Study on Mechanism of *Dendrobium officinale* Kimura et Migo in Preventing and Treating Exercise-induced Muscle Damage (EIMD) in Rats

Peihong CAI, Jiangyan XIONG*, Zelin ZHAO, Sibu MA, Yuanyu LONG, Danguo WEN, Litao XIA, Qinmei ZHU

Guizhou University of Traditional Chinese Medicine, Guiyang 550025, China

Abstract [**Objectives**] This study was conducted to observe the mechanism of *Dendrobium officinale* Kimura et Migo on gastrocnemius muscle in rats with exercise-induced muscle damage (EIMD). [**Methods**] The micro-injury model of skeletal muscle was established by treadmill training. Forty two SD rats were randomly divided into a control group, 1, 12 and 24 h exercise groups, *D. officinale* 2 ml + 1 h exercise group, *D. officinale* 2 ml + 12 h exercise group, and *D. officinale* 2 ml + 24 h exercise group, with 6 rats in each group. Various *D. officinale* groups were given the drug once in the morning and once in the evening at a dose of 2 ml/time, a week in advance. Except for the quiet group, the samples were collected from the 1, 12 and 24 h exercise groups after anesthesia following 1, 12 and 24 h of exercise for the last time, respectively, and the *D. officinale* 2 ml + 1 h exercise group, *D. officinale* 2 ml + 12 h exercise group and *D. officinale* 2 ml + 24 h exercise group were also sampled after anesthesia following 1, 12 and 24 h of exercise for the last time, respectively. The contents of ATP, CK-MM and CK in rat serum were determined by enzyme-linked immunosorbent assay (ELISA). The histopathological changes of gastrocnemius muscle were observed by HE staining. PCR and Western-blot detection were carried out to analyze the effects of *D. officinale* on IGF-1 mRNA and protein expression in gastrocnemius muscle. [**Results**] Compared with the quiet group, the ATP contents in the serum of rats in the 1, 12 and 24 h exercise groups significantly decreased ($P<0.01$), while the CK and CK-MM contents significantly increased ($P<0.01$). The expression of IGF-1 mRNA and protein in the gastrocnemius muscle tissue significantly increased ($P<0.01$). Compared with the 1 h exercise group, the ATP content and IGF-1 protein expression in the gastrocnemius muscle tissue of the *D. officinale* liquid + 1 h exercise group significantly increased ($P<0.05$), while the CK and CK-MM contents significantly decreased ($P<0.01$). Compared with the 12 h exercise group, the *D. officinale* liquid + 12 h exercise group showed a significant increase in ATP content ($P<0.01$), significant increases in IGF-1 mRNA and protein expression in the gastrocnemius muscle tissue ($P<0.01$), and significant decreases in CK and CK-MM contents ($P<0.01$). Compared with the 24 h exercise group, the ATP content and IGF-1 mRNA and protein expression in the gastrocnemius muscle tissue of the *D. officinale* liquid + 24 h exercise group significantly increased ($P<0.01$), while the CK and CK-MM contents significantly decreased ($P<0.01$). From the pathological tissue morphology of the gastrocnemius muscle in rats with EIMD treated with *D. officinale*, it could be concluded that the gastrocnemius muscle of each exercise group was significantly damaged, and the damage was significantly alleviated after administration of *D. officinale* liquid. [**Conclusions**] The effects and mechanism of *D. officinale* on prevention and treatment of EIMD in rats might be related to the promotion of IGF-1 mRNA and protein expression in injured tissues by reducing ATP energy consumption, CK-MM and CK activity.

Key words *Dendrobium officinale* Kimura et Migo; Exercise-induced muscle damage; Gastrocnemius muscle; IGF-1 mRNA

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Exercise-induced muscle damage (EIMD) is a type of exercise that results in changes in the ultrastructure of skeletal muscle membranes, Z lines and other structures caused by high-intensity, high centrifugal exercise, *etc.*[1], leading to a decrease in endurance exercise and degree of joint activity[2-3]. High intensity or unfamiliar centrifugal exercise is the most common form of exercise leading to EIMD. Its main characteristic is delayed muscle soreness, which usually occurs 8 - 24 h after exercise and then reaches its peak at 24 - 48 h, lasting approximately 1 week. Meanwhile, it is also the most common phenomenon in all sports[4]. Studies have shown that[5-6], *Dendrobium officinale* Kimura et Migo has the remarkable characteristics of multiple targets and multiple pathways in the prevention and treatment of exercise-induced skeletal muscle micro-injury, and exhibits remarkable curative effect. *D. officinale* is a perennial herb of *Dendrobium* in Orchidaceae[7], which is a kind of traditional nourishing Chinese medicine in China. It is sweet in taste and slightly cold in nature, and attributive to the stomach and kidney meridians. It is good at nourishing yin and promoting body fluid production, and is used for yin injury, body fluid deficiency, stomach yin deficiency, and muscle weakness. Modern pharmacological research shows that *D. officinale* has the functions of enhancing immunity, resisting fatigue injury and protecting liver[8]. However, the mechanism of EIMD is not clear. Therefore, in this study, the relation between EIMD and IGF-1, CK, CK-MM and ATP was discussed from the overall level, aiming to find out the role and mechanism of *D. officinale*'s intervene in EIMD by using modern molecular biology means.

It has been pointed out that insulin-like growth factor 1 (IGF-1) is a kind of polypeptide, which can promote both anabolism and growth. During the repair of skeletal muscle injury, IGF-1 appears at a high concentration in the body and plays an important role in the repair process of skeletal muscle injury. Meanwhile, muscle satellite cells, myoblasts and others can

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Peihong CAI (1998 -), male, P. R. China, graduate student.

* Corresponding author. Jiangyan XIONG (1982 -), female, P. R. China, associate professor, master, devoted to traditional Chinese medicine nursing and nursing education.

secrete and express IGF-1 in injured skeletal muscle. During vigorous exercise, ATP is the only energy supply substance that muscles can directly utilize, and creatine kinase (CK) also assists in ATP hydrolysis, participating in glycolysis control, muscle contraction energy supply, *etc.* Its isoenzyme CK-MM combines with myofibril M-line structure through lysine residue pairs, and exists in the space of sarcomere I band to combine with various ATP pumps on sarcoplasmic reticulum and regulate various ATP-dependent functions, supporting muscle energy demand. In this study, *D. officinale* was used as the corresponding intervention drug to conduct in-depth research on EIMD, hoping to provide new potential targets and experimental basis for the prevention and treatment of EIMD by *D. officinale*.

Materials and Methods

Materials

Animals Clean SD rats, male, weighing 180 – 220 g each, were purchased under license number: Scxk (Xiang) 2019-0014 from Changsha Tianqin Biotechnology Co., Ltd.

Instruments DHG-9240A electrothermal constant-temperature blast drying oven (Shanghai Yuejin Medical Instrument Factory), rat and mouse rotating rod fatigue tester (XR1514), HB-RO/60 ultrapure water instrument (Millipore, USA), FINESSE E + paraffin microtome and real-time fluorescent quantitative PCR instrument were all purchased from Thermo Company of the United States. Universal Hood II nucleic acid protein gel imager and CCD, T100PCR instrument, Mini Trans Plot® Cell protein wet transfer instrument and Mini Sub Cell GT protein electrophoresis instrument were all purchased from Bio-Rad Company in the United States.

Drugs and reagents The crude drug *D. officinale* was purchased from Guizhou Xingqian Technology Development Co., Ltd., and it was identified by professor Sun Qingwen from Guizhou University of Traditional Chinese Medicine as fresh stems of *D. officinale* Kimura et Migo, an Orchidaceae plant. ATP (enzymatic method) ELISA kit (batch number: RXWB0028), Rat CK-MM ELISA kit (batch number: RX302629R) and Rat CK ELISA kit were all purchased from Ruixin Biotechnology Co., Ltd. Eosin stain (batch number: G1002), hematoxylin stain (batch number: G1004) and PBS (batch number: G0002) were all purchased from Servicebio Technology Co., Ltd. RNAiso Plus (batch number: 9108) was purchased from Takara, Japan, and Golden Star RT6 cDNA Synthesis Kit Ver. 2 (batch number: TSK302M) and 2 × T5 Fast qPCR Mix (SYBR Green I) (article number: TSK302M) were all purchased from Beijing Tsingke Biotech Co., Ltd. ECL enhanced chemiluminescence detection kit (batch number: 34580) and 10% PAGE gel rapid preparation kit (batch number: PG112) were purchased from Epizyme Biotech, China, and IGF-1 antibody (batch number: A11985), β-actin primary antibody (batch number: AC026) and secondary antibody (batch number: AS014) were purchased from ABclonal, China.

Methods

Preparation of drug liquid Clean fresh *D. officinale* was cut into pieces, and a 180 g of sample was accurately weighed and poured into a juicer, and added with 80 ml distilled water to perform juicing. Filtration was performed, and 50 ml of distilled water was added to the filter residue to perform juicing again. After filtration, the two filtrates were combined, and centrifuged to obtain a supernatant. The supernatant was diluted to a *D. officinale* concentration of 0.375 g/ml and stored in a refrigerator at 4 ℃ for later use.

Grouping Forty-two male SD rats were fed adaptively for 3 d and randomly divided into 7 groups according to their body weight, with 6 rats in each group, namely: quiet group, 1 h exercise group, *D. officinale* liquid + 1 h exercise group, 12 h exercise group, *D. officinale* liquid + 12 h exercise group, 24 h exercise group and *D. officinale* liquid + 24 h exercise group. Except for the quiet group, all other groups were subjected to adaptive treadmill training for 2 d. Next, they were subjected to treadmill training for 15 min every day, and the running speed of the treadmill was 15 r/min.

Modeling and drug administration When rats were familiar with treadmill running, referring to the human sports injury model of Armstrong and Tian *et al.* [9-10], the establishment of an EIMD model was started formally. Except the quiet group, rats in other groups were given continuous treadmill running, in which the *D. officinale* liquid + 1 h exercise group, *D. officinale* liquid + 12 h exercise group and *D. officinale* liquid + 24 h exercise group were given 2 ml of *D. officinale* liquid, and the running speed was 15 ml/min. If a rat couldn't persist, the exercise would be continued after 2 min of rest. Each rat undergone a one-time exercise for 60 min/d, and was continuously trained on a treadmill for one week. Muscle stiffness and swelling indicated successful modeling. After 7 d of modeling, samples were taken from the quiet group; samples were taken after anesthesia following 1, 12 and 24 h of exercise in the 1, 12 and 24 h groups, respectively; and samples were taken after anesthesia following 1, 12 and 24 h of exercise in the *D. officinale* liquid + 1 h exercise group, *D. officinale* liquid + 12 h exercise group and *D. officinale* liquid + 24 h exercise group, respectively.

Sample collection and treatment After the end of the last administration, the animals in various groups were fasted for 12 h with free access to water and injected intraperitoneally with 10% chloral hydrate for anesthesia. Blood was taken from the abdominal aorta and centrifuged for 10 min at 3 000 r/min, and serum was taken and stored in a −80 ℃ refrigerator. After blood collection, various groups of rats were dissected immediately to obtain the gastrocnemius muscles, of which the gastrocnemius muscle of the right limb was frozen, and the gastrocnemius muscle of the left limb was fixed with 4% paraformaldehyde.

Index detection

ELISA detection on effects of *D. officinale* on ATP, CK-MM and CK in serum of rats with EIMD ATP, CK-MM, and CK in rat serum were detected according to the instructions of ELISA kits. ① The kits were equilibrated at room temperature. ② Standard and sample wells were set, and 50 μl of standards with different concentrations were added to the standard wells. To sample wells, 10 μl of samples to be tested were added, and 50 μl of sample diluents were then added. ③ To each of the standard and sample wells, 100 μl of detection antibodies labeled with

horseradish peroxidase were added. The plates were sealed with sealing film and incubated at 37 ℃ for 60 min. ④ After the incubation was completed, the liquid in the plates was discarded, and the plates were dried on absorbent paper. Each well was filled with a washing solution, and stood for 1 min, and the washing solution was then discarded, and the plates were dried on absorbent paper. The operation was repeated 5 times. ⑤ To each well, 50 μl of substrate was added, and incubation was then allowed in dark at 37 ℃ for 15 min. And ⑥ after incubation, 50 μl of termination solution was added to each well. The *OD* value of each well was measured at a wavelength of 450 nm within 15 min.

HE staining detection on histopathological changes in gastrocnemius muscle of rats with EIMD induced by *D. officinale*

The pathological tissue was detected by HE staining. The gastrocnemius muscle of the left limb fixed by 4% paraformaldehyde was subjected to dehydration with different gradients of ethanol, transparentizing, paraffin penetration, embedding, slicing, heating, deparaffinage, rehydration, washing with double distilled water for 3 times, staining, dehydration and sealing, and observed under an microscope.

PCR and Western blot detection on effects of *D. officinale* on IGF-1 mRNA and protein expression in the gastrocnemius muscle tissue of rats with EIMD Total RNA was extracted from the gastrocnemius muscle of right limb frozen at −80 ℃. ① The tissue was added in a mortar fully precooled by liquid nitrogen, and added with a proper amount of liquid nitrogen to freeze and grind the tissue into powder, which was then transferred to a precooled 1.5 ml centrifugal tube. ② Next, 1ml of RNAiso Plus lysis solution was added. After fully oscillating and mixing, the sample was stood at room temperature for 10 min. ③ Chloroform was added at a ratio of 1/5 of the volume to the sample, which was then oscillated for 1 min, fully emulsified, and stood at room temperature for 10 min. ④ Centrifugation was performed at 4 ℃ and 13 000 rpm for 15 min. ⑤ The upper water phase was transferred into a precooled 1.5 ml EP tube, and added with equal volume of isopropanol to precipitate RNA by mixing well and standing at room temperature for 10 min. Then, the liquid obtained was centrifuged to get the supernatant, and the precipitate was washed once with 75% ethanol, and dried for 10 min. ⑥ An appropriate amount of DEPC water was added to dissolve RNA. And ⑦ the obtained total RNA was stored at −80 ℃.

Spectrophotometric determination of RNA concentration: First, 2 μl of RNA extract was added to a spectrophotometer, and the absorption peaks at 230, 260 and 280 nm were determined, respectively, and corresponding ratios were calculated. The concentration and purity of RNA in each sample were calculated.

RNA reverse transcription: ① A reaction mixture was prepared in a nuclease-free microcentrifuge tube. ② The reaction mixture was incubated at 42 ℃ for 2 min, then at 60 ℃ for 5 min, and placed on ice for rapid cooling. ③ The above mixture was centrifuged for a second, and then corresponding reagents were added to a final total amount of 20 μl. And ④ reverse transcription was carried out under conditions of 25 ℃ − 10 min, 55 ℃ −50 min, 85 ℃ −5 min and 4 ℃ − +∞. The subsequent qPCR detection included primer synthesis, *etc.*

Total protein was extracted first. ① Ground tissue was transferred into a precooled 1.5 ml centrifugal tube. ② The cell sample in the 1.5 ml centrifugal tube was centrifuged to obtain a precipitate. The precipitate was washed with PBS and centrifuged again, and the operation was repeated twice. ③ A RIPA lysis solution was added. ④ The obtained system was oscillated for 1 min and stood on ice for 10 min, and the operation was repeated thrice to fully lyse the cells. ⑤ Centrifugation was performed at 4 ℃ and 13 000 rpm for 20 min. And ⑥ the supernatant was transferred to a precooled 1.5 ml EP tube and stored at −80 ℃.

WB detection was carried out next. ① Prepared gel was taken out from a refrigerator at 4 ℃ and put into an electrophoresis tank. ② Next, 500 μg of total protein from each sample was mixed with 5 × SDS loading buffer at a ratio of 4 ∶ 1, and the protein concentration after mixing was about 3.3 μg/μl. The obtained protein liquid was denatured by heating in a metal bath at 100 ℃ for 6 min. ③ From each denatured total protein sample, 60 μg was loaded for detection. ④ The samples were electrophoresized. ⑤ The clips were opened to keep the black side horizontal, and a sponge pad, filter paper, gel, a PVDF membrane (activated by methanol), filter paper and a sponge pad were put on it in turn. Meanwhile, the electophoresis buffer was changed into a transfer liquid. ⑥ The current was adjusted to determine the transfer time. ⑦ The membrane was taken out, labeled and cleaned. ⑧ The primary antibodies were diluted, and incubation was carried out at 4 ℃ overnight. ⑨ Washing was carried out thrice with TBST, 10 min each time. ⑩ The secondary antibodies were diluted, and incubation was carried out at room temperature. ⑪ Washing was carried out thrice with TBST, 10 min each time. And ⑫ exposure was finally carried out.

Statistical processing The experimental data were expressed by ($\bar{x} \pm s$), and statistically processed using SPSS 25.0 statistical software, including one-way ANOVA and two independent samples *T*-test, with $P < 0.05$ or $P < 0.01$ indicating statistically-significant differences.

Results and Analysis

Effects of *D. officinale* on ATP, CK-MM and CK in serum of rats with EIMD

Compared with the quiet group, the contents of ATP in the serum of rats in the 1, 12 and 24 h exercise groups decreased significantly ($P < 0.01$), while the contents of CK and CK-MM increased significantly ($P < 0.01$). Compared with the 1 h exercise group, the ATP content in the *D. officinale* liquid + 1 h exercise group increased significantly ($P < 0.05$), while the CK and CK-MM contents decreased significantly ($P < 0.01$). Compared with the 12 h exercise group, the ATP content in the *D. officinale* liquid + 12 h exercise group increased significantly ($P < 0.01$), while the CK and CK-MM contents decreased significantly ($P < 0.01$). Compared with the 24 h exercise group, the ATP content in the *D. officinale* liquid + 24 h exercise group increased significantly ($P < 0.01$), while the CK and CK-MM contents decreased significantly ($P < 0.01$). The results are shown in Table 1.

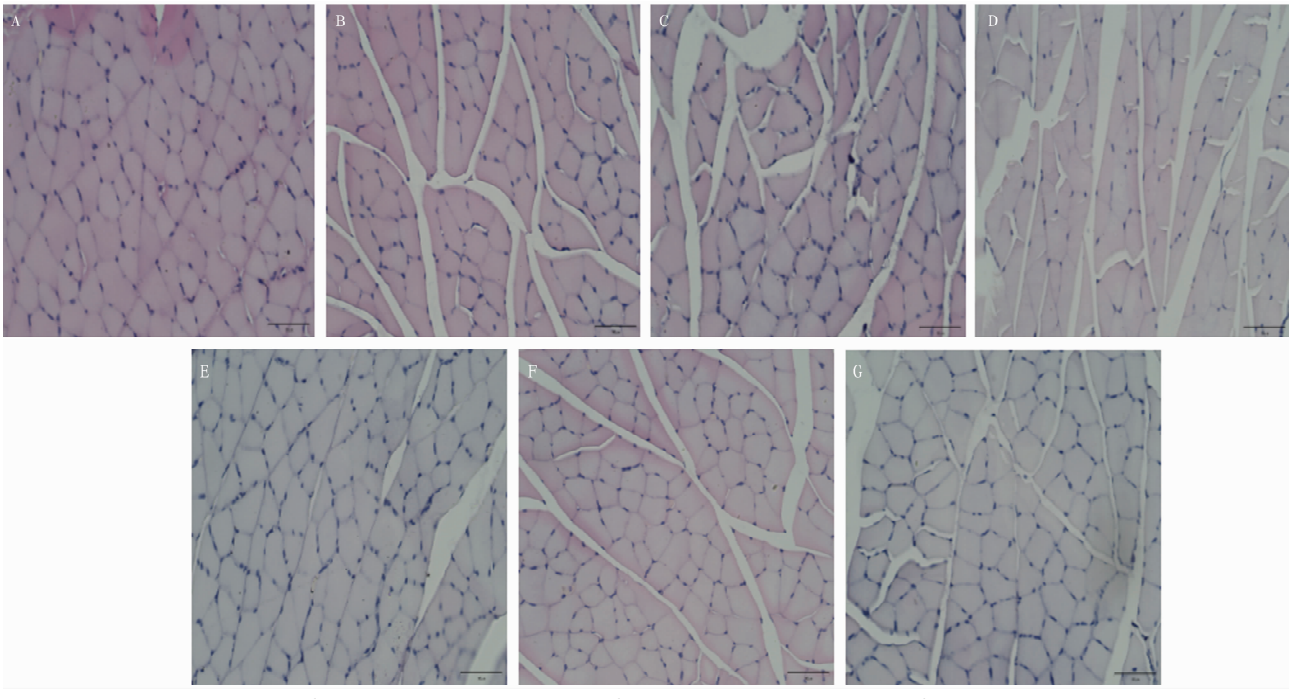
Table 1 Effects of *D. officinale* on ATP, CK-MM and CK in serum of rats with EIMD ($\bar{x} \pm s$, $n=6$)

Group	ATP//μmol/L	CK-MM//μg/L	CK//U/L
Quiet group	106.151 ± 12.427	18.905 ± 3.204	172.645 ± 8.826
1 h exercise group	72.602 ± 8.986**	31.162 ± 2.382**	215.115 ± 14.934**
D. officinale liquid + 1 h exercise group	88.794 ± 13.103#	19.781 ± 1.702##	183.875 ± 15.199##
12 h exercise group	56.515 ± 15.709**	55.266 ± 4.685**	229.993 ± 16.780**
D. officinale liquid + 12 h exercise group	81.280 ± 7.207##	34.858 ± 4.241##	187.930 ± 10.585##
24 h exercise group	37.148 ± 4.169**	71.365 ± 4.635**	254.563 ± 14.195**
D. officinale liquid + 24 h exercise group	71.332 ± 14.395##	42.148 ± 4.491##	196.443 ± 17.503##

Compared with the quiet group: $^{**}P<0.01$, $^{*}P<0.05$; the 1, 12 and 24 h exercise groups were compared with corresponding medicated groups, respectively: $^{\#\#}P<0.01$, $^{\#}P<0.05$.

HE staining detection on histopathological changes in gastrocnemius muscle of rats with EIMD induced by *D. officinale*

In the quiet group, the skeletal muscle structure was normal, showing neatly-arranged muscle fibers and clearly-visible muscle membrane. In the 1 h exercise group, muscle fibers were irregularly arranged and inflammatory cells were visible; and after adding *D. officinale* liquid, inflammatory cells slightly decreased and some muscle fibers arranged neatly. In the 12 h exercise group, the arrangement of muscle fibers was uneven, and local inflammatory cell infiltration increased, accompanied by bleeding; and after adding *D. officinale* liquid, the infiltration of local inflammatory cells decreased and bleeding decreased. In the 24 h exercise group, muscle fiber damage further worsened, and inflammatory cell infiltration, local necrosis of muscle fibers, unclear muscle membrane and severe bleeding were observed; and after adding *D. officinale* liquid, the infiltration of inflammatory cells decreased and bleeding decreased. The results are shown in Fig. 1.



A. Quiet group ×400; B. 1 h exercise group ×400; C. 12 h exercise group ×400; D. 24 h exercise group ×400; E. *D. officinale* liquid + 1 h exercise group ×400; F. *D. officinale* liquid + 12 h exercise group ×400; G. *D. officinale* liquid + 24 h exercise group ×400.

Fig. 1 Histopathology of gastrocnemius muscles in rats with EIMD

Western blot detection on effects of *D. officinale* on IGF-1 mRNA and protein expression in gastrocnemius muscle tissue of rats with EIMD

Compared with the quiet group, the expression levels of IGF-1 mRNA and protein in the gastrocnemius muscle tissue of rats in the 1, 12 and 24 h exercise groups significantly increased ($P<0.01$). Compared with the 1 h exercise group, the expression of IGF-1 protein in the gastrocnemius muscle tissue of rats in the *D. officinale* liquid + 1 h exercise group significantly increased ($P<0.05$). Compared with the 12 h exercise group, the expression levels of IGF-1 mRNA and protein in the gastrocnemius muscle tissue of rats in the *D. officinale* liquid + 12 h exercise group significantly increased ($P<0.01$). Also, compared with the 24 h exercise group, the expression levels of IGF-1 mRNA and protein in the gastrocnemius muscle tissue of rats in the *D. officinale* liquid + 24 h exercise group significantly increased ($P<0.01$). The results are shown in

Table 2 and Fig. 2.

Table 2 Effects of *D. officinale* on IGF-1 mRNA and protein expression in gastrocnemius muscle tissue of rats with EIMD ($\bar{x} \pm s$, $n=6$)

Group	IGF-1mRNA	IGF-1 protein expression
Quiet group	1.000 ± 0.127	0.188 ± 0.013
1 h exercise group	1.512 ± 0.224**	0.313 ± 0.008**
D. officinale liquid + 1 h exercise group	1.884 ± 0.159	0.340 ± 0.010#
12 h exercise group	2.010 ± 0.145**	0.489 ± 0.016**
D. officinale liquid + 12 h exercise group	3.232 ± 0.193##	0.629 ± 0.025##
24 h exercise group	2.521 ± 0.123**	0.651 ± 0.012**
D. officinale liquid + 24 h exercise group	4.404 ± 0.194##	0.787 ± 0.033##

Compared with the quiet group: $^{**}P<0.01$, $^{*}P<0.05$; the 1, 12 and 24 h exercise groups were compared with corresponding medicated groups, respectively: $^{\#\#}P<0.01$, $^{\#}P<0.05$.

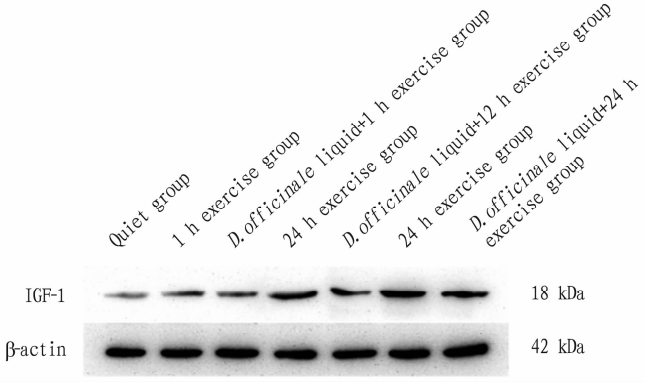


Fig. 2 Western blot analysis of IGF-1 protein expression in gastrocnemius muscle tissue of rats in various groups

Conclusions and Discussion

The motive organs of the human body's motion system are mainly skeletal muscles, which undergo contraction activities under the control of nerves, causing various movements in the body[11]. Skeletal muscle injury belongs to the category of "tendon injury" in traditional Chinese medicine, as stated in *Suwen Yin Yang Yingxiangdalun*: "Qi damage causes pain, shape damage causes swelling". Skeletal muscle damage is extremely common in the field of sports medicine, and it usually undergoes self-healing after injury. Such healing ability largely depends on some undifferentiated skeletal muscle satellite cells in skeletal muscle[12-14]. Muscle satellite cells are important repair cells in the process of skeletal muscle regeneration and repair. However, the repair process after skeletal muscle injury is very complex, requiring not only the activation of muscle satellite cells, but also the participation of various inflammatory factors, growth factors, *etc.* [15-17].

The most direct functional substance in the human body, ATP, has very little content in the body and is the only energy supply substance that can be directly utilized by muscles. Its content in muscles is relatively low, but when the body undergoes intense exercise with high intensity, the metabolic rate of substances in the body increases significantly, requiring a large amount of energy substances. Under normal circumstances, fatigue occurs when ATP production cannot meet the needs of exercise. Creatine kinase is a member of the phosphocreatinase family, whose main function is to assist in ATP hydrolysis, and participate in glycolysis control, muscle contraction energy supply and mitochondrial respiration. It has a significant impact on the metabolism of the human functional system, and thus typically exists in tissues such as the brain and muscles that require high energy. In high-intensity exercise, repeated stretching of muscles and accumulation of metabolites can lead to damage to skeletal muscle cells, resulting in an increase in cell membrane permeability and the infiltration of CK into the serum. Therefore, CK, as a cytoplasmic enzyme, is a key indicator for measuring the degrees of damage, fatigue and recovery of skeletal muscle cells after high-intensity exercise[18-19].

CK in the cytoplasm is composed of two peptide subunits: B (brain type) and M (muscle type), forming three different isoenzymes: CK-BB (brain), CK-MM (skeletal muscle), and CK-MB (myocardium). Related findings indicate that the skeletal muscle typically contains 98% MM and 2% MB subtypes[20]. CK-MM is a response kinase that binds to the M-line structure of myofibrils through lysine residue pairs[21]. It exists in the space of the sarcomere I band and binds to various ATP pumps on the sarcoplasmic reticulum to regulate various ATP dependent functions, providing support for muscle energy demand.

Insulin like growth factor 1 (IGF-1) is an important neurotrophic factor that plays an important role in the repair of skeletal muscle injury. Studies have shown that IGF-1 has the effect of promoting myoblast proliferation, differentiation, and skeletal muscle protein synthesis[22-23]. After skeletal muscle injury, repair can be achieved by supplementing exogenous IGF-1 or overexpressing IGF-1 in cells through transgenic technology[24-26]. Meanwhile, IGF-1 plays an important role in regulating skeletal muscle inflammation[27], skeletal muscle fibrosis[28] and promoting nerves after injury[29]. During the repair of skeletal muscle injury, IGF appears at a high concentration in the body and plays a role as a nutritional factor during the repair process of skeletal muscle injury. IGF can promote the proliferation and differentiation of satellite cells in damaged skeletal muscle, and form myotubes through cell fusion, thereby repairing damaged skeletal muscle.

In this study, the exercise groups and exercise groups treated with *D. officinale* liquid significantly reduced the serum ATP content of rats after exercise. During high-intensity exercise, the metabolic rate of rats increased significantly, requiring the consumption of a large amount of energy substances. After treatment with *D. officinale* liquid, the ATP content increased, indicating that it could delay energy consumption and reduce skeletal muscle

damage. The levels of CK-MM and CK in the serum of rats in the exercise groups significantly increased, and the damage to the skeletal muscle significantly increased with the prolongation of exercise time. After administering *D. officinale* liquid, it could help with the recovery of EIMD. From the analysis of HE results, prolonged exercise time accelerated skeletal muscle damage, and the damage was significantly alleviated after administration of *D. officinale* liquid.

Compared with the quiet group, the expression levels of IGF-1 mRNA and protein in the gastrocnemius muscle tissue of rats in the exercise groups significantly increased. The expression levels of IGF-1 mRNA and protein in the *D. officinale* liquid + exercise groups were higher than those in various exercise groups. The results indicated that the relative expression levels significantly increased with prolonged exercise time, but after administering *D. officinale* liquid, it effectively reduced the relative expression levels and promoted the secretion of IGF-1 in damaged skeletal muscle, thereby promoting the proliferation and differentiation of satellite cells and the repair of damaged skeletal muscle.

In summary, the role and mechanism of *D. officinale* in preventing and treating EIMD in rats might be related to reducing ATP energy consumption, improving the activity of CK-MM and CK, and promoting the expression of IGF-1 mRNA and protein in damaged tissues. This study provides a more scientific and rich theoretical basis for the clinical application of *D. officinale* in preventing and treating EIMD in the later stage.

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