Original Article

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Effect of *Moringa oleifera* Leaf Extract on FGF21 mRNA Expression in Male Wistar Rats' Skeletal Muscle Under Sedentary Condition

Titing Nurhayati,[1] Karen Lolinhandary[2]

[1]Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran, Indonesia
[2]Faculty of Medicine, Universitas Padjadjaran, Indonesia

Abstract

Background: Fibroblast growth factor 21 (FGF21) is a muscle-derived myokine whose expression changes in response to elevated reactive oxygen species (ROS), primarily generated by cytochrome c oxidase complex IV (COX IV) in skeletal muscle. *Moringa oleifera* is known for its antioxidant potential, which may influence FGF21 expression and oxidative stress pathways. This study aimed to evaluate the effects of *Moringa oleifera* leaf extract on FGF21 mRNA expression and mitochondrial oxidative capacity in skeletal muscle under sedentary conditions.

Methods: This semiquantitative analytic study used 10 sedentary male Wistar rats, divided into control and treatment groups. The treatment group was administered 200 mg/kg of *Moringa oleifera* leaf extract, while the control group was given a standard diet and water for 12 weeks. COX IV and FGF21 mRNA levels in the soleus muscle were measured using real-time polymerase chain reaction. Statistical analysis included the Shapiro–Wilk normality test, Levene's homogeneity test, independent t-test, or Mann–Whitney test, with significance set at $p<0.05$.

Results: The treatment group exhibited lower COX IV (0.6414 vs 0.7388) and higher FGF21 (0.9414 vs 0.7157) mRNA levels compared to the control group; however, the differences were not significant ($p=0.354$ and $p=0.170$, respectively).

Conclusions: Although FGF21 may act as a therapeutic response to decreased mitochondrial activity, *Moringa oleifera* supplementation in sedentary conditions shows no significant effect on mitochondrial oxidative function or FGF21 expression. Nonetheless, these findings contribute to understanding metabolic regulation and highlight the potential role of antioxidants and active lifestyle interventions in promoting wellness and preventing muscle decline due to aging.

Keywords: Antioxidants, FGF21, mitochondria, *Moringa oleifera*, sedentary

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Correspondence:
Titing Nurhayati,
Department of Biomedical Sciences, Faculty of Medicine, Universitas Padjadjaran,
Jalan Ir. Soekarno Km. 21,
Jatinangor-Sumedang,
Indonesia

E-mail:
titing.nurhayati@unpad.ac.id

Introduction

Skeletal muscle remains one of the most dynamic tissues in the human body, holding a vital role in both mechanical and metabolic functions.[1] As it works with other organs, skeletal muscle can secrete myokines, which mediate inter-organ communication through metabolic pathways. Among these myokines, fibroblast growth factor 21 (FGF21) has attracted attention by affecting skeletal muscle in various ways, despite being minimally expressed under normal physiological conditions. FGF21 is typically increased during oxidative stress and may serve as a biomarker for certain diseases.[2,3] Oxidative stress in skeletal muscle may be influenced by a sedentary lifestyle, which increases reactive oxygen species (ROS) production and impairs mitochondrial function. This, in turn, stimulate the unfolded protein response in the organ and results in the release of FGF21 to restore the condition back to normal.[4,5] Cytochrome c oxidase complex IV (COX IV), the terminal complex in the mitochondrial oxidative phosphorylation (OXPHOS) pathway,

is considered a key indicator of mitochondrial oxidative capacity, function, and content.[6] COX IV, along with other mitochondrial proteins, is widely used as a marker of mitochondrial biogenesis, referring to the increase in mitochondrial number and size, an important feature of mitochondrial function in skeletal muscle.[7] Given the relationship between oxidative stress and FGF21 expression, as well as the involvement of COX IV in mitochondrial activity, it is important to investigate how these markers interact under sedentary conditions.

Previous studies have not reached a conclusive statement regarding the physiological roles of FGF21 in skeletal muscle in settings.[8] Research has explored FGF21 expression in various organs, including the liver, pancreas, heart and skeletal muscle.[9] Experimental stimuli used in previous studies on rat skeletal muscle include aerobic activity, fasting, insulin administration, and mitochondrial stress; among these, only aerobic activity consistently upregulated expression of FGF21 in skeletal muscles.[9,10] Compounds that have effects increasing, balancing, or decreasing FGF21 expression in skeletal muscles are still unclear. Natural sources of antioxidants are increasingly being studied for their pharmacological potential. One such source is *Moringa oleifera*, often referred to as the "miracle tree" due to its rich nutritional profile and known anti-inflammatory and antioxidant substances.[11] *Moringa oleifera* leaves contain 43 known antioxidant compounds, including rutin, quercetin, apigenin, gallic acid, salicylic acid, and gentisic acid.[12] These compounds are capable of significantly reducing ROS levels and are known to exert effects at the mitochondrial level.[13] Among these, flavonoids and phenolic acids are particularly potent antioxidants and may influence FGF21 expression by modulating oxidative stress pathways.

Although this plant has numerous pharmacological effects, the use of *Moringa oleifera* extract remains limited in clinical practice in Indonesia. Therefore, investigating the antioxidant potential of *Moringa oleifera* in relation to skeletal muscle FGF21 expression is of scientific and clinical interest. This study aimed to evaluate the effects of *Moringa oleifera* leaf extract on FGF21 mRNA expression and mitochondrial oxidative activity, as indicated by COX IV levels, in skeletal muscle under sedentary conditions. More broadly, this study seeks to contribute to the scientific basis for the medical application of *Moringa oleifera* in improving human health.

Methods

This study was a laboratory-based experimental study using stored biological samples from male Wistar rats. Ethical approval for the original experiment was granted by the Research Ethics Committee of Universitas Padjadjaran (No. 377/UN6/KEP/EC/2023), and this study was also approved by the same committee (No. 875/UN6.KEP/EC/2022). All procedures adhered to the ARRIVE guidelines and EU Directive 2010/63/EU for the care and use of laboratory animals. This study used an analytic semiquantitative method, conducted from October to November 2022 at the Central Laboratory of Universitas Padjadjaran, Jatinangor, West Java, Indonesia.

Twelve male Wistar rats were included in the study, with the following inclusion criteria: male sex, age 9–10 weeks, body weight between 250–275 g, and clinically healthy. Exclusion criteria included behavioral abnormalities, physical injuries, illness, changes in hair structure, and watery feces. After an adaptation period, the rats were randomly assigned to either a treatment group or a control group, with a study duration of 12 weeks. The control group received a standard diet and water ad libitum, while the treatment group received *Moringa oleifera* leaf extract.

The *Moringa oleifera* leaf extract used in this study was obtained from *Moringa oleifera* leaf powder provided by PT. Moringa Organik Indonesia (MOI), registered under BPOM RI No. MD 619111001777. The standardized powder was chosen to ensure consistency and quality, as it represents one of the best *Moringa oleifera* leaf products available in Indonesia.

The extract was dissolved in an aqueous solvent, as this solvent yielded the highest total antioxidant capacity. The dissolved extract was administered orally once daily at a dose of 200 mg/kg body weight. This dosage was extrapolated from the standard human dose for a 70 kg adult, using a conversion coefficient of 0.018, resulting in 3.6 mg of *Moringa oleifera* leaf extract per mL of solvent per 200 g rat.[14] The dose was adjusted individually for each rat according to body weight. The selected dose was based on previous studies demonstrating optimal antioxidant activi.[15] As the dosage was weight-based, the percentage yield of *Moringa oleifera* extract varied among individual animals. The procedures are illustrated in Figure 1.

The primary variables measured the mRNA expression levels of FGF21 and COX IV in the soleus muscles. The soleus muscle was chosen

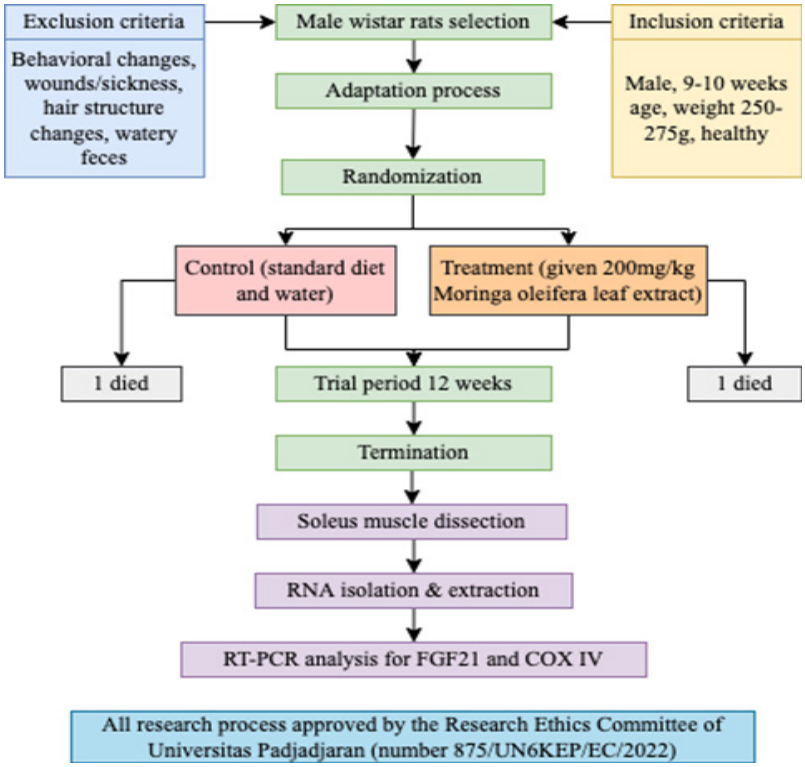

Figure 1 Methods and Experimental Procedure

due to its oxidative nature, which makes it a more sensitive indicator of oxidative stress than the gastrocnemius muscle.[16] It is also widely used in animal studies due to its composition of predominantly oxidative fibers and higher mitochondrial density.[17] The RNA isolation and extraction process were carried out on all 10 soleus muscle samples, and measurements were performed using the real-time polymerase chain reaction (RT-PCR) method.

Following euthanasia, the rats were dissected and soleus muscle tissues were collected and stored for analysis. RNA was isolated using Genezol reagent from all 10 muscle samples. RNA quality was confirmed through spectrophotometric analysis, with all samples meeting purity ratios between 1.9 and 2.1 and concentrations above 1,000 ng/µL. Reverse transcription polymerase chain reaction (RT-PCR; Aria Mix) was performed using specific primers for FGF21 and COX IV (Table 1). Gene expression was normalized to GAPDH as an internal control, and expression levels were calculated using the ΔΔCt method.

Data analysis was conducted using IBM SPSS version 27. The Shapiro–Wilk test was used to assess normality, and Levene's test was used to evaluate homogeneity of variance. Group comparisons were made using the independent t-test for normally distributed and homogeneous data, or the Mann–Whitney U test for non-normally distributed or heterogeneous data. A p-value of <0.05 was considered statistically significant. The results of these analyses are presented in the following sections.

Results

During the trial period, one rat in the control group and one in the treatment group died,

Table 1 Primer sequence used for RT-PCR Analysis of FGF21, COX IV, and GAPDH[7,18]

Gene Symbol	Sense Primer (5′→3′)	Antisense Primer (5′→3′)
FGF21	TTCGGGACTGTGGGTCTGTCTC	TTTGCAGGTGGGCTTCGGTG
COX IV	CTCCCATCTTATGTTGATCG	GTACAATTGGACTTTCTCATCC
GAPDH	GTTACCAGGGCTGCCTTCTC	GATGGTGATGGGTTTCCCGT

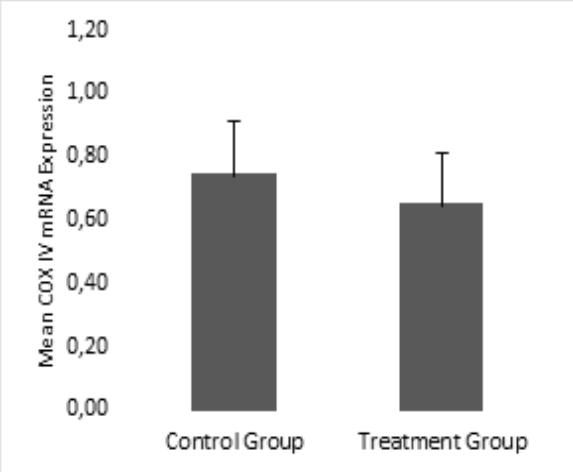

Figure 2 COX IV mRNA Expression in Control and *Moringa olifiera*-treated group. Data are presented as mean±SD

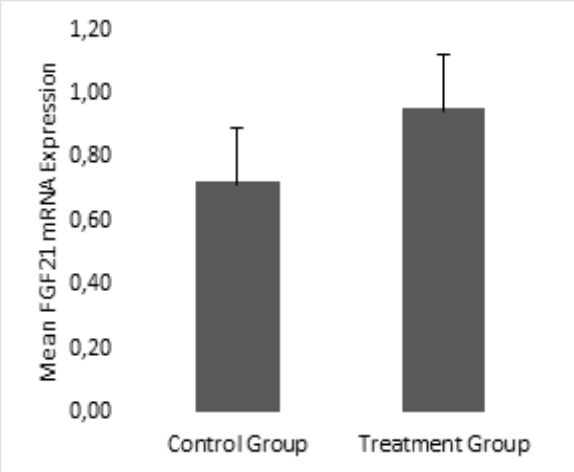

Figure 3 FGF21 mRNA Expression in Control and *Moringa olifiera-t*reated group. Data are presented as mean±SD

leaving five animals in each group for further analysis.

To evaluate the effect of *Moringa oleifera* leaf extract on FGF21 expression in skeletal muscle, COX IV and FGF21 mRNA levels in the soleus muscle of sedentary male Wistar rats were measured. A sedentary lifestyle may increase free radical levels in the body, affect mitochondrial function, and influence FGF21 expression.

The average ddCt values were calculated for both groups. Rats in the treatment group exhibited lower COX IV mRNA expression (mean=0.6414) compared to the control group (mean=0.7388), although the difference was not statistically significant (p=0.354; 95% CI) (Figure 2).

In contrast, FGF21 mRNA expression was higher in the treatment group (mean=0.9414) than in the control group (mean=0.7157), but this difference was also not statistically significant (p=0.170) (Figure 3).

Discussion

Moringa oleifera has been extensively studied in both the nutritional and medical field, due to the pharmacological potential of all its parts. Among these, the leaves are particularly valued due to their substantial nutritional and phytochemical content. *Moringa oleifera* leaf extract contains significantly higher levels of vitamins, minerals, amino acids, and antioxidants compared to many other food sources. For instance, it provides 10 times more vitamin A and 4 times more beta-carotene than carrots, 7 times more vitamin C than oranges, and 4 times more vitamin B1 than pork. It also contains 50 times more vitamin B2 than sardines and 50 times more vitamin B3 than nuts. The mineral profile is equally impressive, with 25 times more iron than spinach, 17 times more calcium than milk, and 15 times more potassium than bananas. Protein content is 9 times higher than yogurt, and the leaves also include 18 amino acids (8 of which are essential). In addition, MO provides 43 different natural antioxidants, 34 anti-inflammatory compounds, and higher quantities of fiber, GABA, and polyphenols compared to common food sources.[19]

Moringa oleifera is widely recognized as a functional food and dietary supplement with the potential to prevent malnutrition. Studies have highlighted its therapeutic benefits including glucose- and lipid-lowering effects, cardioprotective action, and inhibition of cholesterol synthesis. It also possesses anti-hyperglycemic, anti-inflammatory, anti-tumor, anti-cancer, and anti-microbial properties, suggesting potential as both a supplement and a novel herbal therapeutic agent.[20]

The antioxidant capacity of *Moringa oleifera* is largely attributed to its rich polyphenolic content. In this study, the most abundant compounds in *Moringa oleifera* leaf extract were quercetin (137.81 mg/g), kaempferol (106.75 mg/g), and gallic acid (105.67 mg/g), followed by isoquercitrin, quercitrin, chlorogenic acid, rutin, and ellagic acid. Even at lower concentrations, epicatechin and catechin contribute significantly to the plant's

free radical scavenging potential.[21] These compounds not only reduce ROS but also act on mitochondria, making them relevant in studies evaluating mitochondrial oxidative stress.

Antioxidants in *Moringa oleifera* leaves can significantly reduce ROS from hydrogen peroxide and have also been shown to work on mitochondria as their action target.[13] Our recent study also found that *Moringa oleifera* leaf extract is safe to be consumed in a daily dose of 200 mg/kg, as it shows no negative effects on the blood profile.[22] However, the use of *Moringa oleifera* as a daily supplement has not yet been widely studied to this date.

Skeletal muscle expresses FGF21 primarily in response to mitochondrial dysfunction or stress. Its expression increases with exercise,[10] though mitochondrial stress can also be a contributing factor.[23] Among various stimuli, oxidative stress remains the most consistent driver of FGF21 expression in skeletal muscle, while metabolic stimuli dominate its regulation in other organs.[24] Mitochondria are the main producers of reactive oxygen species (ROS) under physiological conditions, and when ROS levels exceed a certain threshold, it can result in muscle dysfunction. Low ROS levels can enhance muscle strength, while overproduction may impair function. With physiological training, muscle adapts to ROS, but antioxidant supplementation, especially under non-stressed conditions may impair this adaptive mechanism.[25]

A sedentary lifestyle increases ROS and disrupts mitochondrial function, causing oxidative stress that elevates unfolded protein levels and activates the unfolded protein response. This, in turn, induces FGF21 expression in skeletal muscle as part of the antioxidant defense mechanism.[4] COX IV is a critical enzyme in the mitochondrial oxidative phosphorylation (OXPHOS) pathway and represents mitochondrial oxidative function and content.[6] Thus, antioxidant interventions may alter COX IV expression depending on their impact on mitochondrial metabolism.

In this study, a nonsignificant decrease in COX IV expression in rats treated with *Moringa oleifera* leaf extract was observed, a result consistent with prior research.[27] This may be due to the antioxidant effects of MO reducing ROS levels, which in turn decreases OXPHOS activity and mitochondrial content, leading to reduced COX IV expression. Under sedentary conditions, antioxidant supplementation may impair basal mitochondrial activity. Our findings support the hypothesis that *Moringa oleifera,* although rich in antioxidants, may not enhance mitochondrial biogenesis under such conditions.

Conversely, the FGF21 expression was found to be higher in the *Moringa oleifera*-treated group, despite the reduced COX IV expression. This inverse relationship suggests that antioxidant-induced mitochondrial impairment may trigger a compensatory increase in FGF21, which is known to respond to deficiencies in OXPHOS.[28] FGF21 enhances oxidative and respiratory capacity, supports mitochondrial biogenesis, and may act as a biomarker for muscle-related mitochondrial disorders,[29] However, its precise role in skeletal muscle remains unclear compared to liver and adipose tissue.

Although the differences between the treatment and control groups were not statistically significant, the trends observed suggest that FGF21 may be induced in response to diminished mitochondrial oxidative capacity. This implies a negative correlation between FGF21 and COX IV levels, possibly reflecting the adaptive response to antioxidant-mediated mitochondrial suppression. Both genes are interconnected in regulating oxidative stress and may be modulated by *Moringa oleifera* supplementation.

This study has several limitations. This study only analyzed two gene markers, which may not provide a comprehensive picture of the oxidative stress pathway and was possible contributing to the insignificant difference of COX IV and FGF21 mRNA expression. Future studies should include additional markers such as PGC-1α (a key regulator of mitochondrial biogenesis) and superoxide dismutase (SOD), to better elucidate the molecular mechanisms involved.

In conclusion, FGF21 appears to act as a therapeutic response to impaired mitochondrial function. Whereas *Moringa oleifera* supplementation at a dose of 200 mg/kgBW does not significantly affect FGF21 or COX IV expression under sedentary conditions, it may have influenced mitochondrial oxidative pathways. Further study is proposed to evaluate different doses and include a broader gene expression profile to understand *Moringa oleifera*'s full pharmacological potential, particularly in aging-related mitochondrial decline.

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