Qianhu. Therefore, one of Qianhu main activities may be to indirectly improve athletic ability.

The primary function of the enzyme CAT is to catalyze the degradation of hydrogen peroxide to eliminate by-products of peroxidative stress found in mitochondria, peroxisomes, and erythrocytes, thereby inhibiting oxidative stress and preventing cell damage by ROS and [27].





**Fig. 6** Compound composition of Qianhu analyzed using HPLC, (A) chromatogram of standard, (B) chromatogram of Qianhu, 1: isochlorogenic acid B, 2: myricetin, 3: baicalin, 4: luteolin, 5: kaempferol

Mammals produce two different SODs, namely SOD1 and SOD2. SOD1 contains  $Mn^{4+}$  and is found in the mitochondria, whereas SOD2 is found in the cytoplasm and contains  $Cu^{2+}$  and  $Zn^{2+}$  ions. Both enzymes greatly reduce in vivo oxidative stress. With aging, after exercise, the body experiences an increase in oxidative stress and releases more free radicals due to liver dysfunction and reduced exercise capacity. Higher activity of the enzymes CAT, SOD1, and SOD2 is required to scavenge free radicals in the body, reduce fatigue, maintain exercise capacity and prevent liver dysfunction [28]. HMOX1 is an important antioxidant enzyme that mainly catalyzes the catabolism of heme into a ferrous ion, carbon monoxide,

and biliverdin, while NFE2L2 can directly regulate the promoter activity of HMOX1 [29]. The NFE2L2-HMOX1 signaling pathway is considered a major cellular defense mechanism against oxidative stress. Upregulation of NFE2L2 expression can accelerate the enzymatic reactions involved in the anti-oxidative stress defense mechanism by inducing the production of antioxidant enzymes and detoxification enzymes in neuronal cells and simultaneously promoting the production of GSH, and other antioxidants to protect tissues [30]. The experimental findings of this study further supported the hypothesis that skeletal muscle and liver tissue produce significant levels of free radicals due to the induction of

various oxidative stress responses. Qianhu can increase the survivability of the enzymes CAT, Cu/Zn-SOD, Mn-SOD, HO-1, and Nrf2, suggesting that Qianhu may slow down the aging process, thereby protecting the body and enhancing the ability of mice to exercise.

The protein encoded by the syncytin-1 gene is involved in immunological regulation and excessive syncytin-1 expression in skeletal muscle results in dysfunctional motor neurons. High syncytin-1 expression in skeletal muscle also causes the formation of oxygen free radicals and mitochondrial damage. Syncytin-1 activation by oxidative stress can result in muscle and nerve damage and impaired motor performance [31]. In this study, Qianhu dramatically reduced syncytin-1 expression, suggesting that Qianhu might reduce oxidative stress, improve free radical scavenging ability, increase the exercise capacity of mice, and alleviate exercise fatigue.

Effective antioxidants include isochlorogenic acid B, myricetin, baicalin, luteolin, and kaempferol, which help prevent oxidative stress-induced tissue damage and slow down aging [32–36]. All of these compounds are flavonoids, which are active key antioxidant ingredients in Qianhu, and similar results have been obtained in previous studies [8, 13]. The combined effects of these active antioxidant ingredients inhibit the exercise capacity-decreasing effect of D-galactose-induced aging.

This study preliminarily demonstrated that Qianhu can improve the ability of mice to exercise through its antioxidant effects. However, the study has certain limitations, which include the requirement for further human clinical studies for in-depth verification, and a more comprehensive analysis of the active ingredients to elucidate the strengthening effect and mechanism of the various active ingredients combined.

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#### Author contributions

Investigation, methodology, writing-original draft: BL; formal analysis, methodology, writing-review, and editing: YNW; conceptualization, supervision, writing-review, and editing: XGY. All authors read and approved the final manuscript.

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None.

#### Availability of data and materials

All data generated or analysed during this study are included in this published article.

#### Declarations

#### Ethics approval and consent to participate

This study was approved by Xi'an University of Science and Technology (Xi'an, China), and the experimental process was followed the 2010/63/EU directive.

#### Competing interests

All authors declare that they have no competing interests.

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