

Original article

The extract of *Ophiocephalus striatus* affect levels of TNF- α , TGF- β 1, IL-17 and suPAR, pulmonary diffusing capacity for carbon monoxide, and health-related quality of life in stable COPD patients with muscle wasting

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Abstract: Objective — This study aimed to evaluate the effect of *Ophiocephalus striatus* extract on body composition, levels of TNF- α , TGF- β 1, IL-17, suPAR and neutrophils, diffusing capacity of the lungs for carbon monoxide (DLCO) and health-related quality of life (HRQoL) in stable COPD patients with muscle wasting.

Methods — Clinical pre- and post- quasi-experimental study of 32 stable COPD patients with muscle wasting from the Pulmonary Outpatient Clinic of Saiful Anwar General Hospital Malang, determined in accordance with the Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2016 and bioelectrical impedance analysis (BIA) measurements (FFM <14.6 kg/m; BMI <18.5 kg/m²). Measurements of the following health-related parameters for the QoL due to COPD were performed before and after the nutritional intervention of 3,000 mg of *O. striatus* extract daily for 12 weeks: TNF- α , TGF- β 1, IL-17 and suPAR levels (by ELISA), neutrophils (by blood test), DLCO (by body plethysmography), and CAT score.

Results — A non-significant reduction of TNF- α (p=0.302), IL-17 (p=0.275), neutrophil (p=0.619), and suPAR (p=0.674) levels, along with an increase in DLCO (p=0.369), occurred after 12 weeks of *O. striatus* extract administration. However, the level of TGF- β 1 declined significantly (p=0.022), followed by an increase in QoL as assessed by the CAT score (p=0.000). There was no significant correlation between inflammatory cytokines and DLCO, nor with the CAT score.

Conclusion — The study results demonstrated a potential role of oral nutritional supplementation in the management of COPD patients with muscle wasting.

Keywords: COPD, muscle wasting, *Ophiocephalus striatus*, TGF- β 1.

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Introduction

Muscle wasting as major comorbidity in Chronic Obstructive Pulmonary Disease (COPD) patients is a significant change in skeletal muscle structure and function associated with complications and mortality. Skeletal muscle weakness is associated with fat-free body mass (FFM) wasting in the limb muscles, respiratory muscles, and diaphragm mass. This causes increased gas-trapping, and a decreased diffusion and exercise capacity [1]. In 1998, Schols demonstrated that 20-35% of patients with mild to severe COPD reported weight loss associated with muscle and fatty tissue wasting [2]. Several factors are involved in the occurrence of muscle wasting in COPD patients, including airway obstruction, hypoxemia, malnutrition, long-term glucocorticoid use, oxidative stress and systemic inflammation. Muscle wasting occurs due to an imbalance between protein synthesis and protein degradation. Studies on muscle wasting in

patients with COPD generally focused their attention on protein degradation factors, while the reduction in protein synthesis in muscle wasting that did not received much attention, albeit it is suspected to be a consequence of chronic inflammation [3, 4].

Proinflammatory cytokines involved in COPD are Interleukin-1 β (IL-1 β), Interleukin-6 (IL-6), C-reactive protein (CRP), Leptin, TNF- α , IL-17, TGF- β 1, and suPAR. These cytokines become markers of tissue inflammation, both locally and systemically. Their levels were found to increase on examination of both bronchoalveolar lavage (BAL) and serum. Their levels increase with exacerbations [5, 6]. Tumor Necrosis Factor Alpha (TNF- α) is a cytokine that has various effects, such as supporting and inhibiting growth, angiogenesis, cytotoxicity, inflammation, and immunomodulation. Along with IL-17, if activated, TNF- α will cause the withdrawal of inflammatory cells, such as neutrophils, in the airway, which causes various pathological processes, both locally and

systemically [7, 8]. Some cytokines, especially TGF- β 1, IL-17, IL-6 and suPAR, are known to be fibrogenic, which triggers the fibrosis of the lung and airways. Chronic respiratory infarction is associated with the severity of COPD patients, as well as other chronic diseases [9, 10]. These conditions affect lung function, which can be measured by examining the diffusing capacity of the lungs for carbon monoxide (DLCO) with body plethysmography. The DLCO enables the transfer of carbon monoxide from the alveolar gas to red blood cells and represents the integrity of the gas exchange process in the alveolar-capillary membrane [11].

COPD is associated with pulmonary and systemic inflammation. The COPD therapy currently used is the result of a long-term study that did not affect the systemic effects of COPD. The combination therapy of inhaled corticosteroids and long-acting beta2-adrenergic agonists, as well as a combination of the long-acting muscarinic antagonist (LAMA) and long-acting beta agonist (LABA), is clinically useful, but still less than optimal, with projected COPD mortality doubling over the next 20 years. Non-pharmacological nutritional treatment and medical rehabilitation therapy are needed.

Ophiocephalus striatus extract contains various nutrients, such as amino acids and fatty acids, various vitamins and minerals, immunoglobulins, omega 3, omega 6, and omega 9. The main fraction (30.2%) contains protein albumin (2.17 ± 0.14 mg/dL) whereas the quality of albumin is better than that of white egg albumin [12]. Also, the essential amino acid leucine (part of the branched-chain amino acid [BCAA] group) is present and involved in preventing muscle atrophy as well as stimulating skeletal muscle synthesis and regulation of blood sugar levels [13]. Polyunsaturated fatty acids (PUFA) are known to decrease inflammatory cytokines through the activation of peroxisome proliferator-activated receptor gamma (PPAR- γ), which prevents NF κ B activity as a transcription factor of proinflammatory cytokines [14]. The association between proinflammatory cytokines, DLCO and nutritional supplements in circulation with muscle wasting loss in COPD are still under investigation. The assessment of muscle wasting is essential in the management of COPD to enable new strategies to be considered as part of the evaluation and management of therapies that deliver better results [15].

Several studies of muscle wasting in COPD patients focused mostly on protein degradation factors, while the decrease in protein synthesis in muscle wasting, which is suspected to be a consequence of chronic inflammation, did not receive proper attention. The purpose of administering *O. striatus* extract is to prevent muscle atrophy and to stimulate skeletal muscle synthesis. Research of administering *O. striatus* extract in COPD patient with muscle wasting was never conducted before. Thus, our study aimed to evaluate the effect of *O. striatus* extract on body composition, levels of TNF- α , TGF- β 1, IL-17, suPAR and neutrophils, DLCO, and HRQoL in stable COPD patients with muscle wasting.

We present this article in accordance with the TREND reporting checklist.

Material and Methods

Instrumentation

Serum tumor necrosis factor- α (TNF- α) was measured using the ELISA Legend MaxTM Human TNF- α with Pre-Coated Plates by BioLegend, with a minimum detected level of 1.6 pg/mL. Serum

tumor growth factor- β 1 (TGF- β 1) was measured using Legend MaxTM Total TGF- β 1 Human ELISA Kit with Pre-Coated Plates by BioLegend, with a minimum detected level of 3.5 pg/mL. Serum interleukin-17 was measured using Legend MaxTM Human IL-17A Elisa Kit with Pre-Coated Plates, with a minimum detected level of 2 pg/mL. Serum soluble urokinase plasminogen activator receptor (suPAR) was measured using MyBioSource Human Soluble Urokinase Plasminogen Activator Receptor (suPAR) Elisa Kit, with a minimum detected level of 0.156 ng/mL.

Patients

The experimental design was as follows. The study was conducted quasi-experimentally on 32 stable COPD patients with comorbid muscle wasting. The participating patients were selected via consecutive sampling based on the exclusion and inclusion criteria for the study, from the pool of patients who were treated at the Pulmonary Outpatient Clinic of Saiful Anwar General Hospital, Malang, Indonesia.

The inclusion criteria for our study were as follows: (1) men; (2) 40-70 years of age; (3) with stable COPD meeting the 2016 GOLD (The Global Initiative for Chronic Obstructive Lung Disease) criteria of A, B, C, and D populations; (4) who underwent spirometry examination and chest X-ray; (5) who received conventional drug therapy; (6) who had comorbid muscle wasting; (7) who were willing to participate in the study. Meanwhile, the exclusion criteria were COPD patients with other illnesses, such as diabetes mellitus, chronic kidney disease, chronic heart failure, malignancy, thyroid dysfunction, liver disorder, and cerebrovascular accident. Also, we excluded from the study those who were currently receiving nutritional or systemic steroid therapy, had exacerbation in the last 12 weeks, or had current symptoms in the respiratory tract. Patients who declined to participate or could not be contacted again during the intervention and observation period were excluded as well.

Data collection

In total, there were 32 study subjects who underwent spirometry examination and bioelectrical impedance analysis (BIA) and completed the COPD Assessment Test (CAT) questionnaire. Their blood samples were sent to the Central Laboratory, and DLCO was examined at Saiful Anwar General Hospital, Malang Indonesia. All procedures were performed at the beginning and in the end of supplementation. Then, supplements of the *O. striatus* extract (2 capsules 3 times per day) were administered for 12 weeks. The routine visits were accomplished monthly. Each month, complaints and responses to supplementation were evaluated. Subjects experiencing exacerbations during treatment were excluded from the study.

Statistical data processing

All data were analyzed by the Kolmogorov-Smirnov test. Statistical comparisons between groups (before and after performing the intervention) were completed using paired t-tests and Wilcoxon tests, and alternatively by the Mann-Whitney U test. A statistical correlation between variables was assessed using the Pearson's correlation or Spearman's rank correlation tests. Data were analyzed using the SPSS v.18.0 for Windows, and $p < 0.05$ was assumed to indicate the statistical significance.

Table 1. Sociodemographic and clinical characteristics of research subjects

Characteristics	N (%)
Age (years)	
45-50	1 (3.13)
51-60	6(18.75)
61-70	25 (78.13)
Job	
Company employer	3 (9.38)
Retirement Civil servant	17(53.13)
Farmer	4(12.50)
Entrepreneur	6(18.75)
Unemployed	2 (6.25)
Brinkman Index	
Mild (0-199)	4(12.50)
Moderate (200-599)	13 (40.63)
Severe (≥600)	15 (46.87)
GOLD stage	
GOLD I	2(6.25)
GOLD II	14 (43.75)
GOLD III	12
GOLD IV	18
Body Mass Index (BMI)	
Underweight (≤18.5)	13 (40.63)
Normal weight (18.5-24.9)	16 (50.00)
Overweight (≥25)	3 (9.38)
Fat-Free Mass Index (FFMI)	
Muscle wasting (≤16)	32(100)
Nonmuscle wasting (>16)	0(0)

Table 2. Effects of supplementing 3,000 mg of *Ophiocephalus striatus* extract for three months on cytokines, lung function, anthropometry and CAT score

Variable	Means		P value
	Pre-Intervention	Post-Intervention	
TNF-a (pg/mL)	153.9 ±62.48	181.8±80.17	0.302
TGF- π (pg/mL)	145.95±50.17	82.75±45.76	0.022*
IL-17 (pg/mL)	20.521±14.526	17.841±9.658	0.275
suPAR (pg/mL)	3.93±2.05	3.77±1.67	0.674
Neutrophils (%)	61.74±11.53	60.89±10.49	0.619
DLCO (% prediction)	53.8±26.52	58.8±33.28	0.298
BMI (kg/m ²)	20.16±3.54	20.30±3.68	0.753
FFMI	10.87±2.02	11.36±1.45	0.447
CAT score	17.91±7.81	11.25±8.28	0.000*

* Statistical significance at $p < 0.05$.

Results

Based on the sociodemographic characteristics listed in [Table 1](#), most of 32 study subjects (n=25) were between 61 and 70 years of age. All subjects were men, 21 were high school graduates, and 17 were retired civil servants. Based on clinical characteristics, the majority of study subjects were heavy smokers (Brinkmann Index ≥ 600 ; n=15), and the highest degree of airflow resistance was GOLD II (in 14 subjects). There were 18 individuals in D population. Based on nutritional status, according to body mass index (BMI), the majority of patients were in the normal weight group, but 16 subjects had muscle wasting, i.e., lower values of the fat-free mass index (FFMI).

TNF- α is one of significant catabolic inflammatory cytokines. It is involved in protein synthesis and in muscle wasting by weakening the Akt signaling pathway, thereby suppressing the process and activating the ubiquitin-proteasome pathway, resulting in muscle protein degradation [16]. However, in this study, the administration of *O. striatus* for 12 weeks in COPD patients did not significantly reduce the level of TNF- α ([Table 2](#)).

Moreover, the mechanism of polyunsaturated fatty acids (PUFA) in *O. striatus* extract decreasing inflammatory cytokines is implemented through the activation of peroxisome proliferator-activated receptor gamma (PPAR- γ), which prevents NF κ B activity as a transcription factor for inflammatory cytokines including IL-17 and neutrophils [14]. suPAR is a biomarker for activating the inflammatory and immune systems. Its levels are positively correlated with proinflammatory biomarkers. In this study, the administration of *O. striatus* extract for 12 weeks in COPD patients did not significantly reduce the content of IL-17, neutrophils and suPAR ([Table 2](#)).

O. striatus extract contains leucine, which can stimulate muscle protein synthesis and inhibit proteolysis. Leucine is an essential amino acid for protein synthesis, tissue regeneration and metabolic processes. Metabolic process of leucine is implemented through the mTOR and AMPK pathways in muscle protein synthesis [17, 18]. However, in our study, we established that administration of *O. striatus* extract for 12 weeks in COPD patients did not significantly increase BMI or decrease FFMI values ([Table 2](#)).

Imbalances of protein metabolism, oxidative stress, and inflammatory processes are involved in the occurrence of muscle wasting, which further affects the structure and function of the muscles. In COPD patients, muscle wasting occurs not only in skeletal muscles but also in the respiratory muscles, thereby affecting respiratory processes and lung function (e.g., DLCO). In our study, the administration of *O. striatus* extract for 12 weeks in COPD patients did not significantly increase DLCO ([Table 2](#)).

The CAT involves unidimensional measurement of 8 items related to impaired health status of COPD patients. Scores range from 0-40, they are very closely related to St. George's Respiratory Questionnaire (SGRQ) and were widely documented in a number of publications. Transforming growth factor- β 1 (TGF- β 1) is involved in the occurrence of fibrosis via inducing extracellular matrix protein deposition, including collagen and fibronectin, as well as mediating inflammation in some tissues including the lungs and muscles. Interestingly, in our study, the administration of *O. striatus* extract for 12 weeks significantly increased CAT score and TGF- β 1 content ([Table 2](#)).

Discussion

All subjects in this study were male, 45-70 years of age, with an assumption that hormonal factors and aging processes did not affect the results of the study [15, 19-21]. The greatest exposure to cigarettes was reported in patients with severe exposure (severe IB) of 46%. Smokers experience an increase in respiratory symptoms and a decrease in lung function each year, which is accompanied by an increase in mortality rates, compared with non-smokers. The same is observed in the population of passive smokers/ETS (Environmental Tobacco Smoke) [15].

Economic and educational factors can affect the occurrence of COPD [22]. Poverty can be a risk factor for COPD due to exposure to indoor and outdoor pollution, overpopulated housing conditions, infectious events, nutritional supplementation, etc. Other factors are type of employment, ability to obtain information, and implementation of healthy living. In this study, the highest employment category was retired civil servants (53.13%), and health services that are more readily available to civil servants/retired civil servants.

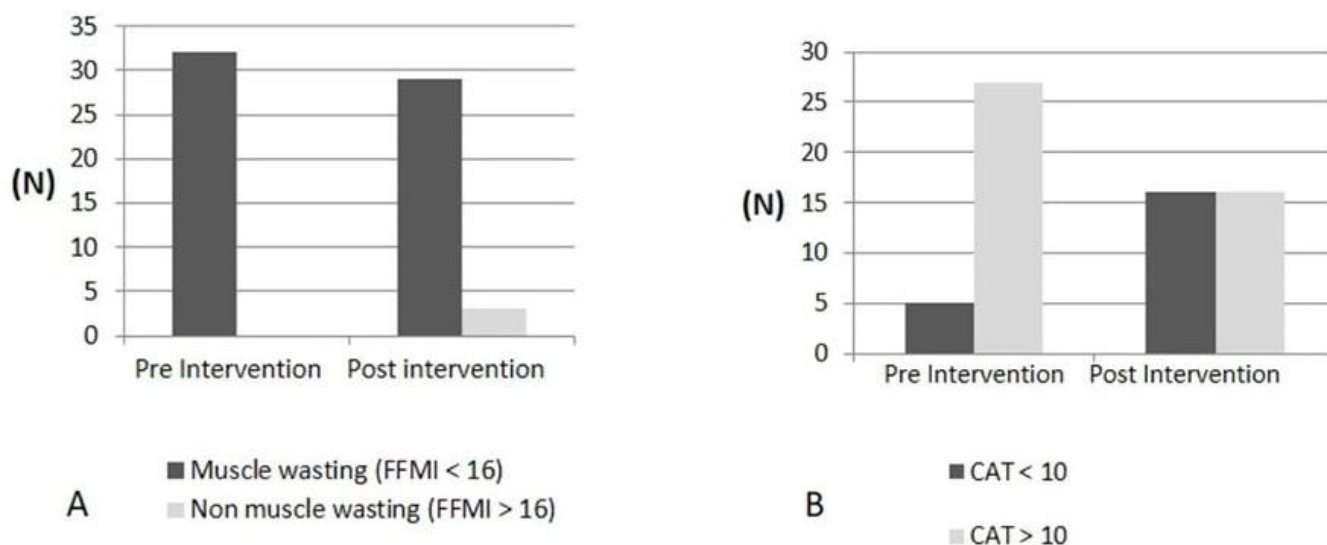


Figure 1. Effect of *Ophiocephalus striatus* A) extract on BMI, B) extract supplementation on COPD population.

As for anthropometry, the majority of subjects were in the standard or underweight BMI categories. In our study, most subjects were in population D, but the average BMI was still in the normal weight category. Despite normal values of BMI, all subjects experienced a reduction in body mass, compared with FFMI values of <16 (Table 2). Approximately 60-80% of muscle mass consists of FFM, meaning that a decrease in body mass is more accurate when measured by FFM because the body mass can be reduced even though the weight is fixed [22]. Approximately 50% of patients with mild to severe weight loss have COPD, which is associated with muscle wasting and fatty tissue depletion [23]. The effect of *O. striatus* extract on BMI and the COPD population is presented in Figure 1.

Effect of *Ophiocephalus striatus* extract on muscle wasting

All subjects in this study (100%) exhibited muscle wasting characterized by reduced fat mass (FFMI <16 kg/m²) and increased body fat, but their BMI did not change. Malnutrition in COPD occurs due to increased energy demand from respiratory muscle work, chronic hypoxemia, and hypercapnia, which causes hypermetabolism. Losing 10 to 20% of the body weight will decrease the ability of the immune system, resulting in morbidity and mortality, and 40% weight loss may lead to death [1].

In this study, the mean BMI of the subjects increased after the administration of *O. striatus* extract for 12 weeks: from 20.16±3.57 to 20.30±3.91 (p=0.125). The number of subjects who experienced malnutrition, i.e., those in the underweight BMI category, declined from 13 patients (40.63%) to only 4 subjects (12.5%), while the number of subjects with normal weight or overweight BMI increased. A study by Raizada et al. [24] showed that the commercial supplementation of nutrients containing carbohydrates, fats, proteins, fiber, vitamins, and minerals with a total energy of 500 kcal over the period of three weeks in stable COPD patients, could significantly increase BMI. It is important to note the tendency to increase fat mass rather than muscle mass in COPD patients when considering the provisioning of nutrition to COPD patients; therefore, it is necessary to take into account the composition of provided food. In this case, amino acids, such as leucine (part of BCAA group), can stimulate the synthesis of muscle

proteins and inhibit proteolysis. In patients with COPD, there is a reduction in plasma leucine levels.

Effect of *Ophiocephalus striatus* extract on the levels of TNF- α , TGF- β 1, IL-17, suPAR and neutrophils

Increased levels of plasma TNF- α and soluble TNF receptor are found in COPD patients, especially in those experiencing weight loss [25]. The provision of nutritional supplementation combined with a low-impact physical exercise was shown to significantly reduce TNF- α levels, as reported by Sugawara [26]. That study found increased TNF- α levels, but this pattern was not statistically significant (p=0.302). Less significant reduction can occur because the provided intervention is exclusively nutritional supplementation not accompanied by physical exercise. However, the results of our study are similar to those of Broekhuizen et al. [27] who established no improvement in inflammatory cytokine levels of TNF- α and IL-6 after eight weeks of supplementation with polyunsaturated fatty acids (PUFAs).

TGF- β 1 is involved in the occurrence of fibrosis via inducing extracellular matrix protein deposition, including collagen and fibronectin, as well as mediating inflammation in some tissues including the lungs and muscle [28, 29]. Administering extracts of *O. striatus* in this study proved to significantly reduce TGF- β 1 level (p=0.022). However, studies conducted on patients with the chronic fibrosis-type disease, such as hepatic cirrhosis, showed similar results. The provision of nutrient supplementation in the form of BCAAs (leucine, isoleucine, and valine) can improve the condition of fibrosis in the liver. Furthermore, BCAAs are known to suppress the regulatory system of TGF- β 1 mRNA expression, which contributes to the process of fibrosis. Furthermore, the intake of omega-3 fish oil extract inhibits the transformation of epithelial cells into myofibroblasts, the processes in which are induced by TGF- β 1 [30].

Hosseini et al. [31] confirmed that the supplementation of 3,000 mg of omega-3 for eight weeks significantly reduced the levels of IL-17, C-reactive protein, and creatine kinase. In our study, the administration of 3,000 mg of *O. striatus* extracts for 12 weeks did not significantly decrease inflammatory markers: IL-17 (p=0.275) and neutrophils (p=0.619). According to the researchers,

this was due to the fact that obtained sample had severe COPD (GOLD 3 or 4), so the level of IL-17 and neutrophils did not decrease significantly. For neutrophils, a review conducted by Alex Pizzini et al. [32], use of Omega 3 supplementation in COPD patients led to decrease in serum neutrophil levels [32, 33].

Effects of *Ophiocephalus striatus* extract on quality of life in COPD patients

Various pulmonary and systemic manifestations that occur in COPD can result in limited daily activity, along with a reduced functional status and HRQoL in patients. Therefore, measurements of the health in COPD patients are necessary. Consequently, various instruments are used to determine the HRQoL in COPD patients. The CAT is a widely used questionnaire in daily clinical practice because it is short and easy to use. It describes the patient's condition adequately and comprehensively [34]. In this study, based on the average CAT scores, there was a significant improvement in the HRQoL ($p=0.000$) in stable COPD patients after 12 weeks of nutritional supplementation. The provision of nutritional therapy, both in the form of a high-protein diet and with the addition of nutritional supplementation, was shown to significantly improve the HRQoL in patients with COPD vs. the patients who did not receive nutritional therapy. The HRQoL, in this case, was assessed using the Seattle Obstructive Lung Disease Questionnaire (SOLDQ). The latter includes measurements of four health-related dimensions: physical, emotional, therapeutic, and coping skills [24].

Effect of *Ophiocephalus striatus* Extract on pulmonary diffusing capacity (DLCO)

Pulmonary function in the study subjects increased insignificantly. In line with another study [35] using nutritional supplementation of Omega-3 PUFA for 6 months, lung function failed to significantly improve, as measured by the forced expiratory volume in the first second (FEV1) and lung diffusion. Imbalances of protein metabolism, oxidative stress, and inflammatory processes are involved in the occurrence of muscle wasting, which further affects the structure and function of the muscles. In COPD patients, muscle wasting occurs not only in skeletal muscle but also in the respiratory muscles, thereby affecting respiratory processes and lung function [35]. Reduced body mass is closely related to lung function, decreased respiratory muscle strength, and lung diffusing capacity for carbon monoxide [36]. Administering 3,000 mg of *O. striatus* for 12 weeks improved the condition of muscle wasting via stopping the progression of airway damage, but it was not possible to restore the damage that has occurred. An insignificant increase in DLCO occurs through the improvement of muscle wasting conditions, which are characterized by increased BMI and FFMI values [36].

This study has several limitations that could possibly cause bias. The intervention was only performed in the form of nutritional supplementation, without medical rehabilitation in the form of physical exercise. There was no control of daily physical activity undertaken by study subjects, and the examination of pulmonary diffusing capacity was new for patients and included maneuvers that were quite difficult to perform. As a result, these maneuvers were often performed below the maximum, especially when taking into account the condition of the patients, since they were elderly with a fairly severe degree of COPD. Further research is needed involving an intervention in the form of nutritional

supplementation combined with a medical rehabilitation via physical exercise. Also, *O. striatus* extract could be administered for a longer period of time, especially if the sample included subjects with severe degrees of COPD.

Conclusion

The administration of 3,000 mg/day of *O. striatus* extract for 12 weeks in COPD patients with muscle wasting may decrease TGF- β 1 and muscle wasting (FFMI and BMI) but not IL-17, suPAR or neutrophil inflammatory markers. It may increase DLCO and TNF- α . Further research is needed with interventions in the form of a combination of nutritional supplementation and medical rehabilitation via physical exercise.

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Ethical statement

The authors are accountable for all aspects of the study in ensuring that questions related to the accuracy or integrity of any part of the research are properly investigated and resolved. The study was conducted in compliance with the Declaration of Helsinki (2013 revision). All procedures performed in studies involving human subjects were in accordance with the ethical standards of the institutional and/or national research committee and with 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee of Saiful Anwar General Hospital (No. 400/23/K.3/302/2016, No. 400/41/K.3/302/2016, and No. 400/47/K.3/302/2016), while individual consent for this study was waived.

Conflict of interest

All authors have completed the ICMJE uniform disclosure form for Potential Conflicts of Interest. The authors have no conflicts of interest to declare.

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