

**USE OF MYCELIUM OF *Phellinus linteus* GKPI IN MANUFACTURE OF
MEDICAMENT FOR TREATING OBESITY AND OBESITY-RELATED
METABOLIC DISORDERS**

BACKGROUND

Field of Invention

[0001] The present invention relates a use of *Phellinus linteus*. More particularly, the present invention relates to a use of a mycelium of *Phellinus linteus* GKPI in the manufacture of a medicament for treating obesity and obesity-related metabolic disorders.

Description of Related Art

[0002] Obesity is a physical condition of an abnormal or excessive accumulation of fat which is positively correlated to the occurrence of many diseases, e.g., metabolic syndrome, cardiovascular diseases and/or diabetes. Moreover, obesity is also positively correlated to the occurrence of the diseases such as musculoskeletal disorders (such as osteoarthritis), some cancers and/or asthma. Furthermore, obesity also affects the process and prognosis of infectious diseases. For example, among the patients with coronavirus disease of 2019 (COVID-19), the rate of hospitalization, critical care requirement and/or the rate of death of an obese patient is higher than that of a nonobese patient.

[0003] When caloric intake exceeds caloric expenditure, the extra calories will be stored in the form of fat. Thus, the essential principle to treat obesity can include but not limited to diet control and exercise, in which exercise can not

only burn calories directly but can also change how a body utilizes fat, e.g., increase the amount of brown adipocytes, which can generate heat by metabolite fatty acid. However, few studies have focus on the active ingredients that can increase of the amount of brown adipocytes.

[0004] In view of above, it is necessary to provide a medicament used for treating obesity and obesity-related metabolic disorders to solve the aforementioned problems.

SUMMARY

[0005] Therefore, one aspect of the present invention provides a use of a mycelium of *Phellinus linteus* GKPI in the pharmaceutical manufacture of a medicament for treating obesity, in which the medicament can include but not limited to an effective dose of an extract of the mycelium of the *Phellinus linteus* GKPI as an active ingredient.

[0006] Another aspect of the present invention provides a use of a mycelium of *Phellinus linteus* GKPI in the manufacture of a medicament for treating obesity, in which the medicament can include but not limited to an effective dose of an hot-water extract of the *Phellinus linteus* GKPI and/or an ethanol extract of the mycelium of the *Phellinus linteus* GKPI as an active ingredient.

[0007] The other aspect of the present invention provides a use of a mycelium of *Phellinus linteus* GKPI in the manufacture of a medicament for treating obesity-related metabolic disorders, in which the medicament can include but not limited to an effective dose of an extract of the mycelium of the *Phellinus*

linteus GKPI as an active ingredient, and the effective dose of the extract can be 150 µg/mL to 250 µg/mL *in vitro*, for example.

[0008] According to the aforementioned aspect of the present invention, a use of a mycelium of *Phellinus linteus* GKPI in the manufacture of a medicament for treating obesity is provided, in which the medicament can include but not limited to an effective dose of an extract of the mycelium of the *Phellinus linteus* GKPI as an active ingredient. The aforementioned mycelium of the *Phellinus linteus* GKPI can be deposited in Patent Microorganisms Depositary of the National Institute of Technology and Evaluation (NITE) on November 12, 2020 with the accession number of NITE BP-03321, for example.

[0009] In one embodiment of the present invention, the extract can include but not limited to a hot-water extract and/or an ethanol extract of the mycelium of the *Phellinus linteus* GKPI. In one embodiment of the present invention, the effective dose of the extract can be 150 µg/mL to 250 µg/mL *in vitro*, for example. In one embodiment of the present invention, the *Phellinus linteus* GKPI treats the obesity by increasing an expression level of peroxisome proliferator-activated receptor gamma coactivator (PGC)-1α, fibronectin type III domain-containing protein (FNDC) 5 and/or uncoupling protein (UCP) 1.

[0010] According to the aforementioned aspect of the present invention, a use of a mycelium of *Phellinus linteus* GKPI in the manufacture of a medicament for treating obesity is provided, in which the medicament can include but not limited to an effective dose of a hot-water extract of the mycelium of the *Phellinus linteus* GKPI and/or an ethanol extract of the mycelium of the *Phellinus linteus*

GKPI as an active ingredient, and the accession number of the *Phellinus linteus* GKPI is NITE BP-03321.

[0011] In one embodiment of the present invention, the effective dose of the hot-water extract and/or the ethanol extract can be 150 µg/mL to 250 µg/mL *in vitro*, for example. In one embodiment of the present invention, the *Phellinus linteus* GKPI treats the obesity by increasing an expression level of PGC-1α. In one embodiment of the present invention, the *Phellinus linteus* GKPI treats the obesity by increasing an expression level of FNDC5 and UCP1.

[0012] According to the aforementioned aspect of the present invention, a use of a mycelium of *Phellinus linteus* GKPI in the manufacture of a medicament for treating obesity-related metabolic disorders is provided, in which the medicament can include but not limited to an effective dose of an extract of the mycelium of the *Phellinus linteus* GKPI as an active ingredient. The effective dose of the extract can be 150 µg/mL to 250 µg/mL *in vitro*, for example, and the accession number of the *Phellinus linteus* GKPI is NITE BP-03321. In one embodiment of the present invention, the extract can include but not limited to a hot-water extract and/or an ethanol extract of the mycelium of the *Phellinus linteus* GKPI.

[0013] By applying the mycelium of *Phellinus linteus* GKPI in the manufacture of a medicament for treating obesity and obesity-related metabolic disorders of the present invention, the *Phellinus linteus* GKPI can increasing an expression level of PGC-1α and its downstream gene (e.g., FNDC5 and UCP1) of a preadipocyte *in vitro*, thereby increasing an amount of the brown adipocytes, and thus can potentially treat obesity and obesity-related metabolic disorders.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] The invention can be more fully understood by reading the followed detailed description of the embodiment, with reference made to the accompanying drawings as follows:

[0015] FIGs. 1A to 1C were microscopic photos of the cells of the blank group, the control group and the experiment group in accordance with one embodiment of the present invention, respectively.

[0016] Fig. 2 was a bar chart illustrating of the relative gene expression levels of PGC-1 α of the cells in different groups in accordance with one embodiment of the present invention.

[0017] FIGs. 3A to 3F were the visible light and blue light microscopic images of the control group, the visible light and blue light microscopic images of the first experiment group as well as the visible light and blue light microscopic images of the second experiment group, in accordance with one embodiment of the present invention, respectively.

[0018] FIG. 4 was a bar chart illustrating the relative gene expression levels of UCP1 in different groups in accordance with one embodiment of the present invention.

DETAILED DESCRIPTION

[0019] As mentioned above, the present invention provides a use of a mycelium of *Phellinus linteus* GKPI in the manufacture of a medicament for treating

obesity and obesity-related metabolic disorders, in which the medicament can include but not limited to an effective dose of the extract of the mycelium of the *Phellinus linteus* GKPI as an active ingredient.

[0020] In one embodiment, *Phellinus linteus* GKPI (also referred to as strain GKPI) is deposited in NITE Patent Microorganisms Depositary of the National Institute of Technology and Evaluation (NITE) on November, 12, 2020 with the accession number of NITE BP-03321. The deposit was made under the terms of the Budapest Treaty.

[0021] In one embodiment, the extract of the mycelium of the *Phellinus linteus* GKPI is obtained by subjecting the mycelium of the *Phellinus linteus* GKPI to a multistep incubation treatment and an extract treatment. The aforementioned multistep incubation treatment can be performed with a known culture method. In one specific embodiment, the multistep incubation treatment can selectively include a solid-state incubation step, a liquid-state incubation step and a fermentation step. In details, the solid-state incubation step can be performed by incubating the mycelium of the *Phellinus linteus* GKPI with a solid-state medium under 15°C to 30°C for 7 days to 14 days, for example to obtain a first culture. The aforementioned solid-state medium can be a known solid-state medium, for example. In one specific embodiment, the solid-state medium is a potato dextrose agar (PDA) containing carbon sources and nitrogen sources.

[0022] A liquid-state incubation step can be performed by incubating the first culture with a liquid-state medium under a condition of 15°C to 30°C, pH 2 to pH 6 and a rotation speed of 110 rpm to 130 rpm for 3 days to 21 days to obtain a second culture, for example. The ingredient of the liquid-state medium is not

limited and can be adjusted depending on the requirement. In one embodiment, based on that the liquid-state medium is 100 weight%, the liquid-state medium can include but not limited to 1.00 weight% to 3.00 weight% mixed carbon-nitrogen resources, 1.00 weight% to 4.00 weight% sugar, 0.10 weight% to 1.00 weight% yeast extract, 0.10 weight% to 1.00 weight% peptone, 0.01 weight% to 0.50 weight% inorganic salt and a balance water. The mixed carbon-nitrogen resources can be grains (e.g., flour) and/or beans (e.g., soybean powder, green bean powder and/or cinnamon powder), for example. The sugar can be glucose, fructose, maltose and/or sucrose, for example. The inorganic salt can be magnesium sulfate, dipotassium hydrogen phosphate, potassium dihydrogen phosphate and/or iron sulfate, for example.

[0023] The fermentation step can incubate the second culture with a fermentation medium under 15°C to 30°C, pH 2 to pH 6 and a rotation speed of 110 rpm to 130 rpm for 3 days to 21 days to obtain a fermented product of the mycelium of the *Phellinus linteus* GKPI (referred to as GKPI fermented product), for example. In one embodiment, a fermentation step can be performed in a fermenter. When a fermentation step is performed, gases are introduced into the fermenter, in which the gases can include but not limited to air, oxygen, carbon dioxide and/or helium, so that the gas pressure in the fermenter can be 0.5 kg/cm² to 1.0 kg/cm², and the ventilation rate of the introduced gases can be 0.1 volume of introduced gases/ volume of the fermentation medium/ minute (vvm) to 1.5 vvm. The ingredients of the fermentation medium is not specially limited and can be the aforementioned liquid-state medium, for example.

[0024] In one embodiment, after the fermentation step, the GKPI fermented product can be selectively subjected to a drying treatment and a grinding treatment to obtain a GKPI desiccate. The drying treatment can be performed by a known drying method, such as a lyophilization method, a vacuum drying method or a spray drying method. The grinding treatment can be performed by a known grinding method, such as mechanical grinding method, a roll mill grinding method or an air grinding method. In one embodiment, the volume-weight ratio of the GKPI fermented product to the GKPI desiccate is 100:3.

[0025] The aforementioned extraction treatment can be performed by known extraction method to obtain the extract of the mycelium of the *Phellinus linteus* GKPI (referred to as GKPI extract herein). In one embodiment, the extraction treatment can extract the GKPI fermented product and/or the GKPI desiccate with 100°C hot water for 30 minutes to obtain a hot-water extract of the mycelium of the *Phellinus linteus* GKPI, for example. In one example, a volume ratio value of the GKPI fermented product and/or the GKPI desiccate to the hot water can be 1/50 to 1/1, 1/30 to 1/10 or 1/20. In one embodiment, the extraction treatment can extract GKPI fermented product and/or the GKPI desiccate with ethanol and optionally with sonication for 60 minutes to obtain an ethanol extract of the mycelium of the *Phellinus linteus* GKPI, for example. In one example, a weight ratio value of the GKPI fermented product and/or the GKPI desiccate to the ethanol can be 1/50 to 1/1, 1/30 to 1/10 or 1/20.

[0026] In one embodiment, the GKPI extract can be selectively subjected to the aforementioned drying treatment and the grinding treatment. In one

embodiment, the GKPI extract can be selectively subjected to a solid-liquid separation treatment and a concentration treatment. The solid-liquid separation treatment can be a concentration step and/or a filtration step, for example. The concentration treatment can be performed by a known concentration method, such as a reduced pressure concentration method, an evaporation concentration method or a membrane concentration method.

[0027] In vitro cell experiments have proven that the GKPI extract can promote the differentiation of the brown adipocytes from the preadipocytes. In details, the preadipocyte can be differentiated into cells with smaller and more lipid droplets therein, an increased gene expression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC)-1 α , an increased promoter activity of fibronectin type III domain-containing protein (FNDC)5 and an increased gene expression of uncoupling protein (UCP)1 after administered with the extract of the mycelium of the *Phellinus linteus* GKPI when compared the preadipocyte not administered with the extract of the mycelium of the *Phellinus linteus* GKPI.

[0028] The aforementioned “preadipocytes” are precursors of adipocytes. In one embodiment, the preadipocytes have no limitations and can be fibroblasts and/or mesenchymal stem cell, for example. The aforementioned adipocytes can include but not limited to white adipocytes and brown adipocytes, in which to increase the amounts of the brown adipocytes indicates to treat obesity. In details, the aforementioned “brown adipocytes” generally indicates the adipocytes with brown adipocyte-like phenotype, in which one of the characteristics of the brown adipocyte-like phenotype can contain numerous

small lipid droplets and more mitochondria for digesting the fat stored in the lipid droplets more efficiently, thereby treating obesity. On the other hand, the white adipocytes contains fewer mitochondria and a single large lipid droplet (i.e., unilocular) to store excess energy in the form of fat. Generally, newborn infants have larger brown adipocyte depots therein, which become smaller after the infancy. However, recent studies have shown that the amounts of brown adipocytes can increase in a human body under specific conditions such as exercising and cold environment. As abovementioned, the GKPI extract has been proven to promote the differentiation of the preadipocytes into the brown adipocytes, and therefore can potentially treat obesity.

[0029] Besides more amounts of brown adipocytes for treating obesity, the mitochondria of the brown adipocytes also features the high expression of UCP1. UCP1 can dissipate the energy stored in the mitochondrial electrochemical gradient, thereby increasing the resting cellular metabolic rate. The expression level of UCP1 is regulated by PGC-1 α and the downstream genes thereof. In details, PGC-1 α is a transcriptional cofactor that can regulate the expression of FNDC5. FNDC5 is the precursor of irisin, which can increase the expression level of UCP1. Thus, the brown adipocyte-like phenotype can be evaluated by the expression levels of PGC-1 α and the downstream genes thereof, in which the downstream genes of PGC-1 α can include but not limited to FNDC5, irisin and UCP1.

[0030] In one embodiment, the expression level can be a gene expression level, a protein expression level and/or a promoter activity, in which the promoter activity can be evaluated by the expression level of the reporter gene operably

linked to the promoter (i.e., at downstream of the promoter). In one embodiment, the reporter gene can be green fluorescent protein (GFP), β -galactosidase and/or luciferase, for example. In one embodiment, the promoter activity can be evaluated by the fluorescence intensity produced by GFP, in which the fluorescence is produced by the GFP after exposed under the light in the range from blue light to ultra-violet light.

[0031] The aforementioned “obesity” can be evaluated by a body mass index (BMI). According to the definition provided by the World Health Organization (WHO), overweight is a BMI greater than or equal to 25, and obesity is a BMI greater than or equal to 30 for an adult. Obesity can increase risks of developing chronic systemic inflammation and insulin resistance, thereby causing obesity-related metabolic disorders. The aforementioned “obesity-related metabolic disorders” can include but not limited to the metabolic syndrome caused by obesity and the diseases caused by the metabolic syndrome. The aforementioned “metabolic syndrome” is a name for a group of risk factors, in which the risk factors includes at least three of abdominal obesity, high blood pressure, high blood sugar, high serum triglycerides, and low serum high-density lipoprotein (HDL), and the disease caused by these risk factors (i.e., the diseases caused by the metabolic syndrome) can include but not limited to cardiovascular disease (e.g., heart disease and/or stroke) and type 2 diabetes. Moreover, these risk factors may foster the development of the diseases such as chronic kidney disease, fatty liver disease, chronic hepatitis, liver cirrhosis and liver cancer.

[0032] The aforementioned “treating obesity” is to reduce the amount of fat accumulated in the body, in which the specific methods to treat obesity can include but not limited to reduce calorie intake and/or increase calorie expenditure. In one embodiment, the aim to treat obesity can be reached by changing how the body use fat, for example, increase the amounts of the brown adipocytes. In one embodiment, obesity can be treated by administering the GKPI extract.

[0033] In one embodiment, the *in vitro* effective dose of the GKPI extract can be 150 µg/mL to 250 µg/mL, for example, to promote the differentiation of the brown adipocytes from preadipocytes. In one embodiment, the extract of the mycelium of the *Phellinus linteus* GKPI can include but not limited to the hot-water extract and/or the ethanol extract. In one embodiment, the weight ratio value of the hot-water extract to the ethanol extract can be 0.1 to 10, 0.5 to 5, or 1, for example.

[0034] 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 followed claims.

EXAMPLE 1. Strain source.

[0035] *Phellinus linteus* GKPI was separated from a fruit body of a wild *Phellinus linteus* found in China, and was deposited in Patent Microorganisms Depositary of the National Institute of Technology and Evaluation (NITE) on November, 12th, 2020 with the accession number of NITE BP-03321, in which the address of the

depository institution was #122, 2-5-8 Kazusakamatari, Kisarazu-shi, Chiba 292-0818, Japan. Noted that although the scientific name *Phellinus linteus* had been commonly used, it was argued that the scientific name should be replaced into *Sanghuangporus Sanghuang* in molecular systematics studies. Please refer to the Taiwan Patent No. TW I729928 B, Taiwan Patent Application No. TW 109136177 and Japan Patent Application No. JP 2021-84633 for the microorganism traits and the culturing method of *Phellinus linteus* GKPI. The patent was cited as a reference herein.

EXAMPLE 2. Preparation of the extract of *Phellinus linteus* mycelium.

[0036] The preparation method of the exact of the mycelium of the *Phellinus linteus* GKPI (referred to as GKPI extract herein) was briefly described as follows: the mycelium of the *Phellinus linteus* GKPI was inoculated on PDA and was incubated under 25°C for 7 days to obtain a first culture. Then, the first culture of the *Phellinus linteus* GKPI was incubated in a liquid medium under the condition of 25°C, pH 4.5 and rotation speed of 120 rpm for 14 days to obtain a second culture. The aforementioned liquid medium included 1 weight% mixed carbon-nitrogen resources, 1.5 weight% sugar, 0.3 weight% yeast extract, 0.3 weight% peptone and 0.1 weight% inorganic salts. The specific ingredients of the mixed carbon-nitrogen resources, sugars and inorganic salts were well-known to a person having ordinary skill in the art and could be arbitrarily adjusted according to the specific need without affecting the fermentation step, and thus would not be elaborated herein.

[0037] Next, the second culture was incubated in the aforementioned liquid medium under a condition of 25°C, pH 6, a rotation speed of 80 rpm and a

ventilation rate of 1.0 vvm for 14 days to obtain the fermented product of the mycelium of the *Phellinus linteus* GKPI. Then, the fermented product of the mycelium of the *Phellinus linteus* GKPI was subjected to a lyophilization to obtain a lyophilized powder of the mycelium of *Phellinus linteus* the GKPI (referred to as GKPI lyophilized powder).

[0038] Then, a hot-water extract and an ethanol extract of the mycelium of *Phellinus linteus* GKPI (referred to as GKPI hot-water extract and GKPI ethanol extract) were respectively prepared. The GKPI hot-water extract was obtained by adding the GKPI lyophilized powder into distilled water to perform a hot-water extract at 100°C for 30 minutes, followed by a lyophilization, in which the volume ratio value of the GKPI lyophilized powder to the distilled water was 1/20. The GKPI ethanol extract was obtained by adding the GKPI lyophilized powder into ethanol and sonicating with ultrasonic waves of 600W, 40 kHz at 25°C for 60 min, followed by a centrifuge to obtain a supernatant and a reduced pressure concentration performed on the supernatant. The weight ratio value of the GKPI lyophilized powder to the ethanol was 1/20. The GKPI hot-water extract and GKPI ethanol extract were dissolved into dimethyl sulfoxide (DMSO) to facilitate the subsequent evaluation.

EXAMPLE 3. Evaluation of the effect of the GKPI extract on increasing the amount of the brown adipocytes.

1. The culture and differentiation of the preadipocytes

[0039] 3T3-L1 cells [the one deposited at American Type Culture Collection (ATCC) with the accession number ATCC CL-17] was derived from the mouse embryo cells NIH 3T3, in which the cell type of the 3T3-L1 cells were fibroblasts

that were able to differentiate into white adipocytes and/or brown adipocytes. Thus, the 3T3-L1 cells could be preadipocytes. The 3T3-L1 cells were seeded into a 6-well cell culture plate, so that the cell density in each well was 2×10^5 cells/mL. The 3T3-L1 cells were cultured at 37°C, 5% CO₂ for 2 days in a cell growth medium [Dulbecco's Modified Eagle Medium (DMEM) including 10% fetal bovine serum and a high concentration of glucose]. The 3T3-L1 cells were categorized into a blank group, a control group and an experiment group when the 3T3-L1 cells reached 70% confluency.

[0040] The 3T3-L1 cells in the blank group were subjected to a subculture treatment, the 3T3-L1 cells in the control group were subjected to a differentiation treatment, and the 3T3-L1 cells in the experiment group were subjected to a GKPI extract treatment. The subculture treatment was to culture the 3T3-L1 cells with the cell growth medium for 8 days. The differentiation treatment was to culture the 3T3-L1 cells for 8 days with a differentiate medium [DMEM containing 10% fetal bovine serum, 0.5 mM isobutyl-1-methylxanthine (IBMX), 1 μ M dexamethasone (DEX), 10 μ g/mL insulin and a high concentration of glucose] containing 0.1% DMSO, which was replaced by fresh differentiate medium containing DMSO every 2 days. The GKPI extract treatment was to culture the 3T3-L1 cells for 8 days with the differentiate medium containing the 200 μ g/mL mixed GKPI extracts (consisting of 100 μ g/mL GKPI hot-water extract and 100 μ g/mL GKPI ethanol extract), which was replaced by fresh differentiate medium containing the mixed GKPI extracts every 2 days. Noted that the total concentration of the DMSO was smaller or equal to 0.1% to prevent the growth of the 3T3-L1 cells from affected by the toxicity of the DMSO.

2. Evaluation of the distribution of the lipid droplet in cells in different groups with Oil-red O stain.

[0041] The Oil-red O dye was used to stain the lipid droplets into red to observe the distribution of the lipid droplet in the cells in different groups. In details, the cells in different groups were washed twice by 1X phosphate buffer saline (PBS) and fixed with 4% formaldehyde for 1 hour. After the excess liquid was removed, the cells in different groups were covered with Oil-red O dye for 1 hour and successively washed by ddH₂O and 40% ethanol solutions once. After the excess liquid was removed, the cells in different groups were covered by adequate water to observe and take photos by an optical microscope.

[0042] FIGs. 1A to 1C were microscopic images of cells in the blank group (FIG. 1A), the control group (FIG. 1B) and the experiment group (FIG. 1C) in accordance with one embodiment of the present invention, respectively. As shown in FIG. 1A, the cells in the blank group had no lipid droplet, indicating that the cells in the blank group were undifferentiated 3T3-L1 cells. As shown in FIG. 1B, the cells in the control group had large lipid droplets, indicating that the 3T3-L1 cells were able to differentiate into white adipocytes after subjected to the differentiation treatment. As shown in FIG. 1C, the cells in the experiment group had small and dispersed lipid droplets, proving that the 3T3-L1 cells were able to differentiate into brown adipocytes after subjected to GKPI extract treatment.

3. Relative gene expression levels of PGC-1 α of cells in different groups by qPCR.

[0043] The commercially available RNA purification kit (GeneJET RNA Purification Kit, Thermo Fisher Scientific, USA) was used to purify mRNA of cells in different groups, and then the commercially available RNA reverse transcription kit (RevertAid H Minus First Strand cDNA Synthesis Kit, Thermo Fisher Scientific, USA) was used to reverse transcribe the mRNA into cDNA. Then, a PGC-1 α upstream primer with a nucleotide sequence shown as SED ID NO: 1 and a PGC-1 α downstream primer with a nucleotide sequence shown as SED ID NO: 2 were used to perform a real-time quantitative polymerase chain reaction (qPCR) on the cDNA, and $2^{-\Delta\Delta C_t}$ method was used to calculate the relative gene expression levels of PGC-1 α of the cells in different groups with the relative gene expression level PGC-1 α of the cells in the control group as 1.0. The experiment was performed in triplicate. A paired t test was used to analyze the experimental results, and the results were recorded in Table 1 and FIG. 2, in which the results in Table 1 were showed in mean $\pm$ standard error of the mean (SEM).

[0044] FIG. 2 was a bar chart illustrating the relative gene expression levels of PGC-1 α of the cells in different groups according to Table 1, in which the horizontal axis represented groups, and the vertical axis represented relative gene expression levels of PGC-1 α . The symbol "***" represented a statistically significant difference ($p < 0.01$) compared to the control group in Table 1 and FIG. 2.

[0045] Table 1

Cell	relative gene expression levels of PGC-1 α
Blank group	1.000 $\pm$ 0.013
Control group	1.042 $\pm$ 0.007
Experiment group	1.409 $\pm$ 0.021**

[0046] As shown in Table 1 and FIG. 2, there was no statistically significant difference between the relative gene expression levels of PGC-1 α of the cells in the control group and experiment group, indicating there was no statistically significant difference between the relative gene expression levels of PGC-1 α of the 3T3-L1 cells not subjected to a differentiation treatment and the 3T3-L1 cells subjected to a differentiation treatment. However, the relative gene expression levels of PGC-1 α of the cells in the experiment group was significantly higher than that of the control group, proving that the GKPI extract was able to promote the differentiation of 3T3-L1 cells into the brown adipocytes and even increase the amount of the brown adipocytes.

4. Evaluation of the promoter activities of FNDC5 of the cells in different groups by the fluorescence intensity produced by the transfected cells.

[0047] The plasmid containing mouse promoter of FNDC5 and GFP gene were transfected into human embryonic kidney (HEK) 293 cells (accession number: ATCC CRL-1573) to obtain transfected cells, in which the GFP gene was operably linked to the promoter of FNDC5. When the activity of the promoter of FNDC5 increased, the expression level of GFP increased, allowing the transfected cells to product a higher intensity of green fluorescence under blue light. The transfected cells were seeded into a 96-well cell culture plate, so that the cell density in each well was 1×10^4 cells/mL to 5×10^4 cells/mL. The transfected cells were cultured at 37°C, 5% CO₂ for 3 days in a cell culture medium (DMEM including 10% fetal bovine serum) to reach 90% confluency.

The mouse promoter of FNDC5 and GFP gene were well-known to a person having ordinary skill in the art and thus would not be elaborated herein.

[0048] Next, the transfected cells were grouped into a control group, a first experiment group and a second experiment group, and the transfected cells were cultured at 37°C, 5% CO₂ for 24 hours, in which the transfected cells in the control group were cultured in the cell culture medium, the transfected cells in the first experiment group were cultured in the cell culture medium containing 200 µg/mL GKPI hot-water extract, and the transfected cells in the second experiment group were cultured in the cell culture medium containing 200 µg/mL GKPI ethanol extract. Noted that the GKPI hot-water extract and GKPI ethanol extract were dissolved in DMSO and then added into the cell culture medium with adequate volumes to reach the abovementioned concentration, and DMSO of the same volume was added into the cell culture medium of the control group.

[0049] Then, the microscopic images of the transfected cells were observed and recorded under visible light and blue light (excitation wavelength of 488 nm) with a fluorescence microscope. Referred to FIGs. 3A to 3F, in which FIGs. 3A to 3F were the visible light (FIG. 3A) and blue light microscopic images (FIG. 3B) of the control group, the visible light (FIG. 3C) and blue light microscopic images (FIG. 3D) of the first experiment group as well as the visible light (FIG. 3E) and blue light microscopic images (FIG. 3F) of the second experiment group, respectively, in accordance with one embodiment of the present invention. As shown in FIG. 3B, the transfected cells in the control group hardly produce green fluorescence under blue light. As shown in FIGs. 3D and

3F, the transfected cells in the first experiment group and the second experiment group produce green fluorescence under blue light at high intensities, proving that the transfected cells in the first experiment group and the second experiment group had better promoter activities of FNDC5.

[0050] The transfected cells in the first experiment group and the second experiment group were lysed by the lysis buffer to obtain cell lysates. Then, the cell lysates were centrifuged at a rotation speed of 6000×g at 4°C to obtain supernatants. Next, the supernatants were collected in a 96-well cell culture plate, and a fluorescence spectrophotometer was used to expose the supernatants with blue light (a wavelength of 488 nm) to detect the intensity of the green fluorescence (a wavelength of 520 nm) produced thereby. The results were recorded in Table 2, in which the symbol “*” represented a statistically significant difference with the control group tested by a paired *t* test.

[0051] Table 2

Supernatant	Relative intensity of fluorescence
Control group	1.00±0.10
First experiment group	1.31±0.16*
Second experiment group	2.96±0.45*

[0052] As shown in Table 2, the relative intensities of fluorescence of the supernatants of the first experiment group and the second experiment group were significantly higher than that of the control group, proving that the GKPI hot-water extract and/or the ethanol extract were able to increase the promoter activity of FNDC5.

5. Evaluation of the relative gene expression levels of UPC1 of the cells in different groups.

[0053] As mentioned above, the cDNA in the blank group was obtained from the 3T3-L1 cells subjected to the subculture treatment, the cDNA in the control group was obtained from the 3T3-L1 cells subjected to the differentiation treatment, and the cDNA in the experiment group was obtained from the 3T3-L1 cells subjected to the GKPI extract treatment. A UCP1 upstream primer with a nucleotide sequence shown as SED ID NO: 3 and a UCP1 downstream primer with a nucleotide sequence shown as SED ID NO: 4 were used to perform a qPCR on the cDNA, and $2^{-\Delta\Delta C_t}$ method was used to calculate the relative gene expression levels of UCP1 of the cells in different groups with the gene expression level PGC-1 α of the cells in the control group as 1.0. The experiment was performed in triplicate. The paired *t* test was used to analyze the experimental results, and the analysis results were recorded in Table 3 and FIG. 4, in which the results were showed in mean $\pm$ SEM.

[0054] FIG. 4 was a bar chart illustrating the relative gene expression levels of UCP1 of the cells in different groups according to Table 3, in which the horizontal axis represented groups, and the vertical axis represented relative gene expression levels of UCP1. The symbol “#” represented a statistically significant difference ($p < 0.05$) compared to the blank group, and the symbol “*” represented a statistically significant difference ($p < 0.05$) compared to the control group of Table 3 and FIG. 4.

[0055] Table 3

Cell	relative gene expression levels of UCP1
Blank group	0.393 $\pm$ 0.144
Control group	5.257 $\pm$ 0.305#
Experiment group	7.187 $\pm$ 0.311*

[0056] As shown in Table 3 and FIG. 4, the relative gene expression levels of UCP1 of the cells in the experiment group was significantly higher than that of the control group, proving that the GKPI extract was able to promote the differentiation of 3T3-L1 cells into the brown adipocytes and even increase the amount of the brown adipocytes.

[0057] In conclusion, the GKPI extract was able to increase the expression level of PGC-1 α , FND C5 and UCP1, proving that the GKPI extract was able to promote the differentiation of the brown adipocytes and even increase the amount of the brown adipocytes, indicating that the GKPI extract had the potential to be the active ingredient of the medicament for treating obesity and obesity-related metabolic disorders since the brown adipocytes were able to burn fat.

[0058] In sum, the use of a mycelium of *Phellinus linteus* GKPI in the manufacture of a medicament for treating obesity and obesity-related metabolic disorders is illustrated by using specific culturing method, the specific process, the specific administration method, the specific experimental model and the specific evaluation method as examples in the present invention, any one of ordinary skills in the art of the present invention can realize that the present invention was not limited thereto, and the present invention can also be implemented by using other culturing methods, other processes, other administration methods, other experimental models or other evaluation methods without departing from the spirit and scope of the present invention.

[0059] Although the present invention has been described in considerable detail with reference to certain embodiments thereof, other embodiments are possible.

Therefore, the spirit and scope of the appended claims should not be limited to the description of the embodiments contained herein.

WHAT IS CLAIMED IS:

1. Use of a mycelium of *Phellinus linteus* GKPI in the manufacture of a medicament for treating obesity, wherein the medicament comprises an effective dose of an extract of the mycelium of the *Phellinus linteus* GKPI as an active ingredient, and the *Phellinus linteus* GKPI is deposited in NITE Patent Microorganisms Depositary of the National Institute of Technology and Evaluation (NITE) on November 12, 2020 with the accession number of NITE BP-03321, and the extract of the mycelium of the *Phellinus linteus* GKPI treats obesity by increasing expression levels of peroxisome proliferator-activated receptor gamma coactivator (PGC)-1 α , fibronectin type III domain-containing protein (FNDC) 5 and uncoupling protein (UCP) 1.

2. The use according to claim 1, wherein the extract comprises a hot-water extract of the mycelium of the *Phellinus linteus* GKPI and/or an ethanol extract of the mycelium of the *Phellinus linteus* GKPI.

3. The use according to claim 1, wherein the mycelium of the *Phellinus linteus* GKPI is obtained by subjecting the *Phellinus linteus* GKPI to a multistep incubation treatment comprising a solid-state incubation step, a liquid-state incubation step and a fermentation step.

4. The use according to claim 3, wherein the solid-state incubation step is performed by incubating the *Phellinus linteus* GKPI with a solid-state medium under 15°C to 30°C for 7 days to 14 days, so as to obtain a first culture.

5. The use according to claim 4, wherein the liquid-state incubation step is performed by incubating the first culture with a liquid-state medium under 15°C to 30°C, pH 2 to pH 6 and a rotation speed of 110 rpm to 130 rpm for 3 days to 21 days, so as to obtain a second culture.

6. The use according to claim 5, wherein the fermentation step is performed by incubating the second culture with a fermentation medium under 15°C to 30°C, pH 2 to pH 6 and a rotation speed of 110 rpm to 130 rpm for 3 days to 21 days, so as to obtain a fermented product of the mycelium of the *Phellinus linteus* GKPI.

7. Use of a mycelium of *Phellinus linteus* GKPI in the manufacture of a medicament for treating obesity, wherein the medicament comprises an effective dose of an hot-water extract of the mycelium of the *Phellinus linteus*

GKPI and/or an ethanol extract of the mycelium of the *Phellinus linteus* GKPI as an active ingredient, the accession number of the *Phellinus linteus* GKPI is NITE BP-03321, and the mycelium of the *Phellinus linteus* GKPI treats obesity by increasing expression levels of PGC-1 α , FNDC 5 and UCP 1.

8. The use according to claim 7, wherein the hot-water extract of the mycelium of the *Phellinus linteus* GKPI is obtained by subjecting the mycelium of the *Phellinus linteus* GKPI to a hot-water extract, and a volume ratio value of the mycelium of the *Phellinus linteus* GKPI to hot water of 100°C is 1/50 to 1/1.

9. The use according to claim 7, wherein the ethanol extract of the mycelium of the *Phellinus linteus* GKPI is obtained by subjecting the mycelium of the *Phellinus linteus* GKPI to an ethanol extract, wherein a weight ratio value of the mycelium of the *Phellinus linteus* GKPI to ethanol is 1/50 to 1/1.

10. The use according to claim 9, wherein the ethanol extract is performed with sonication.