

## Original Article

# Anti-fatigue effects of polysaccharide from *dendrobium officinale* Kimura et Migo

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**Abstract:** *Dendrobium officinale* Kimura et Migo (DOKM) is a traditional Chinese medicine and used for treating fatigue. Polysaccharides are the main constituents in DOKM. However, whether polysaccharides from DOKM are involved in the activity of anti-fatigue was still unknown. To explore the effects of anti-fatigue and the possible molecular mechanisms, DOKM polysaccharides were investigated in the rat models. The results show that DOKM polysaccharides could effectively increase the exhaustive swimming times, decrease the concentrations of BLA and SUN, up-regulate SOD and GSH-Px expression, and down-regulate MDA expression. In addition, DOKM polysaccharides enhanced gene and protein expression of PGC-1 $\alpha$ , Nrf2, and SOD2. These results indicate that DOKM polysaccharides might enhance exercise endurance and ameliorate physical fatigue, which are associated with increased mitochondrial functions and attenuation of oxidative stress.

**Keywords:** *Dendrobium officinale* Kimura et Migo (DOKM), polysaccharides, fatigue

## Introduction

*Dendrobium officinale* Kimura et Migo (DOKM), a medicinal plant widely grown in China, Japan, Australia, and USA, has been documented to exhibit multiple biological functions, including anti-oxidant [1], immune-enhancement [2], hepatoprotective activity [3], and anti-fatigue [3] activities. The effective compounds in DOKM mainly include polysaccharides, phenanthrenes, bibenzyls, alkaloids, and essential oils [3]. Toxicological assays, including acute toxicity test, genetic toxicity test, and 90-day feeding test in rats, have been conducted and showed that DOKM is safe [4]. Polysaccharides are the main constituents in DOKM. The pharmacological activity of DOKM polysaccharides is related to their compositions and contents of monosaccharides [5]. However, the biological functions of DOKM polysaccharides are still unclear, particularly on anti-fatigue.

Fatigue is described as a physiological process that cannot continue its normal functions to a certain extent or maintain the pre-determined exercise intensity [6]. Fatigue may cause homeostatic imbalance in endocrine, immune,

nervous, and bio-regulatory systems [7]. It is quite necessary to explore the therapeutic agents to manage fatigue without producing any obvious side effects. Traditional Chinese medicine (TCM) provides an effective strategy against fatigue. Recently, it has been reported that polysaccharides isolated from Ziyang green tea can significantly ameliorate exercise-induced fatigue [8]. The water-soluble polysaccharides from *Panax ginseng* C.A. Meyer show anti-fatigue activity [9]. However, whether DOKM polysaccharides also exhibit anti-fatigue activity is still unknown. In this study, the anti-fatigue activity of DOKM polysaccharides was investigated *in vivo* and *in vitro*.

## Materials and methods

### Preparation of DOKM polysaccharides

Dried DOKM was obtained from the Chinese herbal medicine market in Zhangshu city and identified by the botanist Qi Jin (College of Pharmacy, Gannan Medical University). Dried DOKM (1 kg) was mashed into powder (200 mesh), which was defatted with chloroform-methanol (1:1, v/v) at 60°C for 6 hours. The

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residue was then extracted for three times with boiling water (1:20, w/v) for 3 hours. The extracts were pooled and concentrated with reduced pressure in a rotary evaporator and de-proteinated by using the Sevag method. Then, they were precipitated with 95% ethanol (3 vol.) at 4°C for 24 hours, and collected by centrifugation (4°C, 5000 rpm, 10 minutes). The precipitate was sequentially washed by 95% ethanol, anhydrous ethanol, and acetone, and finally dried under vacuum to gain polysaccharides. The content of polysaccharides was determined by the phenol-sulfuric acid method, and glucose was used as the standard. Polysaccharides extraction yields (polysaccharide weight/DOKM weight) were 6.21%.

### *Animals and treatments*

The study was approved by the Institutional Animal Care and Use Committee of Gannan Medical University. Male Kunming mice (20 ± 2 grams) were obtained from Hunan Biological Supplier (Changsha city, China). Mice were kept under the standard environment conditions. 80 mice were randomly divided into four groups (20 mice/group). Group I was the negative control (NC) group; Group II was the low-dose DOKM polysaccharides (LDDP) group; Group III was the middle-dose DOKM polysaccharides (MDDP) group; and Group IV was the high-dose DOKM polysaccharides (HDDP) group. DOKM polysaccharides were dissolved in 1.0 mL of distilled water for oral administration, and the NC group received the same volume of distilled water. Mice received orally administration once daily for four weeks. Then, mice were involved in the experimental tests relating to fatigue.

### *Forced swimming test*

The experimental procedures were conducted according to Porsolt [10]. Simply, after the last administration for 30 minutes, 10 mice were placed into a swimming pool (25 ± 1°C) with 30 cm depth of water individually. A lead piece (10% of body weight) was attached on each mouse tail root. Exhaustion was determined by observing failure of swimming, when mice failed to rise to the surface of water to breathe within a 10 second period. The swimming time to exhaustion was considered as the index of the forced swimming capacity.

### *Determination of hepatic glycogen, SUN, and BLA*

The other 10 mice from each group were taken for biomedical parameters determination. After the last administration for 30 minutes, mice were forced to swim without the lead piece on the tails in the swimming pool for 90 minutes. After rest for 60 minutes, mice were anesthetized with 3% pentobarbital sodium. The blood samples were collected in tubes with heparin sodium and without any anti-coagulants and kept under ice cold condition. The content of blood lactic acid (BLA) was determined using the whole blood samples. The content of serum urea nitrogen (SUN) was determined after centrifugation (1500 rpm, 4°C, 10 minutes). Glycogen, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and malonaldehyde (MDA) levels were determined using the liver tissue and the gastrocnemius muscle tissue after homogenation. The determining procedures for the concentration of BLA, SUN, hepatic glycogen, SOD, GSH-Px, and MDA were conducted following the instructions provided by the kits. In addition, some liver tissues were collected and kept for qRT-PCR and Western-blot.

### *qRT-PCR*

Total RNA was extracted with RNeasy mini kit (QIAGEN, Valencia, CA, USA), and the relative changes in PGC-1 $\alpha$ , Nrf2, and SOD2 mRNA expression were determined by quantitative RT-PCR (qRT-PCR). The designed PCR primers were as follows: PGC-1 $\alpha$  forward: 5'-ggcagaa-ggcaattgaagag-3', reverse: 5'-tcaaacggtccct-cagttc-3'; Nrf2 forward: 5'-attcaagccgattagagg-3', reverse: 5'-attgctcctggacatca-3'; HO-1 forward: 5'-atgtcccaggattgtc-3', reverse: 5'-ctgcttgtttcgctct-3'; 18S rRNA forward: 5'-cctgataaccgagctagga-3', reverse: 5'-gcgcgcaatacgaatgccc-3'. The expression of the target genes was normalized to 18S rRNA by using 2<sup>- $\Delta\Delta$ CT</sup> method.

### *Western-blot*

Liver tissues were homogenated and lysed in RIPA buffer on ice. The lysates were centrifuged at 12000 $\times$ g at 4°C for 15 min. BCA assay (Thermo Fisher Scientific, Waltham, MA, USA) was used to determine the protein concentra-

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**Table 1.** Effects of DOKM polysaccharides on body weights in mice

Group	N	Dose (mg/kg)	Body weights (g)	
			Initial	Final
CN	10	0	22.16 ± 0.32	31.89 ± 0.37
LDDP	10	50	21.87 ± 0.29	31.68 ± 0.35
MDDP	10	100	22.05 ± 0.31	32.01 ± 0.32
HDDP	10	200	21.98 ± 0.32	31.95 ± 0.32

tions. The denatured equal protein (30 µg) of each sample was subjected to SDS-PAGE, and proteins were transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA) and subjected to the standard western blot procedures. The primary antibodies against PGC-1α, Nrf2, SOD2, and GAPDH were obtained from Cell Signaling Technology. The enhanced chemiluminescence detection system (Applied Biosystems) was used to detect proteins, and Quantity One software (Bio-Rad) was used to quantify and analyze the bands.

### Statistical analysis

Data are shown as mean ± SD. The results were analyzed by an unpaired t-test.  $P < 0.05$  was considered to be statistically significant ( $*P < 0.05$ ,  $**P < 0.01$ ).

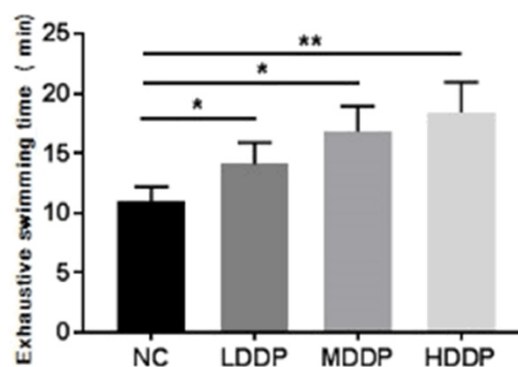
## Results

### Effects of DOKM polysaccharides on body weights in mice

During a 4-week experimental period, body weights of mice were recorded at the initial stage and the final stage of experiments. The changes of body weight are shown in **Table 1**. No significant difference was found between the DOKM polysaccharides-treated groups and the CN group during the initial stage and the final stage of experiments. These indicate that DOKM polysaccharides did not exhibit any significant effects on body weights of mice.

### Effects of DOKM polysaccharides on the exhaustive swimming time in mice

Exercise endurance is a critical indicator for evaluating anti-fatigue effects [11]. Forced swimming test is available for testing exercise endurance, which might be associated with expression changes of some biochemical factors. As shown in **Figure 1**, the exhaustive swi-



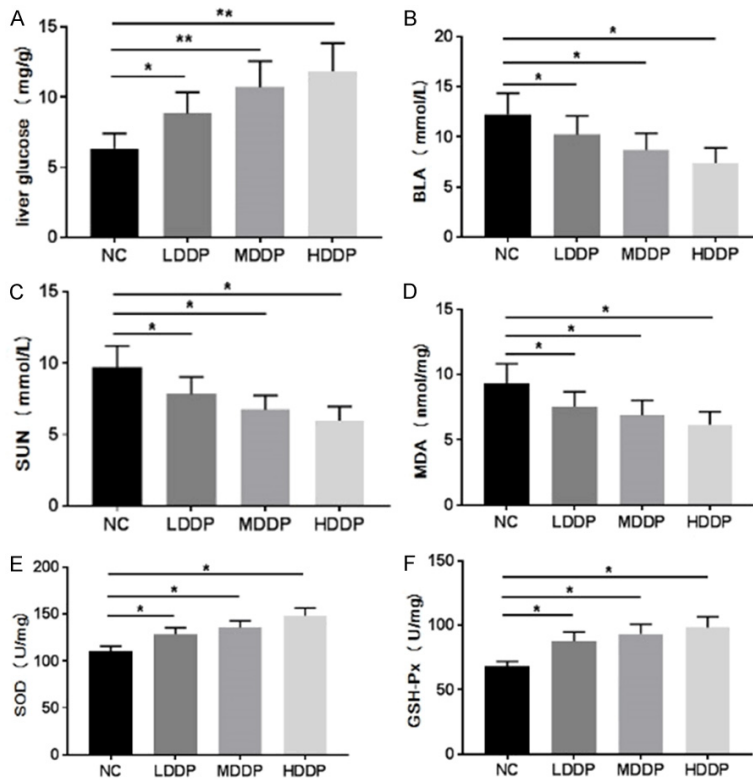
**Figure 1.** Effects of DOKM polysaccharides on the exhaustive swimming time in mice. Data are the mean with their SD represented by vertical bars for 10 replicates.  $P < 0.05$  was considered to be statistically significant ( $*P < 0.05$ ,  $**P < 0.01$ ).

mming times in DOKM polysaccharides-treated groups were demonstrated to be increased in a dose dependent manner than those in the NC group. Therefore, the DOKM polysaccharides might enhance exercise endurance and ameliorate physical fatigue.

### Effects of DOKM polysaccharides on the biochemical parameters after exercise in mice

Fatigue-induced physiological alternations are directly indicated by changes of the energy metabolism. Energy deficiency impairs exercise and endurance capacity. Liver glucose storage might prevent against energy deficiency and enhance endurance capacity. As shown in **Figure 2A**, DOKM polysaccharides could significantly increase liver glucose concentrations. Exercise increases oxygen consumption, which is closely related to the activity of mitochondria. Lactic acid is a product of glycolysis under anaerobic conditions, which is positively correlated with the production of reactive oxygen species (ROS) and oxidative stress [12]. In addition, BLA concentration is also sensitive to fatigue. In **Figure 2B** and **2D-F**, the concentrations of BLA and MDA in DOKM polysaccharides-treated groups were significantly decreased, while the concentrations of SOD and GSH-Px were increased dramatically. Urea nitrogen is also a negative indicator for exercise endurance and body functions. Similarly, the contents of SUN in NC group were much higher than those in DOKM polysaccharides-treated groups (**Figure 2C**). Collectively, DOKM polysaccharides could ameliorate the expression of

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**Figure 2.** Effects of DOKM polysaccharides on the expression of biochemical parameters after exercise in mice. A: Liver glucose; B: BLA; C: SUN; D: MDA; E: SOD; F: GSH-Px. Data are the mean with their SD represented by vertical bars for 10 replicates.  $P < 0.05$  was considered to be statistically significant (\* $P < 0.05$ , \*\* $P < 0.01$ ).

### Effects of DOKM polysaccharides on the gene expression of PGC-1 $\alpha$ , Nrf2, and SOD2

To investigate whether the mitochondrial activity was involved exercise endurance, gene expression of PGC-1 $\alpha$ , Nrf2, and SOD2 in the liver tissues was assessed after exercise. As shown in **Figure 3**, DOKM polysaccharides could significantly up-regulate PGC-1 $\alpha$ , Nrf2, and SOD2 expression in a dose dependent manner. These indicated that DOKM polysaccharides-ameliorated fatigue was, at least partially, associated with the enhanced activity of mitochondria and attenuation of oxidative stress.

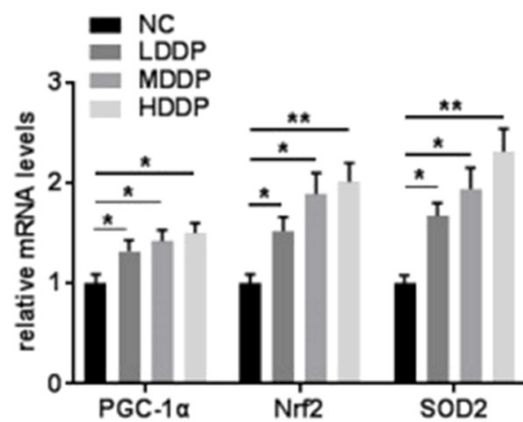
### Effects of DOKM polysaccharides on the protein expression of PGC-1 $\alpha$ , Nrf2, and SOD2

To further explore involvement of the mitochondrial activity in exercise endurance, we determined the proteins expression

of PGC-1 $\alpha$ , Nrf2, and SOD2 in the liver tissues after exercise. As shown in **Figure 4**, the proteins expression of PGC-1 $\alpha$ , Nrf2, and SOD2 in the DOKM polysaccharides-treated groups were significantly up-regulated, compared with those in the NC group. These indicate that PGC-1 $\alpha$ , Nrf2, and SOD2 may play a role in DOKM polysaccharides-ameliorated fatigue.

## Discussion

DOKM has been reported to protect against diabetic cardiomyopathy through decreasing the cardiac lipid accumulation, cardiac fibrosis, oxidative stress, and pro-inflammatory cytokines [13]. In addition, DOKM prevents against insulin resistance by reduction of TLRs and inflammatory responses in rats with diabetic nephropathy [14]. More than 190 compounds have been isolated from DOKM [3], and polysaccharides are the critical effective compounds in DOKM. However, little is known about the functions of DOKM polysaccharides. DOKM polysaccharides could effectively protect ag-

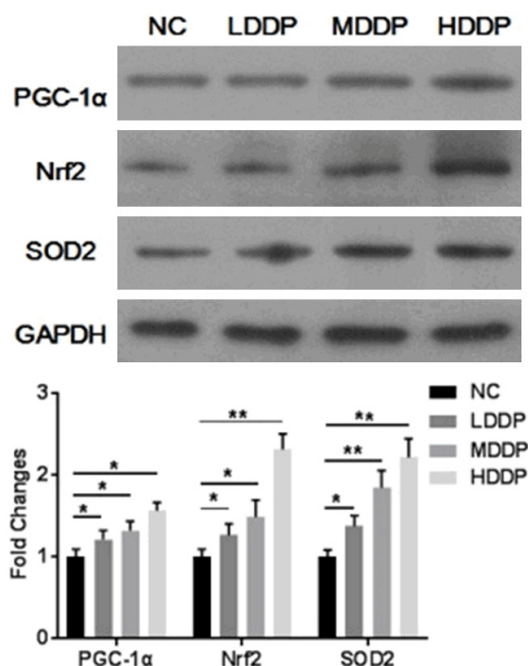


**Figure 3.** Effects of DOKM polysaccharides on PGC-1 $\alpha$ , Nrf2, and SOD2 gene expression. The 18S rRNA was used the internal control genes. Data are the mean with their SD represented by vertical bars for 10 replicates.  $P < 0.05$  was considered to be statistically significant (\* $P < 0.05$ , \*\* $P < 0.01$ ).

fatigue-related biochemical factors BLA, MDA, and SUN, and increase the expression of SOD and GSH-Px.



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**Figure 4.** Effects of DOKM polysaccharides on the protein expression of PGC-1 $\alpha$ , Nrf2, and SOD2. GAPDH was used the internal control genes. Data are the mean with their SD represented by vertical bars for 10 replicates.  $P < 0.05$  was considered to be statistically significant (\* $P < 0.05$ , \*\* $P < 0.01$ ).

against fatigue in rats. The possible mechanisms might be associated with enhancement of mitochondrial activity and attenuation of oxidative stress.

Forced swimming test is a valid model for exploring the anti-fatigue effects of agents [15]. The degree of fatigue is indicated by the duration from the initiation of swimming to exhaustion. The exhaustive swimming time was greatly improved by DOKM polysaccharides. This indicated that DOKM polysaccharides significantly enhanced exercise endurance. This notion was further proved by the changes of the biochemical parameters, including BLA, MDA, SUN, SOD, and GSH-Px, induced by DOKM polysaccharides. BLA is the by-product made by muscle cells, which require efficient energy through glycolysis [16]. Similarly, urea is the main end product of amino acid catabolism [17]. Together, the concentrations of both BLA and SUN are the index for indicating the status of energy supply. Insufficient energy supply may lead cells to activate anaerobic glycolysis, which is accompanied by the up-regulation of ROS [18]. To further investigate the roles of energy supply in exercise endurance, the con-

tents of SOD, MDA, and GSH-Px, the factors relating to redox, were investigated. DOKM polysaccharides administration was found to decrease the concentrations of BLA and SUN and reversed the oxidative stress, as indicated by up-regulation of SOD and GSH-Px and down-regulation of MDA.

Mitochondrion is the main place to produce energy and ROS [19]. PGC-1 $\alpha$  has been demonstrated to be the master in controlling the metabolism of mitochondria [20]. Up-regulation of PGC-1 $\alpha$  expression is positively related to mitochondrial metabolism [21]. In addition, activation Nrf2-SOD2 signaling may balance the micro-environment of mitochondria and improve mitochondrial performance [22]. To investigate whether mitochondrial activity was involved in fatigue during exercise endurance, expression of PGC-1 $\alpha$ , Nrf2, and SOD2 was analyzed. DOKM polysaccharides effectively up-regulated the expression of PGC-1 $\alpha$ , Nrf2, and SOD2 at gene and protein levels. These suggested that DOKM polysaccharides might protect mitochondria under oxidative stress, leading to increased production of energy.

Conclusively, DOKM polysaccharides exhibited anti-fatigue effects, which might be associated with up-regulation of PGC-1 $\alpha$  and Nrf2-SOD2 signaling.

### Acknowledgements

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### Disclosure of conflict of interest

None.

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