USE OF EXTRACT OF *PHELLINUS LINTEUS* GKPI FOR PREPARING MEDICAMENT OF ENHANCING EXERCISE PERFORMANCE AND REDUCING EXERCISE FATIGUE

Field of Invention

[0001] The present invention relates to a use of *Phellinus linteus*, and in particular to a use of an extract of *Phellinus linteus* GKPI in the manufacture of a medicament for enhancing exercise performance and reducing exercise fatigue.

Description of Related Art

[0002] In order to improve the performance of athletes in sports events, more and more researches focus on sports science (such as sports psychology, sports physiology, sports biomechanics, sports nutrition and traditional complementary therapies). Factors affecting exercise performance include physical fitness, sports skills, vision, fatigue, diseases, sports injury, sports nutrition, psychological factors and external factors. Among them, sports nutrition plays an important role in building body composition and providing sufficient energy, and sports nutrition is also one of the most important keys to the improvement of metabolic rate, the body repair rate as well as the immunity of athletes and/or reducing the risk of sports injuries.

[0003] In addition to the ratio and intake timing of carbohydrates, lipids, proteins, vitamins, minerals and water, sports supplements (such as creatine and caffeine) may also result in the improvement of exercise performance and/or the reduction of exercise fatigue. However, some sports supplements are classified as

banned substances since these sports supplements have active substances that can cause side effects or even harm the overall health of athletes. Therefore, finding safe and effective active substances for sports supplements has become an important research direction.

[0004] *Phellinus linteus* is a medicinal fungus belonging to the genus Sanghuangporus in the family Hymenochaetaceae. *Phellinus linteus* is highly safe and has functions such as anti-oxidation, anti-cancer, anti-dementia, anti-inflammation, anti-allergy (such as allergic rhinitis, eczema, rheumatoid arthritis), liver protection, prevention of cardiovascular diseases, sleep-promoting, pain relieving, uric acid inhibition, and skin care, etc. However, currently, there are few researches on the effects of *Phellinus linteus* in enhancing exercise performance and reducing exercise fatigue.

SUMMARY

[0005] Therefore, one aspect of the present invention is to provide a use of an extract of *Phellinus linteus* GKPI in the manufacture of a medicament for enhancing exercise performance, so as to increase the glycogen level of the liver and/or the muscles of a subject after exercise.

[0006] Another aspect of the present invention is to provide a use of an extract of *Phellinus linteus* GKPI in the manufacture of a medicament for reducing exercise fatigue, so as to decrease the blood urea nitrogen level of a subject after exercise.

[0007] According to the above aspects of the present invention, provided is a use of an extract of *Phellinus linteus* GKPI in the manufacture of a medicament for enhancing exercise performance, in which the medicament includes the extract of the *Phellinus linteus* GKPI as an active ingredient, and the extract includes a hot water extract and/or an ethanol extract, so as to increase a glycogen level of a liver and/or muscles. The above-mentioned *Phellinus linteus* GKPI is deposited in the National Institute of Technology and Evaluation (NITE), International Patent Organism Depositary (IPOD) on November, 12th, 2020 under an accession number of NITE BP-03321.

[0008] In one embodiment of the present invention, the hot water extract is to be obtained by subjecting the *Phellinus linteus* GKPI to a hot water extraction treatment with water at 80°C to 120°C for 20 minutes to 40 minutes.

[0009] In one embodiment of the present invention, the ethanol extract is to be obtained by subjecting the *Phellinus linteus* GKPI to an ethanol extraction treatment with ethanol.

[0010] In one embodiment of the present invention, the ethanol extraction treatment selectively includes an ultrasonic step.

[0011] In one embodiment of the present invention, a weight ratio of the *Phellinus linteus* GKPI to ethanol is to be 1/50 to 1/1.

[0012] In one embodiment of the present invention, the *Phellinus linteus* GKPI is to be subjected to a multi-step culture treatment including a solid-state culture step, a liquid-state culture step, and a fermentation step, so as to increase a biomass of the *Phellinus linteus* GKPI.

[0013] In one embodiment of the present invention, the *Phellinus linteus* GKPI is to be selectively subjected to a drying treatment to obtain a dried product of the *Phellinus linteus* GKPI.

[0014] In one embodiment of the present invention, a volume-to-weight ratio of the *Phellinus linteus* GKPI to the dried product is 100:5 to 100:1.

[0015] In one embodiment of the present invention, the medicament is to be a pharmaceutical medicament or a food composition.

[0016] In one embodiment of the present invention, the medicament selectively includes pharmaceutically and/or food acceptable carriers, excipients and/or additives.

[0017] In one embodiment of the present invention, compared with a control subject not administered with the extract of the *Phellinus linteus* GKPI, a subject administered with the extract of the *Phellinus linteus* GKPI has higher exercise duration. Noted that the control subject and the subject do not have muscular dystrophy.

[0018] According to another aspect of the present invention, provided is a use of an extract of *Phellinus linteus* GKPI in the manufacture of a medicament for improving exercise fatigue. The medicament includes an effective dose of the extract of the *Phellinus linteus* GKPI as an active ingredient, and the medicament is to be administered to a subject for at least 28 consecutive days to reduce a blood urea nitrogen level of the subject after exercise. The extract includes a hot water extract and/or an ethanol extract of the *Phellinus linteus* GKPI with an accession number of NITE BP-03321.

[0019] In one embodiment of the present invention, when the subject is a human adult, the effective dose is to be 0.1 g/60 kg body weight (bw)/day to 2.0 g/60 kg bw/day.

[0020] In one embodiment of the present invention, when the subject is a mouse, the effective dose is to be 0.1 g/kg bw/day to 0.5 g/kg bw/day.

[0021] In one embodiment of the present invention, compared with a control subject not administered with the extract of the *Phellinus linteus* GKPI, the subject has a lower blood lactate level after exercise. Noted that the control subject and the subject do not have muscular dystrophy.

[0022] Applying the extract of the *Phellinus linteus* GKPI of the present invention can increase the glycogen levels of a liver and/or muscles, and reduce the blood urea nitrogen level of a subject after exercise, thereby enhancing exercise performance and/or reducing exercise fatigue. Thus, the extract of the *Phellinus linteus* GKPI of the present invention can be used as an active ingredient for preparing various medicaments and compositions.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] The invention can be more fully understood by reading the following detailed description of the embodiment, with reference made to the accompanying drawings as follows:

[0024] FIG. 1 is a bar graph showing time to exhaustion of a mouse in a control group and an experimental group during weight-bearing swimming as shown in Table 1.

[0025] FIG. 2 is a bar graph showing a time to exhaustion during treadmill-running of mice in a control group and an experimental group as shown in Table 2.

[0026] FIG. 3A and FIG. 3B are bar graphs showing the production rate and the clearance rate of the blood lactate of mice in a control group and an experimental group as shown in Table 3, respectively.

[0027] FIG. 4 is bar graph showing blood urea nitrogen levels of mice in a control group and an experimental group as shown in Table 4 before and after long-time swimming.

[0028] FIG. 5 is a bar graph showing glycogen levels in the liver and the muscles of mice in a control group and an experimental group as shown in Table 5.

DETAILED DESCRIPTION

[0029] If the definition or usage of a term in a cited document is inconsistent with or contrary to the definition of the term herein, the definition of the term herein applies instead of the definition of the term in the cited document. Secondly, unless the context otherwise defines, singular terms may include the pluralities and plural terms may also include the singular.

[0030] As previously mentioned, the present invention provides a use of an extract of *Phellinus linteus* GKPI in the manufacture of a medicament for enhancing exercise performance and reducing exercise fatigue. The medicament includes an extract of *Phellinus linteus* GKPI as an active ingredient. The medicament is to be consecutively administered to a subject. After the subject exercises, the extract of *Phellinus linteus* GKPI can increase glycogen

levels in muscles and the liver as well as reduce blood lactate levels and urea nitrogen levels of subjects, thereby enhancing exercise performance and/or reducing exercise fatigue.

[0031] The "subject" herein refers to a healthy person. In some embodiments, the subject refers to a healthy athlete and/or other healthy persons in a training program. In some embodiments, the subject excludes muscle diseases. In some specific examples, the muscle diseases can include but not limited to muscular dystrophy and/or sarcopenia.

[0032] The "exercise performance" herein refers to the speed, strength, skill and/or coordination exhibited by the subject during exercise. The exercise performance of different types of sports can be evaluated in different ways. In some embodiments, the exercise performance includes muscular endurance and/or cardiorespiratory endurance. In some embodiments, the exercise performance can be assessed by aerobic exercise duration and/or glycogen levels in the liver and muscles. In some specific examples, the aerobic exercise duration can be assessed by a time to exhaustion during weight-bearing swimming and/or a time to exhaustion during treadmill running.

[0033] The "exercise fatigue" herein refers to the degree of fatigue of a subject during exercise, after exercise, and at rest after exercise. In some embodiments, the degrees of exercise fatigue can be assessed by blood lactate levels and/or blood urea nitrogen levels after exercise.

[0034] It should be additionally noted that glycogen is one of the energy storage substances in animals. Glycogen is a polysaccharide formed by dehydration and condensation of glucose, and can be produced and stored in liver cells and

muscle cells. When muscles cannot immediately obtain enough energy from glucose in the blood, muscle cells will directly decompose intracellular glycogen, thereby supplying the energy required for the exercising muscle. Secondly, when the blood glucose level decreases, liver cells will also directly decompose glycogen in the cells, so as to replenish the blood glucose level. Therefore, the glycogen levels in the liver and muscles can affect exercise endurance, thereby enhancing exercise performance.

[0035] When the energy produced by aerobic respiration cannot meet the energy required by muscles, the muscles will also obtain sufficient energy through glycolysis as well as produce lactate and hydrogen ions as by-products. Lactate can be transferred to the liver through blood circulation, so that the liver can clear lactate through the Cori cycle and produce glucose. Hydrogen ions can also be removed in the above process. However, when the lactate production rates are greater than the clearance rates, large amounts of lactate and hydrogen ions will accumulate in the muscle, thereby reducing the pH values of muscles and affecting the energy transfer by an enzyme, resulting in exercise fatigue symptoms such as muscle soreness, heaviness, and burning. Therefore, blood lactate levels can be used as indicators of exercise fatigue.

[0036] The body uses energy in the order of carbohydrates (such as glucose and/or glycogen), fats and proteins. Therefore, after exercising for a long time, when the catabolism of carbohydrates or fats cannot provide enough energy, muscles will degrade proteins and amino acids, so as to generate energy and produce urea nitrogen in the liver. The urea nitrogen will be transported to

kidneys through the blood and then excreted through urine. Therefore, blood urea nitrogen (BUN) levels can also be used as an indicator of exercise fatigue.

[0037] In some specific examples, comparing the differences in blood lactate and/or urea nitrogen level before and after exercise respectively can evaluate the accumulation amounts of lactate and/or urea nitrogen after exercise. In some other specific examples, comparing the differences in blood lactate levels after short-time exercise and at rest after short-time exercise respectively can evaluate the clearance amount of blood lactate at rest after exercise.

[0038] It should be noted that the "exercise" described herein can be divided into aerobic exercise and anaerobic exercise. Aerobic exercise refers to moderate-intensity exercise that lasts for a longer time and requires stable and regular muscle contraction and relaxation. The energy of aerobic exercise is mainly produced by decomposing carbohydrates or fats through aerobic respiration. In some specific examples, aerobic exercise can include but not limited to long-distance running, walking, cycling, swimming, aerobic boxing and/or aerobic dancing. Anaerobic exercise refers to high-intensity exercise that lasts for a short time and requires muscles to produce explosive power. The energy of anaerobic exercise is mainly produced by decomposing glycogen through glycolysis. In some specific examples, anaerobic exercise can include but not limited to running and/or weight training and the like. However, in fact, during aerobic exercise or anaerobic exercise, energy will be obtained through aerobic respiration and glycolysis. In some embodiments, the "long-time exercise" mentioned in the present invention means that the above-mentioned

aerobic exercise or anaerobic exercise continues until muscles require the generation of energy through proteins and/or amino acids.

[0039] It has been confirmed by experiments that compared with the control subject not administered with the extract of *Phellinus linteus* GKPI, the subject administered with the extract of *Phellinus linteus* GKPI has a higher exercise duration and higher glycogen levels of the liver and/or muscles, indicating that the extract of *Phellinus linteus* GKPI can indeed improve exercise performance. In the above embodiments, the subjects have undergone sports training for 1 to 10 days, or longer-term sports training. Secondly, compared with the control subject not administered with the extract of *Phellinus linteus* GKPI, the subject administered with the extract of *Phellinus linteus* GKPI has a lower blood lactate level and/or a lower blood urea nitrogen level after exercise, indicating that the extract of *Phellinus linteus* GKPI can indeed reduce exercise fatigue.

[0040] The mycelium of *Phellinus linteus* GKPI (also known as strain GKPI) referred to herein was deposited in National Institute of Technology and Evaluation (NITE), International Patent Organism Depositary (IPOD) (address: #122, 2-5-8 Kazusakamatari, Kisarazu-shi, Chiba 292-0818, Japan) on November, 12th, 2020 under an accession number of NITE BP-03321, as well as the Bioresource Collection and Research Center (BCRC, address: No. 331, Food Road, East District, Hsinchu City, Taiwan, 30062) of the Taiwan Food Industry Research and Development Institute (FIRDI) on July 18, 2019, under an accession number of BCRC 930210. The mycelium of strain GKPI was also deposited in the Patent Microorganisms Depositary (address: #122, 2-5-8 Kazusakamatari, Kisarazu-shi, Chiba-ken, 292-0818, Japan) of the Japan

National Institute of Technology and Evaluation (NITE) on November 12, 2020, with an accession number NITE BP-03321. It should be additionally noted that the present invention requires the specific strain of *Phellinus linteus* (i.e., strain GKPI) to exert the effects of enhancing exercise performance and/or reducing exercise fatigue. The effects of *Phellinus linteus* such as enhancing exercise performance or reducing exercise fatigue cannot be expected if the *Phellinus linteus* is not strain GKPI.

[0041] The extract of strain GKPI described above can be subjected to a multi-step culture treatment to increase biomass. The multi-step culture treatment can be performed using conventional culture methods. In some embodiments, the multi-step culture treatment may selectively include a solid-state culture step, a liquid-state culture step, and a fermentation step.

[0042] In particular, the solid-state culture step can be performed on the mycelium of strain GKPI in a solid-state medium at 15°C to 30°C for 7 days to 14 days, thereby obtaining a first culture. The solid-state medium is not particularly limited in terms of formulation, and can be a conventional solid-state medium. In some embodiments, the solid-state medium can include but not limited to a carbon source, a nitrogen source and potato dextrose agar (PDA).

[0043] The liquid-state culture step can be performed on the first culture with a liquid-state medium for 3 days to 21 days at 15°C to 30°C, pH 2 to pH 6 and an agitation rate of 110 rpm to 130 rpm, thereby obtaining a second culture. The composition of the liquid-state medium is not particularly limited, and can be adjusted according to requirements. In some embodiments, the liquid-state medium may include but not limited to 1.00% to 3.00% by weight of a combined

carbon and nitrogen source, 1.00% to 4.00% by weight of carbohydrates, 0.10% to 1.00% by weight of a yeast extract, 0.10% to 1.00% by weight of peptone, 0.01% to 0.50% by weight of inorganic salts and the balance of water. In some specific examples, the combined carbon and nitrogen source may be, for example, cereals (such as wheat flour) and/or beans (such as soybean flour, mung bean flour and/or cinnamon powder). In some specific examples, the carbohydrates may include but not limited to monosaccharides (such as glucose and/or fructose) and/or disaccharides (such as maltose and/or sucrose). In some specific examples, the inorganic salts may be, for example, phosphates (such as dipotassium hydrogen phosphate, potassium dihydrogen phosphate) and/or sulfates (such as magnesium sulfate and/or iron sulfate).

[0044] The fermentation step can be performed on the second culture in a fermentation medium for 3 days to 21 days at 15°C to 30°C, pH 2 to pH 6, and an agitation rate of 50 rpm to 150 rpm, thereby obtaining a fermented product of strain GKPI (hereinafter referred to as a GKPI fermented product) which contains a mycelium of strain GKPI with increased biomass and growth activity. In some embodiments, the fermentation step can be performed in a fermentation tank. In some specific examples, a tank pressure in the fermentation tank can be 0.5 kg/cm^2 to 1.0 kg/cm^2, and the aeration rate can be 1.0 vvm to 1.5 vvm, in which vvm herein refers to the volume of air introduced/volume of fermentation medium/minute. The composition of the fermentation medium is not particularly limited. In some embodiments, the medicament of the fermentation medium is the same as that of the liquid-state medium described above.

[0045] In some embodiments, after the fermentation step, the GKPl fermented product may selectively be subjected to a drying treatment to obtain a dried product of the mycelium of strain GKPl (hereinafter referred to as a GKPl dried product). The drying treatment can be carried out using conventional drying methods, for example, freeze drying, vacuum drying or spray drying. In some embodiments, the volume-to-weight ratio (unit: L: kg) of the GKPl fermented product to the GKPl dried product may be, for example, 100:5 to 100:1.

[0046] The extraction treatment can be carried out, for example, using conventional extraction methods. In some embodiments, the GKPl extract can include but not limited to a GKPl hot water extract and/or a GKPl ethanol extract. In some embodiments, the GKPl hot water extract can be obtained after the GKPl fermented product and/or the GKPl dried product is subjected to a hot water extraction treatment. In some embodiments, the temperature of hot water used in the hot water extraction treatment may be, for example, 80°C to 120°C. In some embodiments, the time of the hot water extraction treatment may be, for example, 20 minutes to 40 minutes. In some examples, the volume ratio of the GKPl fermented product and/or the GKPl dried product to the hot water may be, for example, 1/50 to 1/1, 1/30 to 1/10, or 1/20.

[0047] In some embodiments, the GKPl ethanol extract can be obtained after the GKPl fermented product and/or the GKPl dried product is subjected to an ethanol extraction treatment. In some embodiments, the time of the ethanol extraction treatment may be, for example, 50 minutes to 70 minutes. In some examples, the weight ratio of the GKPl fermented product and/or the GKPl dried product to the ethanol may be, for example, 1/50 to 1/1, 1/30 to 1/10, or 1/20. In

some embodiments, the ethanol extraction treatment may selectively include an ultrasonic step. In some embodiments, the GKPI hot water extract and/or the GKPI ethanol extract may be selectively subjected to a drying treatment, so as to obtain a dried product of *Phellinus linteus* GKPI.

[0048] It should be noted that the mycelium of *Phellinus linteus* GKPI, and the fermented product and dried product thereof all contain active substances that can improve exercise performance and reduce exercise fatigue. Noted that the extraction treatment can only increase the concentration of these active substances, and is not intended to limit the present invention. In other words, the fermented product and the dried product thereof of the mycelium of *Phellinus linteus* GKPI of the present invention should also have the effects of enhancing exercise performance and reducing exercise fatigue.

[0049] It is worth noting that the extract of *Phellinus linteus* GKPI described in the present invention has not been further purified, and thus, the extract of *Phellinus linteus* GKPI contains a variety of unknown and inseparable ingredients including active substances that can improve exercise performance and reduce exercise fatigue. Secondly, the present invention has proved that the extract of *Phellinus linteus* GKPI as a whole can achieve the effects of enhancing exercise performance and reducing exercise fatigue. Since the extract of *Phellinus linteus* GKPI contains a variety of active substances, it is unreasonable to expect that the effects of enhancing exercise performance and reducing exercise fatigue can still be achieved when a single substance of the extract of *Phellinus linteus* GKPI is used and/or unknown and inseparable ingredients are excluded. Therefore, the extract of *Phellinus linteus* GKPI described in the present invention

cannot be defined in terms of ingredients, properties or parameters, that is, there are situations where it is impossible or impractical to define the present invention in terms of ingredients, properties or parameters.

[0050] The extract of strain GKPI has the effects of enhancing exercise performance and/or reducing exercise fatigue, and can be used in the manufacture of a medicament for enhancing exercise performance and/or reducing exercise fatigue. In some embodiments, the medicament may include but not limited to a hot water extract and/or an ethanol extract of strain GKPI as an active ingredient. In some other embodiments, the active ingredient of the medicament is composed of the hot water extract and the ethanol extract of strain GKPI. In some embodiments, the weight ratio of the hot water extract and the ethanol extract is not particularly limited. In a specific example, the weight ratio of the hot water extract and the ethanol extract is 5:1 to 1:5, or 3:1 to 1:3, or 1:1.

[0051] The medicament for enhancing exercise performance and/or reducing exercise fatigue described above may be, for example, an oral composition. In some embodiments, the medicament may be, for example, a pharmaceutical composition or a food composition. Examples suitable for pharmaceutical compositions may include, but not limited to, pharmaceuticals. Examples suitable for food compositions may include but not limited to general food, health food, beverages, nutritional supplements, sports supplements, dairy products or feeds, etc. In some embodiments, the medicament may selectively include pharmaceutically- and food-acceptable carriers, excipients and/or additives. In other examples, the dosage forms of oral compositions may include, but not limited to, powders, tablets, granules, suppositories, microcapsules, ampoules,

liquid sprays or suppositories. In some embodiments, the medicament is composed of an active ingredient (composed of the hot water extract and the ethanol extract of strain GKPI) and other pharmaceutically- and food-acceptable carriers, excipients and/or additives.

[0052] In use, a medicament containing an extract of strain GKPI may be administered to a subject for a consecutive period. The effective dose and administration period described above depend on the subject and are not particularly limited. For example, when the subject is a human adult, the effective dose of the extract of strain GKPI can be, for example, 0.1 g/60 kg body weight (bw)/day to 2.0 g/60 kg bw/day, preferably 0.5 g/60 kg bw/day to 1.5 g/60 kg bw/day, more preferably 1.0 g/60 kg bw/day. In another example, when the subject is a mouse, the effective dose of the extract of strain GKPI can be, for example, 0.1 g/kg bw/day to 0.5 g/kg bw/day, preferably 0.1 g/kg bw/day to 0.3 g/kg bw/day, more preferably 0.15 g/kg bw/day to 0.25 g/kg bw/day. In other examples, the administration period described above may be, for example, at least 28 consecutive days, or can be longer or shorter.

[0053] Several examples are used below to illustrate the application of the present invention, but they are not used to limit the present invention. Those having ordinary skill in the art of the present invention can make various changes and modifications without departing from the spirit and scope of the present invention.

Example 1. Preparation of GKPI extract

1.1 Source of *Phellinus linteus* GKPI

[0054] The accession number of the mycelium of *Phellinus linteus* strain GKPI used in this example was NITE BP-03321 or BCRC 930210. The strain GKPI was a mycelium isolated from the fruiting body of wild *Phellinus linteus* in China. The microbiological properties and culture methods of *Phellinus linteus* GKPI were recited in Taiwan patent TW I729928 B, Taiwan patent TW I766394 B and Japanese patent JP7250065B2 (publication number: JP 2022067043 A), which were incorporated herein as references.

1.2 Preparation of extract of *Phellinus linteus* strain GKPI

[0055] The mycelium of strain GKPI was inoculated in a potato dextrin agar and subjected to a solid-state culture step at 25°C for 7 days, thereby obtaining a first culture. Then, the first culture was subjected to a liquid-state culture step in a liquid-state medium for 14 days at 25°C, pH 4.5, and an agitation rate of 120 rpm, thereby obtaining a second culture. The above liquid-state medium included 1.0% by weight of a combined carbon and nitrogen source, 1.0% by weight of carbohydrates, 0.3% by weight of a yeast extract, 0.3% by weight of peptone and 0.1% by weight of inorganic salts. The specific formulations of the combined carbon and nitrogen source, carbohydrates and inorganic salts were well known to those having ordinary skill in the art of the present invention, and could be adjusted arbitrarily according to actual needs, and will not be repeated here.

[0056] Then, the second culture was subjected to a fermentation step in the above culture solution for 14 days at 25°C, pH 5, an agitation rate of 80 rpm, a tank pressure of 0.5 kg/cm^2 and an air ventilation rate of 1.0 vvm, thereby obtaining the fermented product of strain GKPI. Then, the fermented product of strain GKPI was freeze-dried to obtain the lyophilized powder of the fermented

product of strain GKPl (hereinafter referred to as a GKPl lyophilized powder). In this example, 3 kg of the GKPl lyophilized powder can be obtained from 100 L of the fermented product of strain GKPl.

[0057] Next, a hot water extract and an ethanol extract of strain GKPl were prepared respectively (hereinafter referred to as a GKPl hot water extract and a GKPl ethanol extract respectively). The GKPl hot water extract was obtained after adding the GKPl lyophilized powder to distilled water, followed by a hot water extraction treatment at 100°C for 30 minutes, where the volume ratio of the GKPl lyophilized powder to the distilled water was 1/20. The GKPl ethanol extract was obtained by adding the GKPl lyophilized powder to ethanol, followed by an ethanol extraction treatment and an ultrasonic step at 25°C for 60 minutes, where the weight ratio of the GKPl lyophilized powder to the ethanol was 1/20. The GKPl hot water extract and the GKPl ethanol extract were mixed in a weight ratio of 1:1, thereby obtaining an extract of strain GKPl (hereinafter referred to as a GKPl extract). The extract of strain GKPl was further subjected to a lyophilization step, so as to obtain a GKPl lyophilized powder.

Example 2. Evaluation of the effects of GKPl extract in enhancing exercise performance and reducing exercise fatigue

2.1 Test animals

[0058] In the example below, twelve 6-week-old male mice of the ICR strain were selected and divided into a control group and an experimental group, 6 in each group. From day 1 to day 28, the mice were orally administered twice with water (for the control group) or the GKPl extract (for the experimental group) at 9 am every day, and the body weight, the weights of the food intake and water

intake of the mice were observed and recorded. The following test or detection was performed on day 29 to day 37: weight-bearing swimming-to-exhaustion test (unit: min), treadmill-to-exhaustion test (unit: min), change rate of blood lactate level before and after short-time swimming and at rest after the short-time swimming, urea nitrogen level (unit: mg/dL) after long-time swimming, and at rest after long-time swimming, and glycogen level in the liver and muscle (mg/g liver or mg/g muscle).

2.2 Weight-bearing swimming-to-exhaustion test

[0059] On day 31, a weight-bearing swimming-to-exhaustion test was performed. Before the test, the mice were fasted for 12 hours. 30 minutes before the test, the mice were administered twice with water (for the control group) and the GKPl extract (for the experimental group). During the test, a lead sheet weighing 5% of the mouse body weight was fixed at the base of the mouse tail, and then the mouse were put into a water tank and forced to swim. The water temperature of the water tank was controlled at 27±1°C, and the test was carried out with a single swimmer to avoid pushing and affecting the experimental results when swimming in a group.

[0060] Throughout the test, it was necessary to ensure that the limbs of the mouse were kept in motion. If the mouse was floating on the water without moving its limbs, a stir bar was used to stir around it. The test was terminated when the mouse lost coordinated movement and was unable to return to the water surface within 7 seconds (i.e., swimming to exhaustion). The time from the start of the test to the end of the test was calculated as the time to exhaustion

of a mouse during weight-bearing swimming (i.e., duration). The experimental results were recorded in Table 1.

[0061] Table 1

Group	Time to exhaustion during weight-bearing swimming (mean ± standard deviation; unit: min)
Control group	5.02±0.83
Experimental group	8.97±0.45***

[0062] FIG. 1 was a bar graph showing the time to exhaustion of a mouse in a control group and an experimental group during weight-bearing swimming as shown in Table 1, where the horizontal axis represented the group, and the vertical axis represented the time to exhaustion during weight-bearing swimming (unit: min), and the symbol "***" indicated a statistically significant difference from the control group ($p<0.001$).

[0063] As shown in FIG. 1, the time to exhaustion of a mouse in the experimental group during weight-bearing swimming was significantly longer than that in the control group (increased by about 78.89%), indicating that the GKPl extract could indeed increase the exercise duration of the mice and had the effect of enhancing exercise performance.

2.3 Treadmill-to-exhaustion test

[0064] On day 31, a treadmill-to-exhaustion test was performed. Before the test, the mice were fasted for 12 hours. 30 minutes before the test, the mice were administered twice with water (for the control group) and the GKPl extract (for the experimental group). During the test, the mice were placed on a conveyor belt of a treadmill with a 5° incline ending at the lower end. An electric

shock grid was arranged at the end of the treadmill, so that an electric shock was applied to the mice when the mice stopped running and a body part touched the electric shock grid. At the beginning of the test, the speed of the treadmill was 10 m/minute. After the test was carried out for 5 minutes, the speed of the treadmill was increased by 2 m/minute until the mice were exhausted, and the test was ended then. The criteria used to indicate an exhausted mouse was defined as follows: the mouse was shocked multiple times, or the mouse was continuously shocked for 5 seconds. The experimental results were recorded in Table 2.

[0065] Table 2

Group	Time to exhaustion during treadmill-running (mean ± standard deviation; unit: min)
Control group	8.25±1.14
Experimental group	13.4±0.94***

[0066] FIG. 2 was a bar graph showing the time to exhaustion during treadmill-running of mice in a control group and an experimental group as shown in Table 2, where the horizontal axis represented the groups, the vertical axis represented the time to exhaustion during treadmill-running (unit: min), and the symbol "***" indicated a statistically significant difference from the control group ($p<0.001$).

[0067] As shown in FIG. 2, the time to exhaustion during treadmill-running of the mice in the experimental group was significantly longer than that in the control group (increased by about 62.42%), indicating that the GKPl extract could increase the exercise duration of the mice and indeed had the effect of enhancing exercise performance.

2.4 Production rate and clearance rate of blood lactate at rest after the short-term exercise

[0068] On day 35, the blood lactate production rates of mice after short-term exercise and the blood lactate clearance rates of mice at rest after the short-term exercise were detected. The mice were administered twice with water (for the control group) and the GKPl extract (for the experimental group) first, and a first blood collection was performed 30 minutes later to obtain blood samples before the mice was going to swim. Then, the mice were allowed to swim in a water tank at a water temperature of 30°C for 10 minutes, and a second blood collection was performed to obtain blood samples after swimming. Next, after the mice rested for 20 minutes, a third blood collection was performed to obtain blood samples of mice at rest after swimming.

[0069] The blood lactate levels in the blood samples before swimming, after swimming and at rest after swimming were detected to obtain blood lactate levels before swimming, blood lactate levels after swimming and blood lactate levels at rest after swimming. The blood lactate levels were detected by an automatic biochemical analyzer (name: HITACHI 7060; manufacturer: Hitachi, Tokyo, Japan).

[0070] Ratios of differences between blood lactate levels after swimming and the blood lactate levels before swimming to the blood lactate levels before swimming were calculated, so as to obtain production rates of blood lactate after swimming. The ratios of the differences between the blood lactate levels after swimming and the blood lactate levels after swimming and at rest after swimming to the blood lactate levels after swimming were calculated, so as to obtain

clearance rates of blood lactate at rest after swimming. The experimental results were recorded in Table 3.

[0071] Table 3

Group	Blood lactate production rate after swimming (mean ± standard deviation; unit: no unit)	Blood lactate clearance rate at rest after swimming (mean ± standard deviation; unit: no unit)
Control group	1.85±0.21	0.11±0.08
Experimental group	1.56±0.14*	0.23±0.08*

[0072] FIG. 3A and FIG. 3B were bar graphs showing the production rate (FIG. 3A) and the clearance rate (FIG. 3B) of the blood lactate of mice in a control group and an experimental group as shown in Table 3, respectively, in which the horizontal axis represented the group, and the vertical axis represented a production rate (FIG. 3A, no unit) and a clearance rate (FIG. 3B, no unit) of blood lactate, and the symbol "*" indicated a statistically significant difference from the control group ($p<0.05$).

[0073] As shown in FIG. 3A, the production rate of the blood lactate of mice in the experimental group was significantly smaller than that in the control group, indicating that the GKPl extract could reduce the accumulation of blood lactate during exercise. Secondly, as shown in FIG. 3B, the production rate of the clearance rate of the mice at rest after swimming in the experimental group was significantly higher than that in the control group, indicating that the GKPl extract could improve the lactate clearance at rest and had the effect of effectively reducing exercise fatigue.

2.5 Blood urea nitrogen level before and after long-time swimming

[0074] On day 37, blood urea nitrogen levels of mice before and after swimming for a long time were detected. The mice were administered twice with water (for the control group) and the GKPl extract (for the experimental group) first, and s first blood collection was performed 30 minutes later to obtain blood samples of mice before swimming for a long time. The mice were allowed to swim in a water tank at a water temperature of 30°C for 60 minutes, and a second blood collection was performed to obtain blood samples of the mice after long-time swimming.

[0075] The blood urea nitrogen levels of the blood samples before long-time swimming and the after long-time swimming were detected to obtain blood urea nitrogen levels before long-time swimming and blood urea nitrogen levels after long-time swimming. The experimental results were recorded in Table 4.

[0076] Table 4

Group	Blood urea nitrogen level before long-time swimming (mean ± standard deviation; unit: mg/dL)	Blood urea nitrogen level after long-time swimming (mean ± standard deviation; unit: mg/dL)
Control group	22.1±0.70	35.2±2.60
Experimental group	22.2±1.20	29.4±1.20*

[0077] FIG. 4 was a bar graph showing blood urea nitrogen levels of mice in a control group and an experimental group as shown in Table 4 before and after long-time swimming, where the horizontal axis represented before long-time swimming and after long-time swimming from left to right, the vertical axis represented blood urea nitrogen levels (unit: mg/dL), bar 410 and bar 420 respectively represented the control group and the experimental group, and the

symbol "*" indicated a statistically significant difference from the control group ($p<0.05$).

[0078] As shown in FIG. 4, before long-time swimming (i.e., under the general activity state), there was no statistically significant difference in blood urea nitrogen level between the mice in the experimental group and the control group, indicating that the GKPl extract did not affect the metabolism of proteins and/or amino acids in the mice under the general activity state. However, after long-time swimming, the blood urea nitrogen level of mice in the experimental group was significantly lower than that in the control group, indicating that the GKPl extract could indeed reduce the accumulation of urea nitrogen and had the effect of effectively reducing exercise fatigue.

2.6 Glycogen level in the liver and muscles

[0079] On day 39, glycogen levels in the liver and muscles of the mice were analyzed. The mice were administered twice with water (for the control group) and the GKPl extract (for the experimental group) first, and the mice were sacrificed 30 minutes later. The liver and hindlimb calf muscles of the mice were removed, washed and dried, and then weighed. Then, the tissues of the liver and hindlimb calf muscles at the same position were removed respectively, and homogenized by a tissue homogenizer (product name: Bullet Blender®; manufacturer: Next Advance, Cambridge, MA, US) using 10% glacial perchloric acid in a volume 5 times the tissue volume as a homogenization solution, thereby obtaining a tissue homogenate.

[0080] The tissue homogenate was centrifuged at 12,000 x g for 15 minutes at 4°C to obtain a supernatant. 30 μL of the supernatant was mixed with 200 μL

of iodine-potassium iodide reagent for 10 minutes to react to glycogen in the supernatant with the iodine-potassium iodide reagent to produce a brown substance. The absorbance of the supernatant at a wavelength of 460 nm was detected by ELISA (product name: Tecan Infinite M200; manufacturer: Tecan Austria, Salzburg, Austria). A standard curve was drawn using standard glycogen (purchased from Sigma), and then the glycogen levels (mg/g liver or mg/g muscle) in the liver tissues and muscle tissues of mice in each group were calculated respectively. The results were recorded in Table 5.

[0081] Table 5

Group	Liver glycogen level (mean ± standard deviation; unit: mg/g liver)	Muscle glycogen level (mean ± standard deviation; mg/g muscle)
Control group	15.8±2.81	1.02±0.15
Experimental group	21.2±4.55*	1.59±0.23*

[0082] FIG. 5 was a bar graph showing a glycogen level in the liver and the muscles of mice in a control group and an experimental group as shown in Table 5, where the horizontal axis represented the parts, which from left to right were the liver and muscles respectively, the vertical axis represented a glycogen level (unit: mg/g liver, mg/g muscle), bar 510 and bar 520 represented the control group and the experimental group respectively, and the symbol "*" indicated a statistically significant difference from the control group ($p<0.05$).

[0083] As shown in FIG. 5, after several days of training, the glycogen levels in the liver and muscles of mice in the experimental group were significantly higher than those in the control group, indicating that the GKPl extract can indeed

increase the glycogen level in the liver and muscle, and had the effect of enhancing exercise performance.

[0084] In summary, the specific culture method, specific extraction method, specific dose, specific dosage form, specific test animal or specific evaluation method described above are only used to illustrate the use of the extract of *Phellinus linteus* GKPI of the present invention in the manufacture of a medicament for enhancing athletic performance and reducing exercise fatigue. However, those having ordinary skill in the art of the present invention should understand that without departing from the spirit and scope of the present invention, other culture methods, other extraction methods, other dosages, other dosage forms, other test animals or other evaluation methods can also be useful in the use in the manufacture of a medicament for enhancing athletic performance and reducing exercise fatigue, and are not limited to the foregoing. For example, the multi-step culture treatment is only to increase the biomass and biological activity of the extract of *Phellinus linteus* GKPI, and when the multi-step culture treatment is carried out under conventional conditions, the obtained extract *Phellinus linteus* GKPI should also have the effects of enhancing exercise performance and reducing exercise fatigue.

[0085] As can be seen from the above examples, the use of the extract of *Phellinus linteus* GKPI of the present invention in the manufacture of a medicament for enhancing exercise performance and reducing exercise fatigue has the advantage of using the extract of *Phellinus linteus* GKPI of a specific strain, so as to increase exercise duration, glycogen levels in the liver and muscle, and reduce blood lactate level and blood urea nitrogen level after

exercise, and thus it can be used as an active ingredient in a medicament for enhancing exercise performance and reducing exercise fatigue.

[0086] It will be apparent to those skilled in the art that various modifications and variations can be made to the structure of the present invention without departing from the scope or spirit of the invention. In view of the foregoing, it is intended that the present invention cover modifications and variations of this invention provided they fall within the scope of the following claims.

WHAT IS CLAIMED IS:

1. Use of an extract of *Phellinus linteus* GKPI in the manufacture of a medicament for enhancing exercise performance, wherein the medicament comprises the extract of the *Phellinus linteus* GKPI as an active ingredient, the extract comprises a hot water extract and/or an ethanol extract, and the *Phellinus linteus* GKPI is deposited in National Institute of Technology and Evaluation (NITE), International Patent Organism Depositary (IPOD) on November, 12th, 2020 under an accession number of NITE BP-03321, so as to increase a glycogen level in a liver and/or muscles.

2. The use according to claim 1, wherein the hot water extract is to obtained by subjecting the *Phellinus linteus* GKPI to a hot water extraction treatment with water at 80°C to 120°C for 20 minutes to 40 minutes.

3. The use according to claim 1, wherein the ethanol extract is to be obtained by subjecting the *Phellinus linteus* GKPI to an ethanol extraction treatment with ethanol.

4. The use according to claim 3, wherein the ethanol extraction treatment further includes an ultrasonic step.

5. The use according to claim 3, wherein a weight ratio of the *Phellinus linteus* GKPI to the ethanol is to be 1/50 to 1/1.

6. The use according to any one of claims 2 to 3, wherein the *Phellinus linteus* GKPI is to be subjected to a multi-step culture treatment including a solid-state culture step, a liquid-state culture step, and a fermentation step, so as to increase a biomass of the *Phellinus linteus* GKPI.

7. The use according to claim 1, wherein the *Phellinus linteus* GKPI is to be further subjected to a drying treatment to obtain a dried product of the *Phellinus linteus* GKPI.

8. The use according to claim 7, wherein a volume-to-weight ratio of the *Phellinus linteus* GKPI to the dried product is 100:5 to 100:1.

9. The use of according to claim 1, wherein the medicament is to be a pharmaceutical composition or a food composition.

10. The use of according to claim 1, wherein the medicament further comprises pharmaceutically and/or food acceptable carriers, excipients and/or additives.

11. The use according to claim 1, wherein compared with a control subject not administered with the extract of the *Phellinus linteus* GKPI, a subject administered with the extract of the *Phellinus linteus* GKPI has higher exercise duration, and the control subject and the subject do not have muscular dystrophy.

12. Use of an extract of *Phellinus linteus* GKPI in the manufacture of a medicament for improving exercise fatigue, wherein the medicament comprises an effective dose of the extract of the *Phellinus linteus* GKPI as an active ingredient, the medicament is to be administered to a subject for at least 28 consecutive days, the extract comprises a hot water extract and/or an ethanol extract of the *Phellinus linteus* GKPI with an accession number of NITE BP-03321, so as to reduce a blood urea nitrogen level of the subject after exercise.

13. The use according to claim 12, wherein when the subject is a human adult, the effective dose is to be 0.1 g/60 kg body weight (bw)/day to 2.0 g/60 kg bw/day.

14. The use according to claim 12, wherein when the subject is a mouse, the effective dose is to be 0.1 g/ kg bw/day to 0.5 g/kg bw/day.

15. The use according to claim 12, wherein a weight ratio of the hot water extract and the ethanol extract of the *Phellinus linteus* GKPI is 5:1 to 1:5.

16. The use according to claim 12, wherein compared with a control subject not administered with the extract of the *Phellinus linteus* GKPI, the subject has a lower blood lactate level after exercise, and the control subject and the subject do not have muscular dystrophy.