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ORIGINAL ARTICLE

Preparation and Anti-fatigue Effects of *Vicatia thibertica* Polysaccharide

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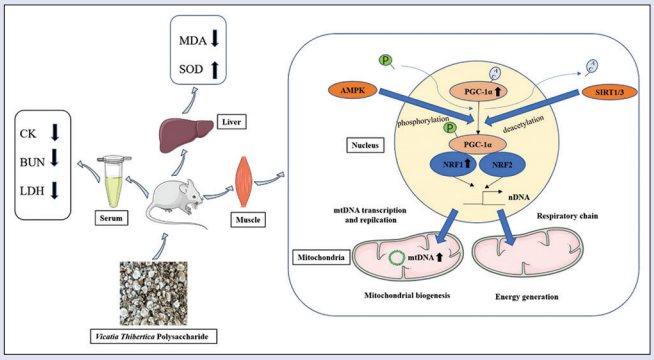
ABSTRACT

Background: Prior studies have revealed that crude *Vicatia thibertica* polysaccharide have the anti-fatigue effect, but the anti-fatigue effect and potential mechanism of purified polysaccharides endure blurred. **Objectives:** We intended to purify and gauge the anti-fatigue effects of a polysaccharide isolated from *Vicatia thibertica* polysaccharide 1 (VTP1). **Materials and Methods:** First, the exercise fatigue model was recognized in Kunming mice. After 14 days of continuous intragastric administration of VTP1 (50, 100, and 200 mg/kg/day), we assessed the anti-fatigue and antioxidant effects of VTP1 on fatigued mice. In addition, the effects of VTP1 on mitochondrial morphology of skeletal muscle of fatigue mice were detected by transmission electron microscopy. Meanwhile, the expression of mitochondrial DNA was noticed. Finally, using Western blot, immunohistochemistry, and real-time quantitative polymerase chain reaction, respectively, to sense the expression of sirttuin1, peroxisome proliferative activated receptor-γ coactivator 1α (PGC-1α), and nuclear respiratory factor 1 (NRF1). **Results:** VTP1 meaningly extends the time to exhaustion during weight-bearing swimming and improves the endurance and bodyweight of fatigued mice. Furthermore, VTP1 exerts antioxidant effects, reduces creatine kinase, blood urea nitrogen and lactate dehydrogenase in the serum, surges superoxide dismutase and cuts malondialdehyde in the liver. Meanwhile, VTP1 diminishes degeneration of mitochondria in the skeletal muscle of mice with exercise fatigue and improves the biosynthetic efficiency of mitochondria in skeletal muscle. Finally, VTP1 can upregulate the mRNA and protein expression of PGC-1α and NRF1. **Conclusion:** VTP1 has an anti-fatigue effect, and the effect may be mediated by the PGC-1α/NRF1 signaling pathway.

Key words: Anti-fatigue, mitochondria, muscle, polysaccharide, *Vicatia thibertica*

SUMMARY

- *Vicatia thibertica* polysaccharide has an anti-fatigue effect, and the effect may be mediated by the peroxisome proliferative activated receptor-γ coactivator 1α/nuclear respiratory factor 1 signaling pathway. The outcomes deliver a scientific basis for the application of *Vicatia thibertica* for the treatment of fatigue.



Abbreviations used: VTP1: *Vicatia thibertica* Polysaccharide 1; mtDNA: Mitochondrial DNA; RT-qPCR: Real-time quantitative polymerase chain reaction; CK: Creatine kinase; BUN: Blood urea nitrogen; LDH: Lactate dehydrogenase; SOD: Superoxide dismutase; MDA: Malondialdehyde; BSA: Bovine serum albumin; HPLC: High-performance liquid chromatography; HPGPC: High-performance gel permeation chromatography; IHC: Immunohistochemical.

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INTRODUCTION

Fatigue is one of the utmost shared physiological reactions.[1] Fatigue often leads to a series of physical and mental disquiets such as muscle soreness, anxiety, and depression.[2-4] A survey has found that more than one in two people feel tired and that more than one in three tired individuals are evidently affected by fatigue and show the condensed quality of life and work efficiency.[5,6] It has been hypothesized that the mechanisms which lead to fatigue lie within one of 2 separate camps: central or peripheral.[7] Central fatigue denotes to fatigue caused by motor stimulation, which leads to lessened functioning of human cerebral cortex cells and extensive inhibition of the cerebral cortex. Peripheral fatigue refers to decreased body functioning due to exercise and occurs in peripheral nerve-muscle contacts, myocyte membranes, myoepithelial networks, mitochondria, and muscle contractile proteins.[8] Fatigue has slowly become a type of "invisible killer." However, few effective pharmacological drugs or therapies are presently obtainable to eradicate fatigue.[9] Research on fatigue has attracted widespread attention.

Increasing indication displays those polysaccharides extracted from plants exhibit low toxicity and exert anti-fatigue effects. For instance,

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the polysaccharides of *Abelmoschus esculentus* and Maca (*Lepidium meyenii* Walp) exert anti-fatigue effects.[10,11] Moreover, a polysaccharide isolated from *Lycium barbarum* can upsurge glycogen reserves, reduce the accumulation of urea nitrogen and lactic acid after exercise and exert anti-fatigue effects in mice.[12]

Vicatia thibertica de Boiss is largely distributed in the cloud forests of Tibet, Sichuan, and the Dali region of Yunnan. It is disbursed by the locals as a nourishing vegetable. It encompasses high levels of sugars and sugar alcohols, and 32.71% of the total contents are reported by polysaccharides.[13,14] Studies have shown that the crude extract exerts anti-hypoxia effects, deliberates resistance to high temperature and can prolong the time to exhaustion due to weight-bearing swimming in mice.[15,16] Furthermore, the crude polysaccharides can significantly decrease serum creatine kinase (CK), blood lactic acid, and blood urea nitrogen (BUN) in mice with exercise fatigue and significantly upsurge liver glycogen levels, which specifies that they have an obvious anti-fatigue effect.[17] However, the molecular structures and anti-fatigue effects of the purified polysaccharide are uncertain. In this study, the crude polysaccharides of *Vicatia thibertica* were isolated and purified to obtain *Vicatia thibertica* polysaccharide 1 (VTP1). The anti-fatigue effects of the VTP1 were elucidated, and the preliminary mechanisms were examined. The results deliver a scientific basis for the application of *Vicatia thibertica* for the treatment of fatigue.

MATERIALS AND METHODS

Materials and chemicals

Vicatia thibertica, the crude material, was composed from Machang Township, Heqing County, Dali Prefecture, Yunnan Province, and was recognized as the root of *Vicatia thibertica* de Boiss, which belongs to the *Umbelliferae* family, by Yue Yang, a senior laboratory scientist at the Department of Biomedicine, College of Pharmacy, Dali University (plant approval number: 2016122801). Anhydrous ethanol was acquired from Qilu Pharmaceutical Co., Ltd. (Jinan, China). Phenol, pentobarbital sodium salt, and sulfuric acid were obtained from Sigma-Aldrich (St. Louis, USA). Macroporous adsorption resin, DEAE-agarose gel FF, acetonitrile, fetal bovine serum albumin (BSA), the glucose standard, glucuronidation, interhydroxybenzidine, and sodium tetraborate were gotten from Solarbio (Beijing, China). Dextran gel G-100 was procured from Guizhou Tiandi Pharmaceutical Co., Ltd. (Guizhou, China). Peroxisome proliferative activated receptor-γ coactivator 1α (PGC-1α) antibody, recombinant nuclear respiratory factor 1 (NRF1) antibody, sirttuin1 (SIRT1) antibody, GAPDH antibody were obtained from Proteintech Group, Inc. (Chicago, USA).

Animals

SPF Kunming mice (20 ± 2 g) aged 6–8 weeks (at a male: female ratio of 1:1) were bought from Hunan Shrek Jinda Experimental Animal Co., Ltd. (license no. SCXK (Xiang) 2016-0002). The animal experimental procedures were in accordance with the National Institutes of Health Guide and the animal experimentation studies were permitted by the Ethics Committee on Animal Research of Dali University (approval number: 2017-P2-12).

Preparation of *Vicatia thibertica* polysaccharide 1

The crude polysaccharides were extracted with hot water, and the proteins were detached using Savage reagent. D101 macroporous adsorption resin and DEAE-agarose gel were employed to decolorize and separate the polysaccharides with an FF column to get *Vicatia thibertica* Polysaccharide 1 (VTP1). After dialysis and freeze-drying, VTP1 was purified with a dextran gel chromatography column.

Determination of physicochemical properties of *Vicatia thibertica* Polysaccharide 1

The total carbohydrate content was measured using the phenol-sulfuric acid method with glucose as the standard (purity: ≥98%).[18] The meta-hydroxy-diphenyl method was applied to determine the uronic acid content of the polysaccharides, and bovine D-glucuronic acid was used as the standard (purity: ≥98%).[19] The Bradford method with BSA as the standard (purity: ≥98%) was exploited to determine the protein content of the polysaccharides.[20]

Monosaccharide composition determination

Mannose (Man, purity: ≥98%), glucose (Glc, purity: ≥98%), ribose (Rib, purity: ≥98%), glucuronic acid (GlcA, purity: ≥98%), arabinose (Ara, purity: ≥98%), rhamnose (Rha, purity: ≥98%), and galactose (Gal, purity: ≥98%) were measured reference substances. A high-performance liquid chromatography (HPLC, Tomy Digital Biology Co., JP) system was used for the monosaccharide composition determination of VTP1 after precolumn derivatization. For sample hydrolysis, 14 mg of VTP1 was located in an ampoule bottle, and 2 mL of 4 mol/L trifluoroacetic acid was then added. A small amount of methanol was added after 8 h later, and the mixture was alienated into 120 sealed tubes for hydrolysis and dried. Subsequently, trifluoroacetic acid was evaporated, and the sample hydrolysate was dissolved in water. For the derivation of the samples, 100 μL of the hydrolyzed sample solution was found according to the above method and filtered through a microporous membrane. The HPLC conditions were as follows: column, waters C18 column (250 mm × 4.6 mm, i.d. 5 μm); mobile phase, 0.1 mol/L phosphate (pH 6.7) buffer with acetonitrile (volume ratio 83:17); column temperature, 35°C; detection wavelength, 250 nm; flow rate, 0.8 mL/min; and volume, 20 μL.

Homogeneity and molecular weight distribution of *Vicatia thibertica* polysaccharide 1

VTP1 was mixed with 0.2 M NaCl solution to a concentration of 5 mg/mL and filtered, and 20 μL polysaccharide solutions were detected and analyzed by high-performance gel-permeation chromatography (HPGPC).

Fourier transform infrared spectroscopy analysis

Infrared spectra of VTP1 were measured by attenuated total reflection Fourier transform infrared spectroscopy (Tomy Digital Biology Co., JP). VTP1 were pretreated at 40°C for 24 h under vacuum before analysis. All spectra were attained using 64 scans in the frequency range of 400–4000/cm. The samples were investigated as KBr pellets.[21]

Experimental animals and treatment

Seventy-two mice were sustained in an ambient temperature of 22°C ± 2°C and 50%–80% humidity. After 3 days of adaptation, the mice were arbitrarily separated into six groups: normal group (CON), model group (MOL), coenzyme Q10 group (CQ, 2.7 mg/kg), high-VTP1-dose group (HVTP1, 200 mg/kg), medium-VTP1-dose group (MVTP1, 100 mg/kg), and low-VTP1-dose group (LVTP1, 50 mg/kg). The normal control group and the model control group were given water by gastric perfusion, while the other groups were treated with the conforming drugs by gastric perfusion once daily for 2 weeks. There were 12 mice in each group. 30 min after the last administration, various indexes were distinguished.

Forced swimming test

The mouse weight-bearing swimming experiment was achieved according to the literature.[22] Based on 5% of the bodyweight of the mice,

the lead block was tied to their tail at a distance equal to 1/3 from the root. Briefly, the mice were independently placed into a 30 cm diameter glass jar containing 25 cm deep water maintained at 22°C ± 2°C. The criterion for exercise fatigue was the time from the initiation of swimming to exhaustion, that is, the time until the head sank into the water and endured underwater for 8 s. Forced swimming capacity was restrained by the swimming time record. The body weight and general state of the mice were logged every day for 14 days.

Determination of physiological and biochemical indexes in mice

Mice were anesthetized with intraperitoneal injection of 30 mg/kg pentobarbital sodium salt after the mice exhausted, the blood samples were composed from the orbital vein. The lactate dehydrogenase (LDH) levels in the blood and the BUN and serum CK levels were measured using LDH detection kit (Guizhou Tiandi Pharmaceutical Co., Ltd.), BUN detection kit (Guangdong Guanghua Technology Co., Ltd.) and CK activity test kit (Beijing Solarbio Technology Co., Ltd.). The livers of the mice were poised, and malondialdehyde (MDA) and superoxide dismutase (SOD) contents were determined using MDA content detection kit (Beijing Solarbio Technology Co., Ltd.) and SOD activity test kit (Beijing Solarbio Technology Co., Ltd.).

Histopathological analysis

After comprehensive swimming, the gastrocnemius muscles extracted from mice were expurgated into 1 mm tissue blocks and fixed in 40 mg/mL paraformaldehyde overnight, and dehydrated in a series of fractionated ethanol solutions. Afterward, the tissues were embedded in paraffin and cut into 4-μm thick sections. After the sections were double-stained with uranium and lead, they were endangered to routine morphological analysis by transmission electron microscopy (Hitachi, Co., USA), micrographs were verified at ×20,000–×50,000.[23]

Immunohistochemical analysis

The skeletal muscle tissue samples were collected. Subsequently, the muscle tissue samples were entrenched in paraffin and cut into 10 μm-thick slices for further analysis. The tissue samples were deparaffinized and rehydrated through a xylene and ethanol gradient to confiscate picric acid for optimal preservation and immunohistochemical detection. Following antigen retrieval using a microwave, the tissue was treated with 0.997 mol/L H_2O_2 in methanol for 10 min to quench endogenous peroxides and nonspecific binding was congested using 2% rabbit serum for 30 min. SIRT1, PGC-1α, SIRT1 antibodies (dilution: 1:250) were incubated overnight at 4°C in a humid chamber. The antigen-antibody complex was perceived using horseradish peroxidase-conjugated secondary antibody and visualized with a diaminobenzidine substrate chromogen solution (Millipore, Co., USA).[24]

Real-time quantitative polymerase chain reaction analysis

Total RNA was extracted using a TRIzol kit (Tiangen Biochemistry Technology Co., Ltd., Beijing). Reverse transcription was carried out using 1 μg of total RNA with 5 × HiScript II qRT SuperMix for Quantitative Polymerase Chain Reaction (qPCR) (Solarbio Technology Co., Ltd., Beijing) in a volume of 10 μL. 1 μL of cDNA was amplified with specific primers and quantified on a StepOne RT-PCR system (ABI) using 2 × ChamQ SYBR qPCR Master Mix (Tiangen Biochemistry Technology Co., Ltd., Beijing), with normalization to GAPDH. Primer sequences are recorded in Table 1. Briefly, after an initial denaturation step at 95°C for 30 s, the amplifications were achieved through 40 cycles of 95°C (the melting temperature) for 25 s and 60°C (the annealing temperature) for 30 s. β-actin was employed as a reference for the analysis of mRNA, and the data were intended using the $2^{-\Delta\Delta}$Ct method.

Table 1: Primers used for the reverse transcription-quantitative polymerase chain reaction

Taget gene	Primer sequence (5′-3′)	Bases	Sequence length (bp)
GAPDH			
Forward	GGCTGTATTCCCCTCCATCG	20	154
Reverse	CCAGTTGGTAACAATGCCATGT	22	154
mtDNA			
Forward	CCTCCACCGACTTGCTGTTGAC	22	121
Reverse	TGCCCGCTGCGTTCTGTTTG	20	121
SIRT1			
Forward	CCAGACCTCCCAGACCCTCAAG	22	120
Reverse	GTGACACAGAGACGGCTGGAAC	22	120
PGC-1α			
Forward	GTGCCACCGCCAACCAAGAG	20	141
Reverse	TTCCTCGTGTCCTCGGCTGAG	20	141
NRF1			
Forward	TCTGCTGTGGCTGATGGAGAGG	22	83
Reverse	GATGCTTGCGTCGTCTGGATGG	22	83

mtDNA: Mitochondrial DNA; SIRT1: Sirttuin 1; PGC-1α: Peroxisome proliferative activated receptor-γ coactivator 1α; NRF1: Nuclear respiratory factor 1

Western blot analysis

The skeletal muscle tissue samples were homogenized in 200 μL RIPA lysis fluid (Solarbio Technology Co., Ltd., Beijing) using a homogenizer, and the supernatants were composed. The protein concentration was determined by a BCA protein concentration assay kit (Beyotime Institute of Biotechnology, Shanghai). Equal amounts of protein (20 μg/well) were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and the resolved proteins were transferred onto polyvinylidene-difluoride membranes. The membranes were incubated for 1 h with 50 mg/mL of skim milk in the Tris-buffered saline containing Tween 20 to block nonspecific binding. The membranes were then distinctly incubated with the suitable primary antibodies against SIRT1, PGC-1α, NRF1 at 4°C overnight. Then, the secondary antibody was added and incubated for 2 h. The blots were envisaged with an enhanced chemiluminescence detection system (BIO-Tek, Inc., USA) according to the procedure.[23]

Statistical analysis

Statistical analysis was accomplished with GraphPad Prism v. 7.0a (GraphPad software, San Diego, USA). The data were articulated as the means ± standard deviation. The data were analyzed using one-way analysis of variance, and differences were measured significantly and extremely noteworthy at $P < 0.05$ and $P < 0.01$, respectively.

RESULTS

Physicochemical properties and structural characterization of *Vicatia thibertica* polysaccharide 1

The total sugar content of the crude polysaccharides was 36.1%, and the total sugar content after deproteinization was 60.5%. The D101 macroporous adsorption resin and DEAE-agarose gel were employed to decolorize and distinct the polysaccharides in an FF column to get the VTP1 component, which corresponded to 90.03% of the total polysaccharide content. After dialysis and freeze drying, the component

was purified based on the peak gained with dextran gel G-100. The calculated recovery rate was 86%, and the total sugar content was 90%.

Monosaccharide composition of *Vicatia thibertica* polysaccharide 1

Determination of the monosaccharide composition of VTP1 by high-performance liquid chromatography presented that VTP1 contained mannose, ribose, rhamnose, anhydrous glucose, galactose, and arabinose, which were found at a molar ratio of 1.14:1.12:0.48:2.83:6.12:2.56:1.20 [Figure 1a and b].

High-performance gel permeation chromatography of *Vicatia thibertica* polysaccharide 1

A single sharp symmetrical peak shape specified a low impurity content in VTP1. The result of HPGPC exposed that the molecular weight of VTP1 was 1.53 × 106 Da [Figure 2].

Infrared spectroscopy of *Vicatia thibertica* polysaccharide 1

Fourier transform infrared spectroscopy was applied to describe the structure of VTP1, the infrared spectra have typical absorption peaks of polysaccharides. IR (OH) cm^{-1}: 3386.88, 3385.01, 3363.65; IR (CH2) cm^{-1}: 2929.29, 2928.60, 2927.13cm^{-1}; IR (C-H) cm^{-1}: 1419.54, 1417.64, 1413.21 [Figure 3].

Anti-fatigue effect of *Vicatia thibertica* polysaccharide 1

Vicatia thibertica polysaccharide 1 improved the general state and weight loss of fatigue mice

As shown in Figure 4, the weight of the mice in the MOL group was meaningfully lower than that of the mice in the CON group ($P < 0.01$). However, VTP1 administration knowingly abridged this loss of body weight, particularly in HVTP1 and MVTP1 groups ($P < 0.01$). In addition, the mice in the MOL group exhibited mental retardation, dry hair without luster, and loose stools, and compared with the mice in the CON group, they exhibited less movement, a lighter auricle and tail color, and condensed food intake. These effects could be lessened by the polysaccharide, as shown in the numerous VTP1 dose groups [Figure 4].

Vicatia thibertica polysaccharide 1 prolonged weight-bearing swimming time in mice

The time to exhaustion due to swimming imitates the tolerance of the mouse body. Compared with the CON group, the MOL group had a meaningly shorter time to exhaustion due to negative-gravity swimming ($P < 0.01$). Compared with the MOL group, the positive control group had a suggestively protracted time to exhaustion due to weight-bearing swimming ($P < 0.01$). The time to exhaustion due to weight-bearing swimming was continued in the various VTP1 dose groups compared with the MOL group, and a dose-effect relationship was detected [$P < 0.01$; Figure 5].

Vicatia thibertica polysaccharide 1 - An antioxidant effect on mice with exercise fatigue

Effect of Vicatia thibertica polysaccharide 1 on serum biochemical indexes

The serum CK activity of the MOL group was pointedly higher than that of the CON group ($P < 0.05$), and the activity in each of the HVTP1 and MVTP1 groups was meaningfully lower than that in the MOL group [$P < 0.01$; Figure 6a]. The serum BUN content of fatigued mice

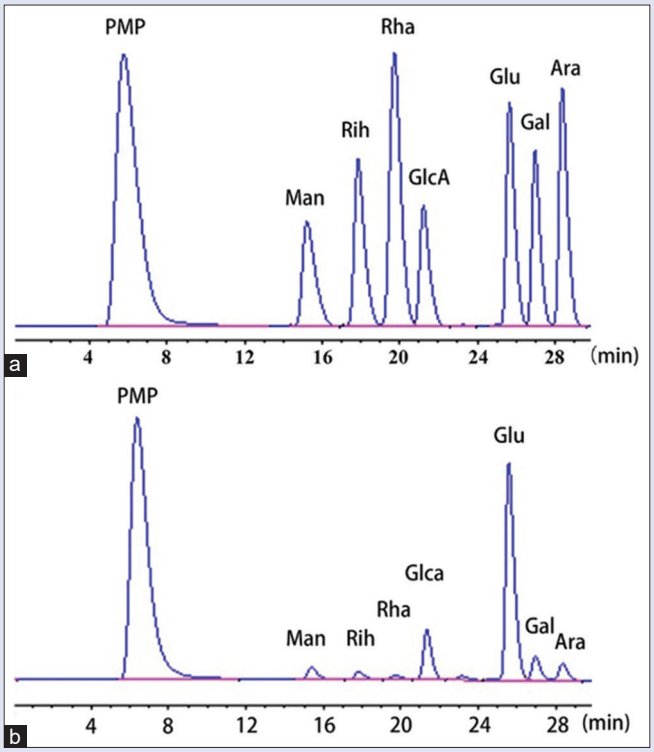

Figure 1: High performance liquid chromatography of VTP1. (a) Standard high performance liquid chromatography; (b) high performance liquid chromatography of VTP1. VTP1: *Vicatia thibertica* polysaccharide 1

in the model group was higher than that in the CON group ($P < 0.05$), and the serum BUN content of the mice in each of the positive control, HVTP1 and LVTP1 groups was significantly inferior than that of the mice in the MOL group [$P < 0.01$; Figure 6b]. The mice in the MOL group had significantly advanced serum LDH activity than those in the CON group ($P < 0.01$). However, VTP1 administration declined serum LDH activity, expressly in the MVTP1 group [$P < 0.01$; Figure 6c].

Effects of Vicatia thibertica polysaccharide 1 on malondialdehyde and superoxide dismutase in the liver

Compared with the mice in the CON group, the mice in the MOL group unveiled a suggestively higher liver MDA content ($P < 0.01$). The level of liver MDA in fatigued mice belonging to the HVTP1 group was significantly lesser than that in the MOL group [$P < 0.01$, Figure 6d]. Compared with the MOL group, the HVTP1 group exhibited augmented SOD activity in the liver ($P < 0.01$); improved SOD activity was also found in both the MVTP1 and LVTP1 groups relative to the MOL group, but the differences were not important [Figure 6e].

Vicatia thibertica polysaccharide 1 reduced mitochondrial damage and inhibited the downregulation of mitochondrial DNA expression caused by fatigue

Ultrastructural changes in Vicatia thibertica polysaccharide 1 in the mitochondria in skeletal muscle of mice with exercise fatigue

Mitochondria are the chief source of ATP to preserve locomotion performance. Therefore, transmission electron microscopy was employed to examine mitochondrial ultrastructure in gastrocnemius muscles. Compared with the MOL group, in the MVTP1 and HVTP1 groups, the mitochondria were somewhat amassed and swollen, some

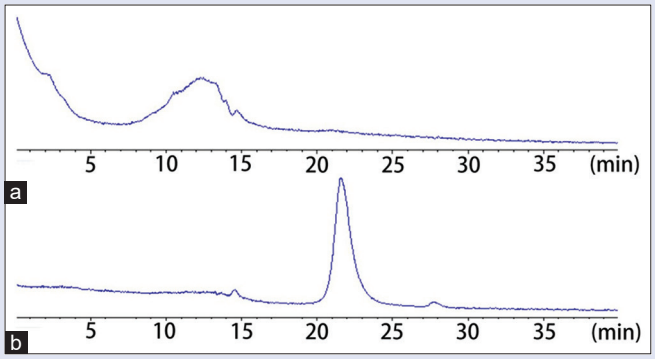

Figure 2: High-performance gel chromatography of VTP1. (a) High-performance gel chromatography of *Vicatia thibertica* crude polysaccharides; (b) high-performance gel chromatography of VTP1. VTP1: *Vicatia thibertica* polysaccharide 1

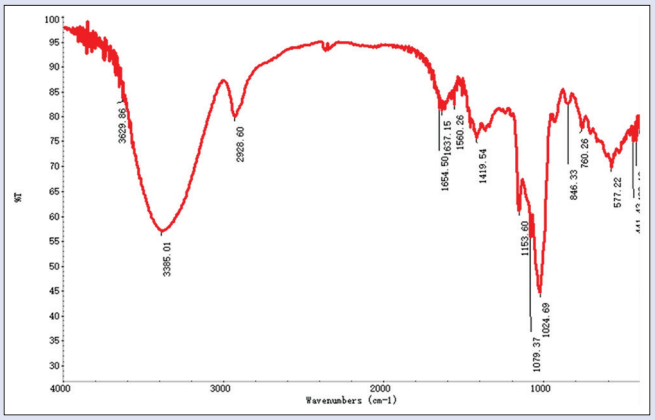

Figure 3: The infrared spectrum of VTP1. VTP1: *Vicatia thibertica* polysaccharide 1

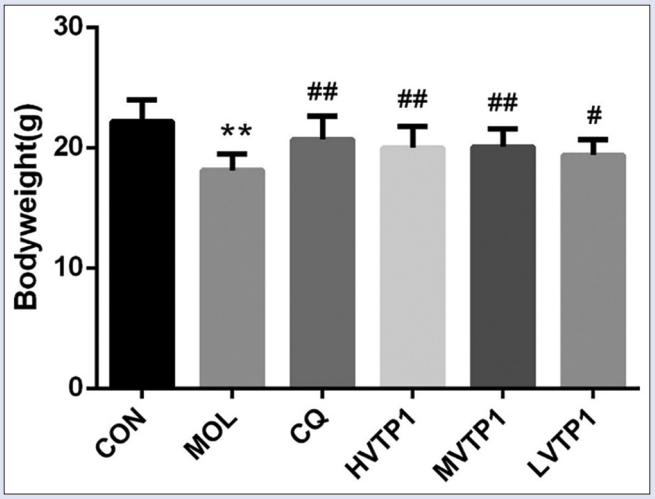

Figure 4: Body weight of mice subjected to different treatments. The data are expressed as means ± SEM (n = 12) and analyzed using a one-way ANOVA. $^{**}P < 0.01$, compared with CON, $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$, compared with MOL. SEM: Standard error of the mean; ANOVA: Analysis of variance

were enlarged, the membrane was intact, and the ridge structure was standard. In the LVTP1 group, the number of mitochondria was increased. Some local aggregation, serious swelling, and clearly amplified matrix dissolution were detected. The mitochondrial ridge was broken and eventually disappeared. In conclusion, VTP1 can diminish the degree of mitochondrial functional injury in skeletal muscle [Figure 7a].

Changes in skeletal muscle mitochondrial DNA expression

The mitochondrial DNA (mtDNA) expression in fatigued mice belonging to the MOL group was knowingly lower than that in the CON group ($P < 0.01$), which directed that exercise fatigue can lead to a significant lessening in mtDNA expression in skeletal muscle tissue. The expression of mtDNA was upregulated in the HVTP1 group and the CQ group ($P < 0.01$). The mtDNA expression level in the HVTP1 group was advanced than that in the LVTP1 group ($P < 0.05$), which recommends that VTP1 can inhibit the downregulation of mtDNA expression caused by fatigue [Figure 7b].

Effects of *Vicatia thibertica* polysaccharide 1 on the Regulation of the sirttuin1-peroxisome proliferative activated receptor-γ coactivator 1α-nuclear respiratory factor 1 pathway in skeletal muscle of fatigued mice

Changes in the expression of immunohistochemically positive materials

The expression of SIRT1 in the HVTP1 group was suggestively higher than that in the MOL group. Comparisons among the numerous dose groups presented that the expression level of SIRT1 in the HVTP1 group was higher than that in the other two VTP1 dose groups. Compared with the MOL group, among VTP1-dose groups exhibited augmented expression of NRF1 immunoreactive substances with dose dependent. However, we found no noteworthy difference in the expression of PGC-1α among groups [Figure 8a].

Gene expression changes

The level of SIRT1, PGC-1α, NRF-1 mRNA in the MOL group was inferior than that in the CON group ($P < 0.01$). In addition, the expression of SIRT1 was upregulated after treatment compared with the expression found in the MOL group, but the changes were not significant [$P > 0.05$; Figure 8b]. The level of the PGC-1α in skeletal muscle was significantly upregulated in mice fitting to the HVTP1, MVTP1, and CQ groups compared with the MOL group ($P < 0.01$ or $P < 0.05$). Pairwise comparisons naked no momentous differences among the several VTP1 groups [Figure 8c]. Moreover, the level of NRF-1 in the skeletal muscle cells of fatigued mice going to the CQ group and HVTP1 groups was upregulated compared with that in the MOL group ($P < 0.01$). Moreover, the level of NRF-1 in the HVTP1 was upregulated compared with that in the MVTP1 and LVTP1 groups [$P < 0.01$; Figure 8d].

Changes in protein expression

The expression level of PGC-1α, NRF-1 protein in the MOL group was meaningly lower than that in the CON group ($P < 0.01$), which designated that weight-bearing swimming to exhaustion can lead to an important diminution in PGC-1α, NRF-1 protein expression in skeletal muscle tissue. Compared with the MOL group, the expression of PGC-1α and NRF1 protein in skeletal muscle was significantly upregulated in the mice belonging to the HVTP1, MVTP1and LVTP1 groups ($P < 0.01$). Moreover, the expression level of PGC-1α and NRF1 protein in skeletal muscle was enlarged in fatigued mice of HVTP1 groups relative to the mice in the LVTP1 group [$P < 0.01$; Figure 8e-h].

DISCUSSION

Due to the subjectivity of clinical appearances, unclear pathogenesis and dearth of effective intervention drugs, an animal model of exercise fatigue has not been documented. Therefore, based on a review of the literature, a model of exercise fatigue due to weight-bearing swimming to exhaustion in a water environment was employed to study fatigue in mice. Motor fatigue is a predominantly complex physical state caused by the incessant excessive physical movement that leads unswervingly to failures in self-motivation and physical activity as well as other clear symptoms, such as peripheral and central metabolic disorders. In exercise fatigue, exhaustion due to weight-bearing swimming is the core index for assessing body tolerance.

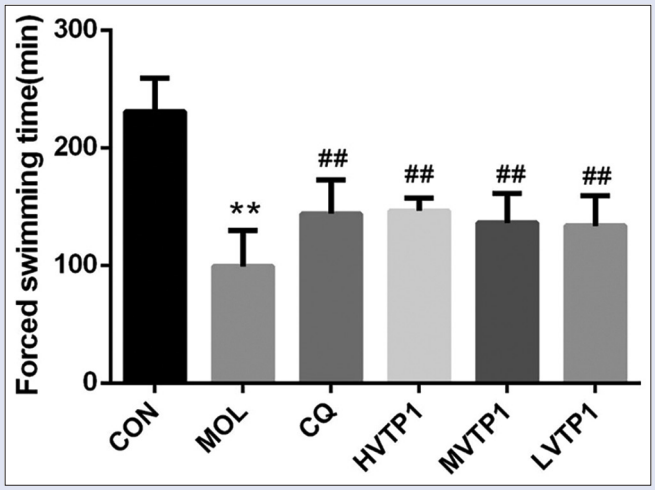

Figure 5: Effect of VTP1 on the time to exhaustion due to weight-bearing swimming found for the mice. The data are expressed as means ± SEM (n = 12) and analyzed using a one-way ANOVA. $^{**}P < 0.01$, compared with CON, $^{\#\#}P < 0.01$, compared with MOL. VTP1: *Vicatia thibertica* polysaccharide 1; SEM: Standard error of the mean; ANOVA: Analysis of variance

Although polysaccharides have a comparatively large molecular weight and encounter a series of barriers when fleeting through the intestinal wall, there are still pertinent studies confirming that polysaccharides can be absorbed into the blood through the intestinal wall.[25-28] The intestinal mucosa has a big surface area, rich in intestinal villi and intestinal capillaries, and there are transporters in the gastrointestinal tract. These structural features aid nutrients absorb into the blood. Therefore, VTP1 in this study may also be unswervingly absorbed through the small intestine, but the precise absorption pathway and mechanism still need to be further considered.

Alteration in body weight can reproduce the level of physiological functioning of mice and plays significant role in the measurement of body growth and development. The results showed no momentous difference in body weight between the treatment groups and the model group. Regarding appearance, the hairs of the mice in the numerous treatment groups were smooth and lustrous. The bodyweight of mice depends mainly on food intake and energy consumption. Exercise fatigue can cause decays in physical function, cognitive damage, and negative emotions and thus affect the appetite of mice. A comprehensive assessment presented that VTP1 improves appetite and decreases consumption in mice with exercise fatigue. In addition, exercise fatigue can lead to a failure in physical activity, and physical tolerance in the contemporary study was echoed by the time to exhaustion due to weight-bearing swimming.[29] In this study, VTP1 significantly augmented the time to exhaustion due to weight-bearing swimming in mice, which indicated that VTP1 can improve muscle tolerance and improve somatic activity.

The energy metabolism in exercise fatigue is thoroughly connected to the oxidation of cells.[30] Exercise fatigue can decrease muscle strength and work efficiency through oxidation. CK, BUN, and LDH are significant indicators of somatic activity. BUN is the main reactive protein in metabolism, and the CK and LDH contents reproduce skeletal muscle function.[31] As the body loads surges, the energy demands upsurge evidently, the fat and sugar supplies become inadequate, protein breakdown and consumption and rises in urea production leading to an increase in BUN content.[32] In this study, the BUN level of mice in the model group augmented significantly, which indicates that the fat

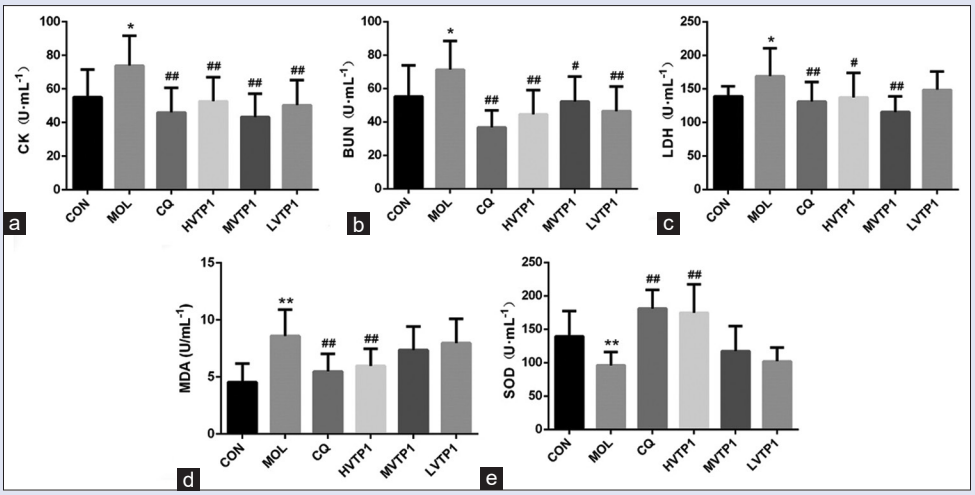

Figure 6: Antioxidant effect of VTP1. (a) Effects of VTP1 on CK in serum; (b) effects of VTP1 on BUN in serum; (c) effects of VTP1 on LDH in serum; (d) effects of VTP1 on MDA in the liver; (e) effects of VTP1 on SOD in the liver. The data are expressed as means ± SEM (n = 10) and analyzed using a one-way ANOVA. $^{*}P < 0.05$ and $^{**}P < 0.01$, compared with CON; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$, compared with MOL. VTP1: *Vicatia thibertica* polysaccharide 1; SEM: Standard error of the mean; ANOVA: Analysis of variance; BUN: Blood urea nitrogen; LDH: Lactate dehydrogenase; MDA: Malondialdehyde; SOD: Superoxide dismutase; CK: Creatine kinase

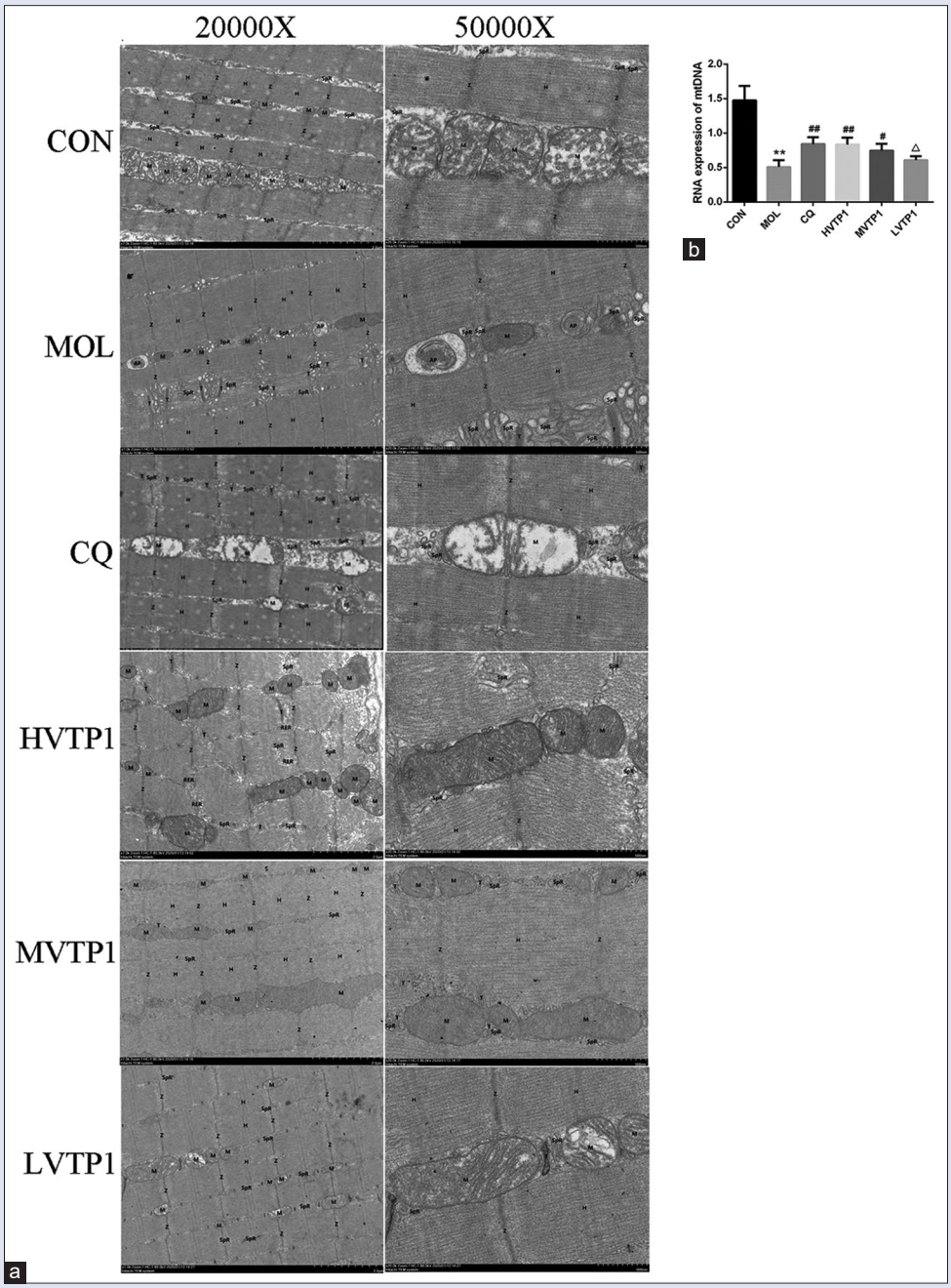

Figure 7: Effects of VTP1 on mitochondrial morphology and mtDNA expression in the skeletal muscle of fatigued mice. (a) Electron microscopy of skeletal muscle of mice at 20,000 and 50,000 (b) effects of VTP1 on mtDNA expression in skeletal muscle of fatigued mice. The data are expressed as means ± SEM ($n = 3$) and analyzed using a one-way ANOVA. $^{**}P < 0.01$, compared with the CON; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ compared with MOL, $^{\triangle}P < 0.05$, compared with the HVTP1. mtDNA: Mitochondrial DNA; VTP1: *Vicatia thibertica* polysaccharide 1; SEM: Standard error of the mean; ANOVA: Analysis of variance

and sugar sources were insufficient and that their consumption was too high under conditions leading to fatigue, which induced the initiation of protein decomposition to provide energy. The BUN content in the serum of mice was condensed in the various VTP1 groups, which directed a reduction in the degree of protein decomposition and the acceleration of BUN elimination.

LDH and CK levels are usually employed to assess the degree of intracellular injury. LDH and CK are macromolecules that cannot enter the system directly. When the body is tired, the structure and composition of the cell membrane are degraded, which ultimately leads to the exposure of CK and LDH and their entry into the blood through the lymphatic system, snowballing the levels of these indexes in serum. Moreover, CK is the main protease controlling the ATP-PC system, and LDH is the main reaction enzyme in glucose metabolism.[33] In the contemporary study, noteworthy differences in serum CK content were found between numerous VTP1 groups and the MOL group, which shown that VTP1 exerts a significant effect on recovery and deliberates functional protection after cell injury. The serum LDH levels in the mice fitting to the various VTP1 groups were lower than the level in the MOL group, which showed that the degree of cell injury in mice with exercise fatigue was mild or moderate.

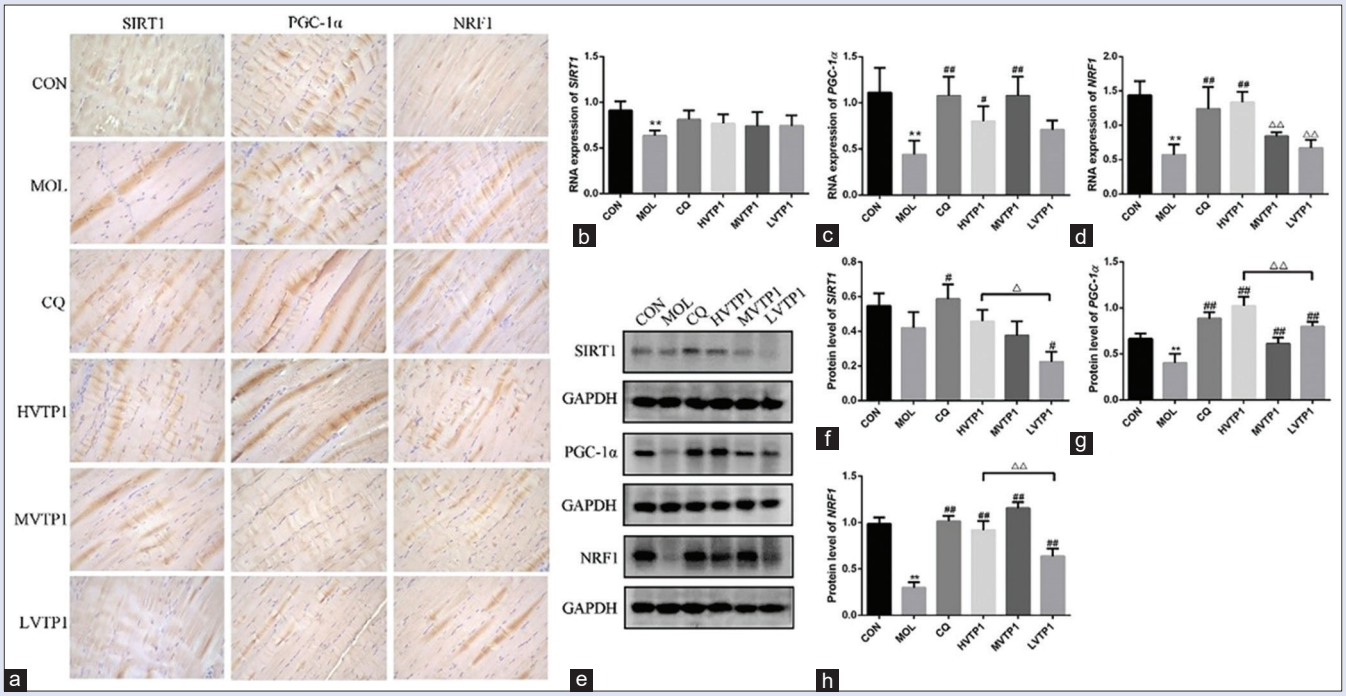

Figure 8: VTP1 upregulated the expression level of SIRT1, PGC-1α, and NRF1 on skeletal muscle in fatigued mice. (a) Effects of VTP1 on skeletal muscle-related enzymes in fatigued mice; (b-d) effects of VTP1 on related genes in skeletal muscle of fatigued mice; (e-h) effects of VTP1 on related proteins in skeletal muscle of fatigued mice. The data are expressed as means ± SEM ($n = 3$). And analyzed using a one-way ANOVA. $^{**}P < 0.01$, compared with the CON; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$, compared with the MOL; $^{\triangle}P < 0.05$ and $^{\triangle\triangle}P < 0.01$, compared with the HVTP1. VTP1: *Vicatia thibertica* polysaccharide 1; SEM: Standard error of the mean; ANOVA: Analysis of variance; PGC-1α: Peroxisome proliferative activated receptor-γ coactivator 1α; SIRT1: Sirttuin1; NRF1: Nuclear respiratory factor 1

The liver is the key metabolic organ of the human body, and the aggravation of oxidative stress can cause fatigue.[34] SOD is an important antioxidant stress reaction enzyme in the human body that can avert damage due to oxidative stress.[35] When the human body experiences exercise fatigue, peripheral and central oxidative phosphorylation increases, resulting in the production of a huge number of oxygen free radicals. Thus, a large number of antioxidants are needed to eliminate these radicals to avoid oxidative damage to the body; excessive amounts of free radicals result in cell structure and function damage. Liver MDA is a product of prostaglandin and lipid peroxidation, and MDA levels vary with the degree of oxidative damage.[36] SOD can scavenge free radicals, lessen lipid peroxidation and maintain the balance between oxidation and anti-oxidation. The results from this study display that the mouse model of exercise fatigue was considered by a significant cut in SOD activity and an increase in MDA content, which specified the aggravation of oxidative damage. The administration of VTP1 to the mice amended SOD activity and reduced the MDA content, which indicated that VTP1 can reduce antioxidant stress.

The operative functioning of skeletal muscle mitochondria depends mostly on their glucose metabolism ability. Mitochondria are primarily produced via the oxidative phosphorylation of ATP and are widely dispersed in the heart, the brain, and skeletal muscle. Structural variations in mitochondria are carefully related to their biosynthesis efficiency and can directly reflect mitochondrial function. By using transmission electron microscopy, the morphology and distribution of mitochondria and the degree of mitochondrial damage can be envisioned. In the moderate- and high-VTP1-dose groups, the mitochondria were somewhat accumulated and swollen, some were enlarged, the membrane was intact, and the ridge structure was usual. In the low-VTP1-dose group, the number of mitochondria was augmented; some local aggregation, serious swelling, and obviously amplified matrix dissolution were observed; and the mitochondrial ridge was broken and eventually vanished. In conclusion, VTP1 can reduce the degree of mitochondrial functional injury in skeletal muscle.

Mitochondrion harbors its own DNA (mtDNA), which encodes countless critical proteins for the assembly and activity of mitochondrial respiratory complexes. Defects or mutations of mtDNA result in a range of ailments.[37] In the contemporary study, the expression of mtDNA in the model and normal groups was meaningfully diminished. The various VTP1 groups exhibited significantly advanced expression of mtDNA in skeletal muscle, which indicated an increase in the biosynthesis efficiency of mitochondria in skeletal muscle of the fatigued mice in the VTP1 groups. Among the numerous VTP1 groups, the effects found in the HVTP1 group were significant.

As an imperative method for recovering after mitochondrial function injury, mitochondrial biosynthesis plays a dominant role in the destruction of mitochondrial morphology and the repair of mtDNA injury after exercise fatigue. In this study, variations in the morphology and function of skeletal muscle mitochondria were detected in the MOL group. Treatment with VTP1 alleviated the morphological damage to mitochondria in skeletal muscle, upregulated the mtDNA content in skeletal muscle, and clearly restored the mitochondrial morphological and functional damage caused by fatigue, which recommended that VTP1 can promote mitochondrial mtDNA biosynthesis and improve mitochondrial function and morphology in mouse skeletal muscle by snowballing the transcription and replication of mtDNA in skeletal muscle.

Mitochondrial efficiency unswervingly affects the metabolism and oxidation function of skeletal muscle. When the body is under pressure and fatigue, the morphology, structure, and function of the mitochondria transformation. Mitochondrial biosynthesis in skeletal muscle is regulated and controlled by many factors, among which the PGC-1α-NRF1 pathway is the most vital regulatory pathway. PGC-1α is a key regulator of mitochondrial respiration, production and function in skeletal muscle, and its aptitude to act as a coactivator depends on its ability to regulate PGC-1α, particularly the nuclear genes involved in regulating mitochondria. PGC-1α affects mitochondrial homeostasis in skeletal muscle, and transcriptional activators are meticulously related to the biosynthesis of motor-induced organelles. NRF1 is the downstream persuading factor of PGC-1α, which mainly moves the expression of mitochondrial riboprotein by activating oxidative phosphorylation-related enzymes. Mitochondrial biosynthesis is caused by SIRT1 agitation, which stimulates PGC-1α activation and then acts on NRF1 to recurrently affect the mitochondrial biosynthesis process.[38-40] The fallouts exposed that mRNA and protein expression of PGC-1α and NRF1 was upregulated in the groups administered numerous doses of VTP1, which enhanced the biosynthesis efficiency of mitochondria in fatigued mice and eased the functional damage to skeletal muscle mitochondria caused by exercise fatigue.

CONCLUSION

In summary, in mice with exercise fatigue, VTP1 can meaningly extend the time to exhaustion, recover the endurance and bodyweight of fatigued mice, exert antioxidant effects, improve the degeneration of skeletal muscle, augment the biosynthetic efficiency of skeletal muscle mitochondria and upregulate the mRNA and protein expression of numerous molecular targets in the PGC-α-NRF1 pathway. These results authenticate the theoretical basis for its use to lessen fatigue. However, the bioavailability of VTP1 by oral administration is small. We will further travel how to improve the bioavailability of VTP1 in the future.

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Conflicts of interest

There are no conflicts of interest.

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