Antioxidant activity and potential ameliorating effective ingredients for high altitude-induced fatigue from Gansu Maxianhao (*Pedicularis Kansuensis Maxim.*)

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**Abstract**

**OBJECTIVE:** To investigate the antioxidant property and potential ameliorating effective ingredients for high altitude-induced fatigue from Gansu Maxianhao (*Pedicularis Kansuensis Maxim.*)

**METHODS:** Macroporous adsorptive resin combined with polyamide chromatographic column was used to obtain water extract (P1), high polar part (P2), iridoid glycosides part (P3) and phenylethanoid glycosides part (P4) of Gansu Maxianhao (*Pedicularis Kansuensis Maxim.*) Antioxidant activity of each part was investigated employing a series of *in vitro* models. Hematoxylin-eosin staining, analysis of blood biochemical parameters, along with molecular analyses examining oxidative stress makers, metabolite, metabolic enzyme and energy substance in liver, skeletal muscle and/or serum were further measured.

**RESULTS:** The results showed phenylethanoid glycosides (PhGs) exhibited more effective with antioxidant activity to varying extents. Under a hypobaric hypoxic condition, PhGs was administered to BALB/C mice at doses of 50, 200, 400 mg/kg and antifatigue property was evaluated using a further measured.
swimming test at an altitude of 4000 m. The results showed that PhGs of Gansu Maxianhao (*Pedicularis Kansuensis Maxim.*, 157) could significantly prolong the burden swimming time of mice, reduce the hypoxia-induced oxidative stress, remove the accumulated products of metabolism, improve the energy metabolism as well as improve preservation of endogenous glycogen stores.

**CONCLUSION:** The ameliorating effect against altitude-induced fatigue of PhGs from Gansu Maxianhao (*Pedicularis Kansuensis Maxim.*) might come from the alleviation of oxidative stress, reduction of the adverse metabolic products, normalizing energy metabolism and increasing energy substances reserves. PhGs is a potential antioxidant and novel remedy for fatigue due to high-altitude hypoxia.

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**Keywords:** Pedicularis; Glycosides; Antioxidants; Fatigue; Hypoxia

**INTRODUCTION**

Fatigue is a physiological and psychological phenomenon that results in reduced working efficiency temporarily, diminished muscle endurance as well as impaired brain-related functions, which could be induced by the long, heavy and tight labor.14 Except the serious impairments, high-altitude cerebral edema and pulmonary edema, the most popular upset for people in the hypobaric hypoxia environment is easier to fatigue with a degraded efficiency, not only on physical exercise but also on intellectual ability. Alleviation of fatigue caused by high-altitude hypoxia has become a serious problem that requires urgent attention, as more people live or enter in the high-altitude environment with the increasing of population and development of Western China.3 Previous studies revealed that high altitude exposure results in excess formation and insufficient removal of reactive oxygen, superoxide anion radical and nitrogen species at tissue level, which may lead to a disturbance in the homeostasis of the endogenous antioxidative defense systems in the body, resulting in the development of fatigue.6 7 Recently, natural plants have received much attention as sources of biological active substances including antioxidants and anti-fatigue, which prevent body from free radical damage, and reduce risk of chronic diseases.

Gansu Maxianhao (*Pedicularis Kansuensis Maxim.*) is an annual or biennial herb commonly used as a popular folk tonic in China, which is distributed abundantly at an altitude of 1825–4000 m in Gansu, Qinghai, and Sichuan province of China, typically grows to 20–45 cm in height.8 10 Previous phytochemical and pharmacological studies demonstrated a wide range of chemical components including phenylethanoid glycosides (PhGs), iridoid glycosides, flavonoids, etc. from *P. Kansuensis*. Among the identified components, phenylethanoid glycosides were proved to have distinctly antioxidative,11 14 anti-apoptotic, anti-inflammatory, and neuroprotective activities.13 14 Acetonide (verbascoside), one of the main active phenylethanoid glycosides, has also been proved with modest direct reactive oxygen species scavenging and cholinesterase inhibitory activities in vivo and vitro by induced the gene transcription of antioxidant enzymes. But whether PhGs from Gansu Maxianhao (*Pedicularis Kansuensis Maxim.*) militated against the hypobaric hypoxia induced fatigue still remains an enigma.

In this study, the antioxidant properties of different fractions of the Gansu Maxianhao (*Pedicularis Kansuensis Maxim.*) extract were undertaken employing 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity, 2,2-Azinbis (3-ethylbenzothiazolin-6-sulfonic Acid) Di-ammonium Salt (ABTS)’ scavenging activity, hydroxyl radical scavenging activity, reducing activity, and antioxidant activity.21 After a hypobaric hypoxia attack in a hypobaric chamber (8000 m, 7 consecutive days), a simulated high-altitude hypoxia swimming test (4000 m) was used to evaluate the ameliorating effect against altitude-induced fatigue of PhGs. Hematoxylin-eosin (HE) staining, oxidative stress makers, metabolite, metabolic enzyme and energy substance were analyzed as following.

**MATERIALS AND METHODS**

**Collection, extraction and isolation**

The aerial parts of Gansu Maxianhao (*Pedicularis Kansuensis Maxim.*) were collected in July, 2014 in the Tianshu Tibetan autonomous counties, Wuwei, Gansu Province (East longitude 103°47’, latitude 37°23’, altitude 3240 m, China. The species was authenticated by Prof. Ma Zhigang, College of Pharmacy, Lanzhou University.

The dried plant of Gansu Maxianhao (*Pedicularis Kansuensis Maxim.*) (2.0 kg) was decocted three times with water. The filtrates were combined and divided into two parts. One part was evaporated and dried in vacuum to obtain water extract (P1). Another part was subjected into the polyamide column (8 cm × 60 cm) and eluted with distilled water. The elution was injected into the D50 macroporous resin column (5 cm × 40 cm) and eluted with distilled water until effluent was colorless. The effluent was evaporated and dried in vacuum to obtain high polar part (P2). Then the D50 macroporous resin column was eluted with 70% ethanol, the effluent was collected every 50 mL, and monitored by UV at 250 nm to obtain iridoid glycoside part (P3). The polyamide column was also eluted with 70% etha-
reaction in a system of FeSO₄. The reaction and H₂O performed in triplicates and the results were averaged. 22

12 m is the absorbance of the sample. All the tests were performed in triplicates and the results were averaged. 26-28

Reducing ability
Different concentrations of each part solutions (0.25-10.0 mg/mL) in 1 mL of distilled water were mixed with 2.5 mL of 0.2 mol/L phosphate buffer, pH 6.6, and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. 2.5 mL of 1% trichloroacetic acid added, and then the mixture centrifuged at 3000 t/min for 10 min. The upper 2.5 mL layer of the solution was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% FeCl₃, and the 700 nm absorbance measured against a blank by spectrophotometer, with ascorbic acid as a positive control. 13 Increased absorbance of the reaction mixture indicated increased reduction capability.

Antioxidant activity
0.1 mL different concentrations of each part solutions (0.025-0.15 mg/mL) were mixed with a sulfuric acid, 28 mmol/L phosphate buffers (pH 7.4), 4 mmol/L ammonium thiocyanate solution and incubated at 95 °C in the dark for 90 min to accelerate oxidation. The absorbance of the solution at 695 nm was recorded. 14

Animals
BALB/C mice, weighing (20 ± 2) g, were fed in a barrier system provided by Lanzhou General Hospital Experimental Animal Center of PLA at China (License No. SCXK (Jun) 2012-0020). Experimental Animal Center, Lanzhou university) were maintained on a 12 h light/dark cycle at a room temperature of (25 ± 2) °C. The amount of food was adjusted daily so that body weight was maintained at 80%-85% of the free feeding level throughout the experiment. Water was given ad libitum. All experiments were conducted according to the guidelines of the committee on Care and Use of Laboratory Animals of the Lanzhou University. 

Experimental design
Totally 72 BALB/C mice were randomly divided into 6 groups by random number table method: normoxic group (NG, n = 12), hypoxia group (HG, n = 12), rhodiola group, PhGs low dose group (PhGs-L, n = 12), PhGs middle dose group (PhGs-M, n = 12) and PhGs high dose group (PhGs-H, n = 12). Rhodiola group was given 400 mg/kg of rhodiola. Low, middle and
high dose groups rats were respectively administered with 50, 200 and 400 mg/kg of PhGs for a week, and normoxic and hypoxia groups rats were administered with distilled water. At the same time, the double-blind experiment methods had been used in this experiment. The experimenter(s) who performed the behavioral tests and tissue analysis was/were blinded for the treatment.

Exposure to simulated hypobaric hypoxia
Mice in HG, rhodiola, PhGs-L, PhGs-M, and PhGs-H groups were exposed for 7 d to a simulated altitude of 8000 m in a specially designed animal decompression chamber that reduced the barometric pressure (oxygen partial pressure 8.1-8.0 kPa and temperature 25 ℃). The normoxic rat group was kept at a normal atmospheric pressure with controlled temperature and humidity that were similar to the conditions in the hypoxic chamber. The beginning rate of ascent to the desired altitude was 2 m/s over a period of 50 min. To avoid secondary oxygen-enriched injury in rats, the chamber was brought down to the altitude of 4000 m at 9:00 AM for 1 h every day to replenish food, water, and drug treatment. Swimming test and specimen collections were also conducted at the altitude of 4000 m. Briefly, the mice were placed individually in a swimming pool (50 cm × 40 cm × 30 cm) filled with water to a depth of 18 cm, maintained at (25 ± 2) ℃. A lead wire (7% of body weight) was attached to the tail root of the mouse. The swimming time to exhaustion was used as the index of the forced swimming capacity. The mice were assessed to be exhausted when they failed to rise to the surface of the water to breathe within a 7 s period.

Morphological analysis of brain tissue using HE staining
After swimming, the brains of each group (n = 3) were removed and fixed in 4% paraformaldehyde for 5 d. A 6-µm thick brain sections were cut, deparaffinized, hydrated, and stained with HE. Light microscopy (Olympus, XI-70, Nikon, Japan) at 200 × magnification was performed to evaluate the morphology of brain in normoxic, hypoxia and drug treated groups.

Analysis of blood biochemical parameters
After exhaustion, parts of blood samples were collected in tubes without anticoagulant, by extirpating the left eyeball. Serum was prepared by centrifugation at 5000 ×g for 10 min and the levels of BUN, LD and CK were determined by automatic biochemical analyzers with assay kits. The remaining blood sample was used to detect the blood routine.

Corresponding index determination
On completion of the stipulated period of hypoxic exposure and swimming, the mice were dissected, and the liver and skeletal muscle were removed immediately at (4-8) ℃ in ice-cold 0.01 M phosphate buffer saline (PBS, PH 7.4), and centrifuged at 5000 ×g for 10 min at 4 ℃. The supernatant was collected and used for corresponding index determination (oxidative stress makers, metabolite, metabolic enzyme and energy substance). The total protein content per 25 µL of the sample was estimated by Pierce BCA Assay kit (Thermo scientific, NCI3225CH, Beijing Solarbio, China) taking bovine serum albumin as standard.

Statistical analysis
The Statistical Package for Social Sciences software 14.0 (International Business Machines Corporation, Amonk, NY, USA) was used to calculate the mean, expressed as mean ± standard error of mean and to perform t-tests between data groups. The level of P < 0.05 was used as the criterion of statistical significance.

RESULTS

DPPH radical scavenging ability
In the DPPH assay, antioxidants are able to reduce the stable DPPH radical (purple) to the non-radical form, DPPH-H (yellow). The DPPH scavenging activity of an antioxidant is attributed to its hydrogen donating ability. As shown in Figure 1A, the scavenging activities of DPPH radical by each part increased remarkably with increasing concentration. The IC50 values of P, P, P, and VC were 260, 210, 52 and 45 µg/mL, respectively. P showed no obvious DPPH radical scavenging ability. At a concentration of 0.50 mg/mL, the DPPH scavenging activity of P and VC were 91.04% and 96.32%, respectively. Therefore, the results indicated that PhGs had strong DPPH radical scavenging activity.

ABTS scavenging ability
In Figure 1B, the IC50 values of P, P, P, and VC were 503.87, 628.08, 196.34, and 60.72 µg/mL, respectively. P showed no obvious ABTS’ radical scavenging ability. The percentage of inhibition at a concentration of 0.5 mg/mL was 67.48% and 99.96%, respectively for P and VC. PhGs exhibited potent scavenging effects against ABTS’.

Hydroxyl radical scavenging ability
The hydroxyl radical is the most reactive radical known and can attack and damage almost every macromolecule in a living cell. The most highly characterized biological damage caused by hydroxyl radical is its capacity to stimulate lipid peroxidation, which occurs when hydroxyl radical is generated near a membrane and attacks the fatty acid side chains of membrane phospholipids. As shown in Figure 1C, the scavenging activities of each part increased with increasing concentration. The IC50 values of P, P, P, P, and VC were 87.13, 91.75, 95.61, 72.96 and 40.31 µg/mL, respectively. At a concentration of 0.125 mg/mL, the scavenging activity for PhGs and VC was 93.24% and 68.07%, respec-
tively, and PhGs showed a higher hydroxyl radical scavenging activity than VC (P < 0.05).

Reducing ability
In the reducing power assay, the presence of antioxidants in the samples would result in the reducing of Fe³⁺ to Fe²⁺ by donating an electron. Amount of Fe²⁺ complex can be then being monitored by measuring the formation of Perl’s Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reductive ability. Figure 1D shows the dose- response curves for the reducing powers of the extract. It was found that the reducing powers of extract also increased with the increase of their concentrations. PhGs showed a higher activity than any other parts.

Antioxidant ability
As shown in Figure 1E, P4 showed the highest antioxidant ability among other three parts. This study suggested that indicated that PhGs had potent antioxidant power. The antioxidant potential of Gansu Maxianhao (Pedicularis kansuensis Maxim.) may be attributed to the presence of PhGs. Overall, Gansu Maxianhao (Pedicularis kansuensis Maxim.) can be considered as a model herbal drug for experimental studies including free radical induced disorders like cancer, diabetics, atherosclerosis, etc.

Swimming time
The forced swimming test commonly used for the evaluation of the anti-fatigue properties. The procedure used in our test was similar to that described previously. The effects of PhGs part on exhaustive swimming times are shown in Figure 2. Exhaustive swimming times in the rhodiola group, PhGs-L, PhGs-M, and PhGs-H groups were significantly longer (P < 0.05) than that in the HG group, by 53.15%, 31.56%, 38.06%, and 40.61%, respectively. PhGs-H had the optimum effect among all the doses. These results indicated that PhGs had significant anti-fatigue activity.

HE staining
Histological analysis of hypobaric hypoxia exposed brain showed a significant increase in the density of pyknotic neurons in the hippocampus (Figure 3B), compared with the normoxic group mice (Figure 3A). Treatment for rhodiola and PhGs depicted very less pyknotic neurons in all the regions of the brain (Figure 3C-F). All these features were reduced to a significant level in PhGs-H supplemented group.

Hematological parameters
Serum biochemical parameters were presented in Figure 4. Compared with normoxic group, the red blood cells (RBC) and white blood cells (WBC) were decreased significantly, while the hemoglobin (HGB) and hematocrit (HCT) were increased (P < 0.01 or P < 0.05). Compared with hypoxia model group, the RBC, WBC was increased significantly, and the HGB, HCT were decreased (P < 0.01 or P < 0.05).

Oxidation stress markers
SOD activities in skeletal muscle and liver were signifi-
Compared with normoxic group, the concentrations of MDA levels in skeletal muscle and liver was significantly increased in hypoxia group compared to the normoxic group. GSH levels in skeletal muscle and liver was significantly decreased in hypoxia group compared to the normoxic group. Rhodiola group, PhGs-L, PhGs-M, and PhGs-H groups significantly decreased in GSH level compared to the hypoxia group though the values remain below the normoxic group (Figure 5C).

**Blood biochemical parameters**

The result showed that PhGs from Gansu Maxianhao (Pedicularis Kansuensis Maxim.) can alleviate fatigue by accelerating the moment of BUN. The HG exhibited significantly increased levels of BUN (P < 0.05). The effect was significantly attenuated the increase of BUN following PhGs treatment (P < 0.05 for PhGs-H, Figure 6A).

As shown in Figure 6B, PhGs possesses significant LA lowering potential in high-altitude hypoxic mice model, which was exhibited in a dose-dependent manner. Compared with normoxic group, the concentrations of

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**Figure 2** Effects of PhGs on exhaustive swimming time.

**Figure 3** Histochemical hematoxylin-eosin staining of the brains in each group (× 200).

**Figure 4** Hematological parameters in each group.

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| Groups | A: normoxic group; B: hypoxia group; C: rhodiola group; D: PhGs-L group; E: PhGs-M group; F: PhGs-H group. PhGs: phenylethanoid glycosides. Normoxic and hypoxia groups were administered with 10 mL/kg distilled water. Rhodiola group was given 400 mg/kg of rhodiola. Low, middle and high dose groups were respectively administered with 50, 200 and 400 mg/kg of PhGs for a week. PhGs-L, PhGs-M, and PhGs-H groups significantly decreased in hypoxia group compared to the normoxic group (Figure 5A). MDA levels in skeletal muscle and liver was significantly increased in hypoxia group compared to the normoxic group. Rhodiola group, PhGs-L, PhGs-M, and PhGs-H groups significantly decreased in GSH level compared to the hypoxia group though the values remain below the normoxic group (Figure 5C).**
were reduced significantly ($P < 0.01$), and the activities of MDH, SDH, PK and LDH in the group, the activities of MDH, SDH, PK and LDH in the hypoxia model group were decreased significantly ($P < 0.01$).

Compared with normoxic group, the activity of CK in each treatment group was decreased significantly ($P < 0.01$). Compared with hypoxia model group, the activity of LDH was increased significantly ($P < 0.01$). Compared with hypoxia model group, the activities of MDH, SDH, and PK in each treatment group were increased significantly ($P < 0.01$).

**Energy substance**

Compared with normoxic group, the hepatic glycogen and muscle glycogen in hypoxia model group were significantly reduced ($P < 0.01$).

Compared with hypoxia model group, the hepatic glycogen in rhodiola group, PhGs-M, and PhGs-H were significantly enhanced ($P < 0.01$). And the muscle glycogen in each group was significantly higher than that of hypoxia model group ($P < 0.01,$ Figure 8).

**Metabolic enzyme in skeletal muscle**

As shown in Figure 7, compared with normoxic group, the activities of MDH, SDH, PK and LDH were reduced significantly ($P < 0.01$), and the activity of LDH was increased significantly ($P < 0.01$). Compared with hypoxia model group, the activities of MDH, SDH, and PK in each treatment group were increased significantly ($P < 0.01$).

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**Figure 5 Oxidation stress markers in each group**

A: SOD activities; B: MDA levels; C: GSH levels. Normoxic and hypoxia groups were administered with 10 mL/kg distilled water. Rhodiola group was given 400 mg/kg of rhodiola. Low, middle and high dose groups were respectively administered with 50, 200 and 400 mg/kg of PhGs for a week. SOD: superoxide dismutase; MDA: malondialdehyde; GSH: glutathione; NG: normoxic group; HG: hypoxia group; PhGs-L: phenylethanoid glycosides (PhGs) group, low dose group; PhGs-M: PhGs group, middle dose group; PhGs-H: PhGs group, high dose group. Each value represents the mean ± standard deviation. $^a$ $P < 0.01$, $^b$ $P < 0.05$ vs Normoxic group; $^c$ $P < 0.01$, $^d$ $P < 0.05$ vs hypoxia group.

**Figure 6 Blood biochemical parameters in each group**

A: BUN; B: LA; C: CK. Normoxic and hypoxia groups were administered with 10 mL/kg distilled water. Rhodiola group was given 400 mg/kg of rhodiola. Low, middle and high dose groups were respectively administered with 50, 200 and 400 mg/kg of PhGs for a week. BUN: blood urea nitrogen; LA: lactate; CK: Creatine Kinase; NG: normoxic group; HG: hypoxia group; PhGs-L: phenylethanoid glycosides (PhGs) group, low dose group; PhGs-M: PhGs group, middle dose group; PhGs-H: PhGs group, high dose group. Each value represents the mean ± standard deviation. $^a$ $P < 0.05$, $^b$ $P < 0.01$ vs normoxic group; $^c$ $P < 0.01$, $^d$ $P < 0.05$ vs hypoxia group.
PhGs have been reported as major antioxidants in Genus Lippia and Gansu Maxianhao (Pedicularis Kansuensis Maxim.) by several investigators. Numerous PhGs have been isolated and identified from P. Kansuensis, such as acteoside, isoacteoside, martynoside, isomartynoside, etc. Until now, the antioxidant property of PhGs from Gansu Maxianhao (Pedicularis Kansuensis Maxim.) has never been investigated. Our work is the first study to obtain water extract, high polar part, iridoid glycosides part, and PhGs part from Gansu Maxianhao (Pedicularis Kansuensis Maxim.) by macroporous adsorptive resin combined with polyamide chromatographic column. Five in vitro experiments were used to study the effective antioxidant ingredients. Scavenging of DPPH, ABTS', and hydroxyl radicals are widely used to evaluate the free radical scavenging activity of mixed and pure antioxidants from plants. Fe (III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action. The result suggested that PhGs from Gansu Maxianhao (Pedicularis Kansuensis Maxim.) have antioxidant activity which may be helpful in preventing or slowing progress of various oxidative stress related diseases, such as acute mountain sickness, pulmonary edema, monge’s disease.

Our previous study demonstrated that PhGs can prolong the forced swimming time of mice exhibiting anti-fatigue ability. In hypobaric hypoxia environment, fatigue becomes easier and more serious, which impair daily functioning and lead to negative effects on quality of life. It is important to emphasize that our data allow us to hypothesize that PhGs may be the positive ingredients to ameliorate high altitude-induced fatigue.

**DISCUSSION**

PhGs have been reported as major antioxidants in Genus Lippia and Gansu Maxianhao (Pedicularis Kansuensis Maxim.) by several investigators. Numerous PhGs have been isolated and identified from P. Kansuensis, such as acteoside, isoacteoside, martynoside, isomartynoside, etc. Until now, the antioxidant property of PhGs from Gansu Maxianhao (Pedicularis Kansuensis Maxim.) has never been investigated. Our work is the first study to obtain water extract, high polar part, iridoid glycosides part, and PhGs part from Gansu Maxianhao (Pedicularis Kansuensis Maxim.) by macroporous adsorptive resin combined with polyamide chromatographic column. Five in vitro experiments were used to study the effective antioxidant ingredients. Scavenging of DPPH, ABTS', and hydroxyl radicals are widely used to evaluate the free radical scavenging activity of mixed and pure antioxidants from plants. Fe (III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action. The result suggested that PhGs from Gansu Maxianhao (Pedicularis Kansuensis Maxim.) have antioxidant activity which may be helpful in preventing or slowing progress of various oxidative stress related diseases, such as acute mountain sickness, pulmonary edema, monge’s disease.

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After 8000 m exposing for 7 consecutive days in a hypobaric chamber, a simulated high-altitude hypoxia swimming test (4000 m) result indicated that swimming capacity time was significantly shortened. PhGs-H (400 mg/kg) had the optimum effect among all the doses and was capable of elevating the exercise tolerance in mice, indicating that the fatigue could be relieved by PhGs. The present results of biochemical alterations were insured by histopathological examination of the brain in mice, which revealed that the hypoxia model group had a significant decrease of MDA and subsequent increase of LA level and the antioxidant status of the body. Antioxidant enzymes, such as SOD and GSH, are responsible for cell damage and muscle fatigue. MDA is one of the end products resulting from degradation of cell membrane by radicals. The accumulation of oxygen free radicals is one of the risk factors which are responsible for oxidative stress and muscle fatigue. Exposure to hypoxia resulted in an increase of MDA levels in plasma and tissues and a concurrent decrease of SOD and GSH. PhGs can successfully prevent or reduce oxidative stress and further improve exercise performance. There was a significant decrease of MDA and subsequent increase of SOD and GSH levels. 5,7,28

BUN is the final product of protein metabolism. Under normal physiological conditions, there is a dynamic equilibrium between the production and the excretion of BUN. High intensity exercise for a long time, physical exertion has resulted in the enhancement of protein catabolism and will find the obvious change of BUN. As a common marker of muscle fatigue, LA is an important intermediate product in energy supply system of glucose metabolism. It is both the end product of glycolysis energy supply systems, as well as an oxide matrix in aerobic energy supply system. In experiment of hypoxia swimming, LA accumulated in body, lowered the pH value, and caused muscle spasms and pain. At sites of high energy consumption, the presence of CK plays an enzymatic role in regenerating ATP even under strenuous conditions. Serum CK is an important clinical biomarker for muscle damage, such as muscular dystrophy, severe muscle breakdown, myocardial infarction, and acute renal failure. During intense exercise the body consumes more energy, blood LA level increases causing CK levels to also increase. Increased CK levels occur because the energy derived from aerobic metabolism is not sufficient to support energy needs of the tissue and anaerobic metabolism is initiated, leading to tissue damage and fatigue. 29 In this study, PhGs significantly lowered blood BUN, LA and CK levels in a dose-dependent manner.

MDH is a key enzyme of the Krebs cycle in biological tissue. When the mice reached exhaustive swimming state, along with the consumption of body’s energy substances, energy supply and the enzymatic activity decreased. After intervened by giving PhGs, the MDH activity become higher than that of the hypoxia model group. SDH is a polymer enzyme which combines with mitochondrial inner membrane and plays a key role in Krebs cycle. 30,31 The level of SDH activity may indirectly reflect the energy metabolism and mitochondrial function status. When the activity of SDH is increased, the rate of citric acid cycle is accelerated, the ability of aerobic oxidation is improved, and the supply of energy is improved. PK can accelerate glycogen synthesis, speed up glucose oxidation and decomposition. Given different doses of PhGs can make SDH and PK activity increased. LDH is a sign of anaerobic oxidation enzyme used to evaluate the capacity of anaerobic metabolism. 32 PhGs can enhance the activity of LDH, reduce the production of LA, speed up the elimination of LA, then reduce the accumulation of LA in the body, ultimately improve endurance capacity, and delay the occurrence of the fatigue.

Glycogen, a principal storage form of carbohydrate, serves as a readily mobilizable source of energy to control vital functions in muscles. 33,34 Glycogen level in liver and skeletal muscles is an important biochemical parameter directly reflects fatigue symptoms. Mice administrated with PhGs had even more liver and skeletal muscles glycogen stores compared with hypoxia group. This suggests that administration of PhGs can significantly improve muscle and liver glycogen reserves, improve the utilization of glycogen in vivo, reduce the degree of anaerobic glycolysis and the consumption of protein, and thereby increase the fatigue resistance of animals. 35
The study provided preliminary evidence on the antioxidant activity and potential ameliorating effect of PhGs from Gansu Maxianhao (*Pedicularis kansuensis Maxim.*). Despite this potential, further researches are needed to conclusively elucidate the mechanisms underlying its protective effects and to verify their safety and efficacy in treatment of a number of different diseases. In conclusion, the present study has demonstrated that PhGs possesses potent antioxidant activity. *In vivo* results showed that the PhGs can ameliorate high altitude-induced fatigue. The mechanism may be closely related to the attenuation of the oxidative stress, reduction of the adverse metabolic products, enhancement of the activity of energy metabolism enzyme and increasing energy substances reserves. The findings of this study corroborate previous results that demonstrate that PhGs is a safe anti-fatigue tonic supplement. However, further studies are required to elucidate the exact underlying mechanisms at cellular and molecular levels and its therapeutic benefits in bioactive compounds warrant further investigation. The antioxidant and anti-fatigue activity of PhGs strongly attenuate many of the processes driving disease. Taken together, these data strongly support the use of PhGs from Gansu Maxianhao (*Pedicularis kansuensis Maxim.*) as a treatment for fatigue-associated disorders.

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