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Short Communication

Effects of *Moringa oleifera* extract on inflammaging markers, muscle mass, and physical endurance in geriatric mice model

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Abstract

A comprehensive approach to managing frailty is required due to the increasing number of elderly. Physical frailty, associated with inflammatory processes and a loss of muscle mass, can significantly impair health status. The aim of this study was to determine the effect of *Moringa oleifera* Lam. leaf extract on frailty in geriatric mice model. An experimental study was conducted using a post-test-only control group design using *Mus musculus* Balb/C mice aged eight weeks. The animals were divided into five groups: negative control group received intraperitoneal injections of normal saline (0.5 cc/day) for eight weeks, positive control group received D-galactose (150 mg/kg body weight (BW)/day) only, and three treatment groups treated with 150 mg/kg of D-galactose intraperitoneally for eight weeks followed by 100 mg/kg BW, 200 mg/kg BW, and 400 mg/kg BW of *M. oleifera* leaf extract orally for five weeks. At the end of the study, the blood levels of malondialdehyde (MDA), transforming growth factor-beta (TGF-β) and caspase-3, as well as caspase-3 expression in skeletal muscle, skeletal muscle mass fibrosis, and physical endurance were measured. The results showed that a dose of 400 mg/kg/day of *M. oleifera* leaf extract had the most significant effect on lowering MDA, TGF-β, and caspase-3 expression in skeletal muscle tissue, skeletal muscle fibrosis, and improved physical endurance compared to other groups ($p<0.001$). This study highlights that *M. oleifera* leaf extract reduced frailty in geriatric mice model-induced using D-galactose by reducing inflammaging factors, thereby improving physical endurance.

Keywords: Frailty, geriatric, inflammaging, *Moringa oleifera*, muscle mass

Introduction

According to the United Nations, the global elderly population was estimated at 901 million in 2015 and projected to reach 2 billion by 2050 [1]. This change brings several problems, especially frailty, which is common in older people. Frailty is characterized by a progressive decline in physiological reserves across multiple organ systems, increasing vulnerability to adverse health outcomes. Age, functional status, poor nutritional status, reduced quality of life, and decreased walking speed are major predictors of frailty [2]. Early detection and intervention are critical to mitigate the adverse impacts, which include malnutrition, functional and cognitive decline, immobilization, elevated risk of falls, heightened morbidity, and mortality. These outcomes impose substantial burdens on individuals, families, communities, and healthcare systems.

Frailty encapsulates the biological aging process, driven by molecular and cellular mechanisms. Central to this phenomenon is inflammaging, a chronic, low-grade inflammatory

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state marked by elevated oxidative stress and the release of markers such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), transforming growth factor-beta (TGF-β), C-reactive protein (CRP), and malondialdehyde (MDA) [3,4]. These processes contribute to physiological decline, manifesting as sarcopenia, an essential component of physical frailty. Sarcopenia is identified by reductions in truncal and appendicular muscle mass, coupled with diminished muscle function, such as weakened grip strength and slower walking speed [5]. Studies, including the Atherosclerosis Risk in Communities (ARIC), have outlined a significant association between frailty and abnormalities in heart structure and function, suggesting a relationship between decreased skeletal and cardiac muscle mass during aging [6,7]. However, further studies are needed to elucidate these mechanisms and validate the associations.

The loss of muscle mass in aging is driven by factors such as oxidative stress, inflammation, cellular senescence, proteostasis imbalance, and muscle cell apoptosis [8]. Addressing frailty requires a comprehensive approach that includes medical care, personalized exercise plans, proper nutrition, and minimized polypharmacy to maintain the elderly functional status and quality of life [9,10]. Experimental studies have emphasized the importance of investigating therapies targeting inflammaging to develop effective interventions for frailty [10,11].

Bioactive compounds, such as curcumin, resveratrol, and flavonoids, have shown anti-aging properties, supported by studies on antioxidant-rich foods, including ginseng, turmeric, and berries [12]. Vitamin D and metformin have also been explored for the potential to enhance muscle mass and mitigate frailty, though careful consideration of side effects is warranted, given the susceptibility of the elderly to iatrogenic complications [13].

Moringa oleifera Lam. (MO) is a plant widely consumed in Indonesia due to affordability, accessibility, and perceived health benefits. The leaves, rich in phenols such as alkaloids, tannins, saponins, and flavonoids, have shown antioxidant and anti-aging properties [14,15]. Studies have reported the anti-inflammatory effect and topical applications in skin aging [16-18]. Despite these results, studies exploring the systemic effect of MO leaf on inflammaging or its role in enhancing skeletal and cardiac muscle mass during aging are limited. Therefore, the aim of this study was to analyze the effects of MO leaf extract on frailty in geriatric mice model.

Methods

Study design and setting

This experimental study used a post-test-only control group design with 30 male Balb/c mice divided into five groups. The negative control group (group C) was given an intraperitoneal injection of 0.5 cc normal saline for eight weeks, while positive control group (group G) was given an intraperitoneal injection of D-galactose 150 mg/kg/day for eight weeks. The treatment group consisted of T1, T2, and T3, which received injections of D-galactose (150 mg/kg/day) for eight weeks, followed by oral administration of MO leaf extract at doses of 100, 200, and 400 mg/kg for five weeks. At the end of the study, mice underwent a swimming test to measure physical endurance. Subsequently, 4 mL of mouse blood was collected through the orbital vein for examination of MDA, TGF-β, and caspase-3 using the enzyme-linked immunosorbent assay (ELISA) method. Mice were euthanized by decapitation after anesthesia using chloroform inhalation. Muscle caspase-3 expression was assessed using immunohistochemical examination. Masson's Trichrome staining was performed to assess the amount of fibrosis in the skeletal muscles (**Figure 1**). To ensure unbiased results, the authors were blinded to the group assignments, and all mice were treated, monitored, and handled identically throughout the experiment.

Animal preparation and eligibility

The inclusion criteria for the animals were male mice, BALB/c strain, aged eight weeks, body weight ranged 20–40 g, no physical disability, and normal activity. Mice that did not survive during the treatment period were excluded. The mice were fed a diet containing 6% fat from LabDiet 5K52 feed (LabDiet, Missouri, USA). The room followed a 12-hour light/dark cycle, with a maintained temperature of 23–26°C, ensuring a stable environment for acclimatization. This setup helped the mice adjust to the experimental conditions, including their biological rhythms

and surroundings. Factors such as water availability, cage density, temperature, and bedding changes were kept consistent across all groups. The cages, measuring 28×30×12 cm, provided enough space for movement to minimize stress.

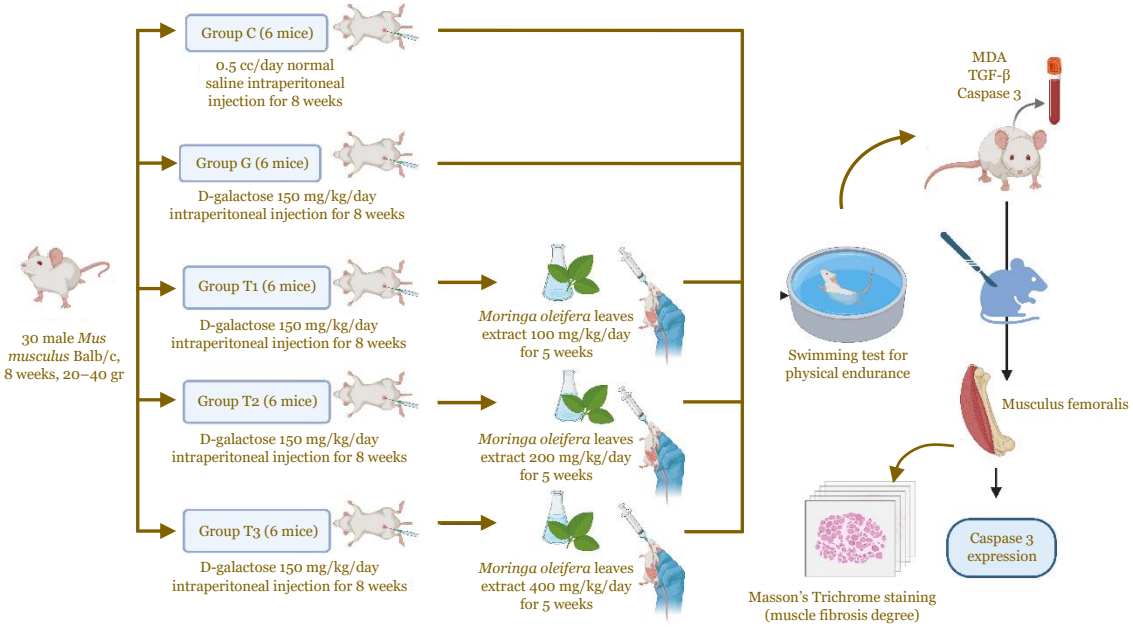

Figure 1. Overview of study design and experimental setting. This figure illustrates the overall study design, including the experimental timeline, grouping of subjects, intervention details, and measured key outcomes.

Sample size and randomization

Mice were randomly selected using the simple random sampling method and then divided into five groups. The sample size was determined by Federer's formula ((n – 1)(t – 1) >15); hence, the required minimum sample size was five mice for each group. One mouse was added to each group to anticipate mortality, making the final sample size 30.

Animal model

To understand the aging process and prevent the effect of aging on organs, D-galactose was administered artificially. This is possible because D-galactose, apart from causing mitochondrial dysfunction, could also increase oxidative stress, inflammation, and apoptosis, which will reduce the functional status of experimental animals. D-galactose administration has been used for various studies related to aging [19]. Furthermore, long-term, low-dose D-galactose has been widely used in animals to study the aging process and test anti-aging drugs [20,21]. The average recommended dose to induce aging in mice is 100–150 mg/kg subcutaneously/intraperitoneally over eight weeks [19-21]. In this study, D-galactose at a dose of 150 mg/kg/day was given through peritoneal injection for eight weeks.

Plant extract preparation

The MO leaf extract was sourced from the Borobudur Natural Herbal Industry in Magelang, Indonesia. The leaves extracts were washed with tap water and dried at 24°C for one day and subsequently oven-dried for two consecutive days at 45°C. The leaves were then grounded using a mechanical blender and stored in a vacuum container. To prepare the extract, 10 g of dried MO leaf powder was combined with 100 mL of distilled water, followed by a 24-hour infusion period at ambient temperature. Subsequently, the extract was stored at 4°C. The mixture was filtered twice using Whatman No. 42 filter paper (Sigma-Aldrich, Darmstadt, Germany) with a 2 µm pore size to remove particulates. The resulting aqueous extract stock solution (100 mg/mL) was maintained at 4°C for a maximum of five days. The total flavonoid content, quantified as quercetin equivalent (QE), was determined to be 10.73±0.31 mg QE per gram of dry weight of MO leaf. The active compounds in the extract were further analyzed by Markherb (EBM Saintifik dan

Teknologi, Bandung, Indonesia) for composition verification using high-performance liquid chromatography with ultraviolet detection (HPLC-UV) method for the effective analysis of polyphenols in MO leaves extract.

Study intervention

The treatment groups (T1, T2, and T3) initially underwent the same D-galactose injection protocol as group G (150 mg/kg/day for eight weeks) to induce aging-related changes. Following this period, the mice in these groups received oral administration of MO leaf extract at doses of 100 mg/kg (T1), 200 mg/kg (T2), and 400 mg/kg (T3) for an additional five weeks. This phase aimed to evaluate the potential protective and therapeutic effects of MO against D-galactose-induced aging.

Study outcomes

After five weeks of treatment, the blood levels of MDA, TGF-β and caspase-3, as well as the skeletal muscle caspase-3 expression and the degree of skeletal muscle fibrosis, were measured. Additionally, physical endurance during swimming was assessed.

MDA, TGF-β, and caspase-3 levels

About 4 mL of blood was collected through the orbital vein and the levels of MDA, TGF-β, and caspase-3 were measured using FineTest MDA ELISA Kit (Wuhan Fine Biotech, Wuhan, China), FineTest TGF-β ELISA Kit (Wuhan Fine Biotech, Wuhan, China), FineTest Caspase-3 ELISA Kit (Wuhan Fine Biotech, Wuhan, China), respectively. The measurement units of MDA, TGF-β, and caspase-3 were nmol/mL, pg/mL, and ng/mL, respectively.

Skeletal muscle caspase-3 expression

The expression of caspase-3 in skeletal muscle tissue (femur caspase-3) was measured using immunohistochemistry (IHC) based on antigen-antibody interactions targeting specific caspase-3 markers. The expression of caspase-3 was measured using caspase-3 Rabbit pAb kit (ABclonal Tech, Woburn, United States). Staining intensity and extent were manually quantified and converted into histology percentage scores, reflecting the levels of caspase-3 expression. The measurement unit of caspase-3 expression in femoral skeletal muscle is percentage (%), which shows the comparison between cells stained with caspase-3 and those unstained in the entire visual field.

Skeletal muscle mass fibrosis

Skeletal muscle tissue was fixed in 4% paraformaldehyde to preserve cellular morphology, followed by staining with Masson's Trichrome reagent (Bio Optica Milano, Milano, Italia) to determine collagen fibers and distinguish areas of fibrosis. Histological sections were then analyzed under a light microscope by a trained histopathologist. The degree of fibrosis was graded on a scale from 0 to 3, where grade 0=normal tissue without fibrotic changes, 1=mild fibrosis, 2=moderate fibrosis, and 3=severe fibrosis. For semiquantitative assessment, fibrosis extent was visually evaluated and categorized as follows: score 0=no fibrosis, +=fibrosis affecting less than 30% of the examined muscle tissue, ++=fibrosis in 30–60% of muscle tissue, and +++=fibrosis in more than 60% of the examined muscle area. The measurement unit of skeletal muscle mass fibrosis was percentage (%), showing a comparison between muscle tissue that stained positive for fibrosis (green region) and the entire visual field.

Physical endurance

To assess physical endurance, a swimming test was conducted on mice. A block-shaped water tank (50 cm long, 25 cm high, and 30 cm wide) was used for this purpose. Briefly, mice swam until signs of fatigue were observed, including submersion of the head for 5 to 7 seconds, a bent torso, a stretched tail, and immobility in all four limbs. The time taken for each mouse to reach exhaustion was recorded. Afterward, the mice were promptly removed from the water, dried with a towel, and warmed using a hair dryer to ensure recovery. Physical endurance was measured in minutes.

Statistical analysis

Levene's test was used to assess the homogeneity of variances, while the Shapiro-Wilk test was conducted to check for normality. For data that met the assumptions of normality and homogeneity, a one-way analysis of variance (ANOVA) was performed to evaluate the effect of MO. To determine differences between groups, the least significant difference (LSD) post-hoc test was applied. For data that did not meet normality and homogeneity criteria, non-parametric tests were used instead. For comparisons between two groups, the Mann-Whitney U test was applied, while for comparisons involving more than two groups, the Kruskal-Wallis test was performed. A *p*-value of less than 0.05 was considered statistically significant. SPSS version 25 for Windows (IBM, New York, USA) was used for statistical analysis.

Results

Effect of *Moringa oleifera* extract on malondialdehyde (MDA) levels

The results of MDA levels based on the treatments are presented in **Table 1**. The lowest MDA level was in group C and the highest MDA level was in group G. In the treatment group, the MDA level was lowest in group T3, suggesting the treatment of MO 400 mg/kg had the best results. There was a significant difference in the MDA levels between the treatments, $p<0.001$ (**Table 1**).

Table 1. Effect of *Moringa oleifera* extract on malondialdehyde (MDA) levels

Group	MDA level (nmol/mL)	*p*-value
	Mean±SD	
C (normal saline, negative control)	0.96±0.19	<0.001*
G (D-galactose only, positive control)	10.71±0.32	
T1 (D-galactose+100 mg/kg *Moringa oleifera* extract)	6.27±0.23	
T2 (D-galactose+200 mg/kg *Moringa oleifera* extract)	4.93±0.30	
T3 (D-galactose+400 mg/kg *Moringa oleifera* extract)	4.22±0.35	

Analyzed using Kruskal-Wallis test
*Statistically significant at $p=0.05$

Based on the ANOVA test results, there was a significant difference in MDA ($p<0.01$). Post hoc LSD was conducted, and the results indicated that group G differed significantly from all treatment groups (T1, T2, and T3), suggesting all treatments significantly reduced MDA levels. Group C showed a significant difference from all treatment groups (T1, T2, and T3) with a $p<0.001$, suggesting MDA levels were reduced by all treatments (**Figure 2**).

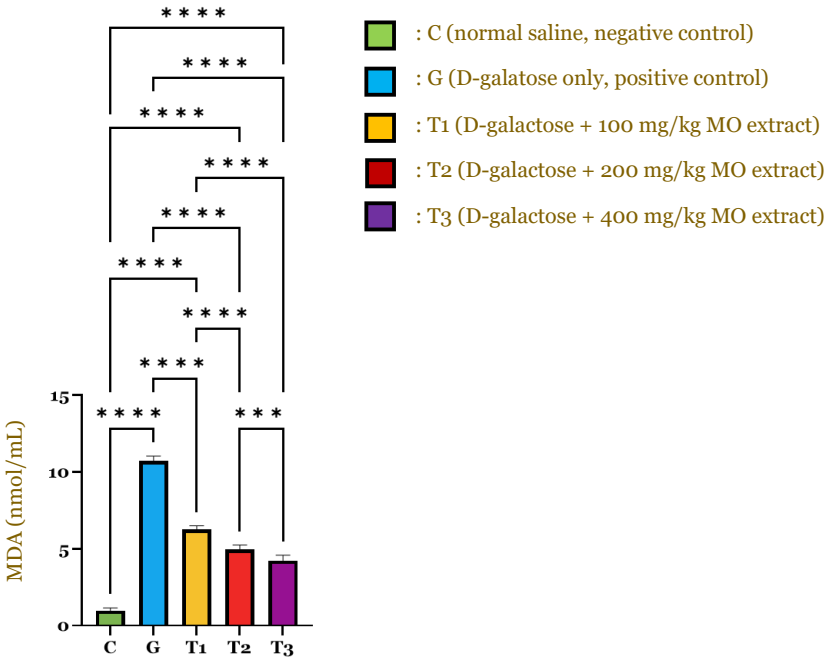

Figure 2. Comparison of malondialdehyde (MDA) levels between study groups. Statistical analysis was performed by post hoc LSD test. Significant at ***$p<0.001$; ****$p<0.0001$.

Effect of *Moringa oleifera* extract on tumor growth factor-beta (TGF-β) levels

The results of TGF-β levels based on the treatments are presented in **Table 2**. The lowest TGF-β level was in group C and the highest MDA level was in group G. In the treatment group, the TGF-β level was lowest in group T3, suggesting the treatment of MO 400 mg/kg had the best results. There was a significant difference in the TGF-β levels among the study groups, $p<0.001$ (**Table 2**).

Table 2. Effect of *Moringa oleifera* extract on transforming growth factor-beta (TGF-β) levels

Group	TGF-β level (pg/mL) Mean±SD	*p*-value
C (normal saline, negative control)	5.14±0.31	<0.001*
G (D-galactose only, positive control)	22.57±0.50	
T1 (D-galactose+100 mg/kg *Moringa oleifera* extract)	15.78±1.12	
T2 (D-galactose+200 mg/kg *Moringa oleifera* extract)	11.81±0.41	
T3 (D-galactose+400 mg/kg *Moringa oleifera* extract)	9.56±0.48	

Analyzed using Kruskal-Wallis test
*Statistically significant at $p=0.05$

Post hoc LSD was conducted, and the results indicated that group G differed significantly from all treatment groups (T1, T2, and T3), suggesting all treatments significantly reduced the TGF-β levels (**Figure 3**). Group C showed a significant difference from all treatment groups (T1, T2, and T3), with a $p<0.001$, suggesting TGF-β levels were reduced by all treatments (**Figure 3**).

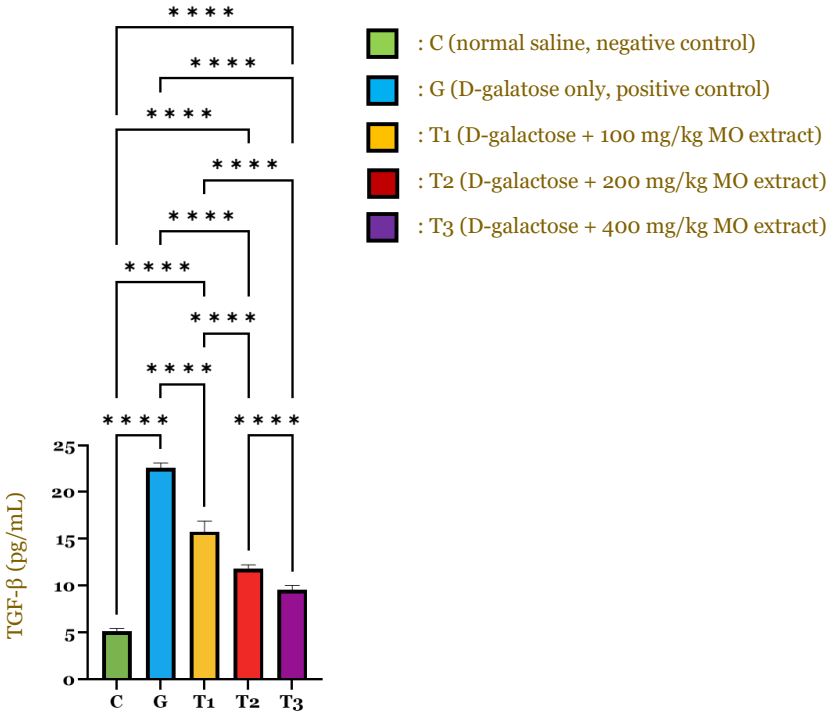

Figure 3. Comparison of TGF-β levels between study groups. Statistical analysis was performed by post hoc LSD test. Significant at ***$p<0.001$; ****$p<0.0001$.

Effect of *Moringa oleifera* extract on blood caspase-3 levels

The results of caspase-3 levels based on the treatments are presented in **Table 3**. The lowest caspase-3 value was in group C and the highest caspase-3 level was in group G. Among the treatment groups, the lowest caspase-3 level was in group T3, suggesting the treatment of MO 400 mg/kg had the best results. There was a significant difference in the caspase-3 levels among study groups, $p<0.001$ (**Table 3**).

Table 3. Effect of *Moringa oleifera* extract on caspase-3 levels

Group	Caspase-3 level (ng/mL) Mean±SD	*p*-value
C (normal saline, negative control)	1.37±0.03	<0.001*
G (D-galactose only, positive control)	7.86±0.29	
T1 (D-galactose+100 mg/kg *Moringa oleifera* extract)	4.99±0.32	
T2 (D-galactose+200 mg/kg *Moringa oleifera* extract)	3.99±0.25	
T3 (D-galactose+400 mg/kg *Moringa oleifera* extract)	2.19±0.19	

Analyzed using Kruskal-Wallis test
*Statistically significant at p=0.05

Post hoc Dunn test was conducted, and the results indicated that group G differed significantly from all treatment groups (T1, T2, and T3), suggesting all treatments significantly reduced caspase-3 levels groups (**Figure 4**). Group C had a significant difference from the treatment groups (T1 and T2) with a p<0.001 but not from group T3, suggesting MO 400 mg/kg had the fastest effect in lowering caspase-3 levels compared to other groups (**Figure 4**).

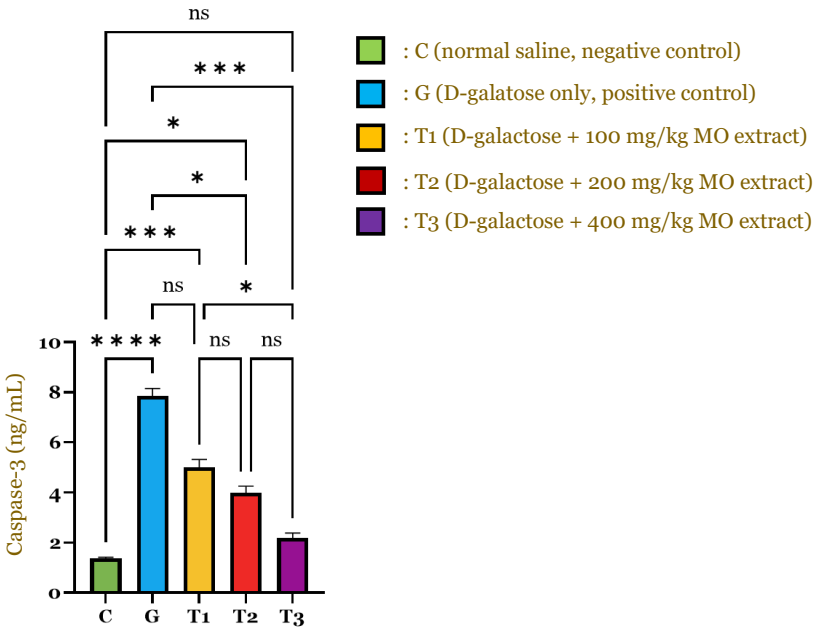

Figure 4. Comparison of caspase-3 levels between study groups. Statistical analysis was performed by post hoc Dunn test. ns: not significant. Significant at *p<0.05; ***p<0.001; ****p<0.0001.

Effect of *Moringa oleifera* extract on femur caspase-3 expression

The results of femur caspase-3 expression based on the treatments are presented in **Table 4**. Among treatment groups, the lowest caspase-3 expression was in group T3, suggesting the treatment of MO 400 mg/kg had the best effect. There was a significant difference in the caspase-3 expression among treatment groups, p<0.001 (**Table 4**).

Table 4. Effect of *Moringa oleifera* extract on femur caspase-3 expression

Group	Femur caspase-3 (%) Mean±SD	*p*-value
C (normal saline, negative control)	1.66±2.58	<0.001*
G (D-galactose only, positive control)	45.83±11.14	
T1 (D-galactose+100 mg/kg *Moringa oleifera* extract)	38.33±7.52	
T2 (D-galactose+200 mg/kg *Moringa oleifera* extract)	30.00±0.00	
T3 (D-galactose+400 mg/kg *Moringa oleifera* extract)	28.33±4.08	

Analyzed using Kruskal-Wallis test
*Statistically significant at p=0.05

Post hoc Dunn test was conducted, and the results indicated that the expression within the T2 and T3 treatment groups was significantly lower compared to group G, suggesting both

treatments significantly decreased femur caspase-3 expression. Supplementation with MO 200 mg/kg and 400 mg/kg significantly decreased femur caspase-3 expression in the geriatric mice model, while a lower dose of 100 mg/kg had no effect. The MO 400 mg/kg treatment produced the best result in lowering femur caspase-3 levels compared to the other groups (**Figure 5**).

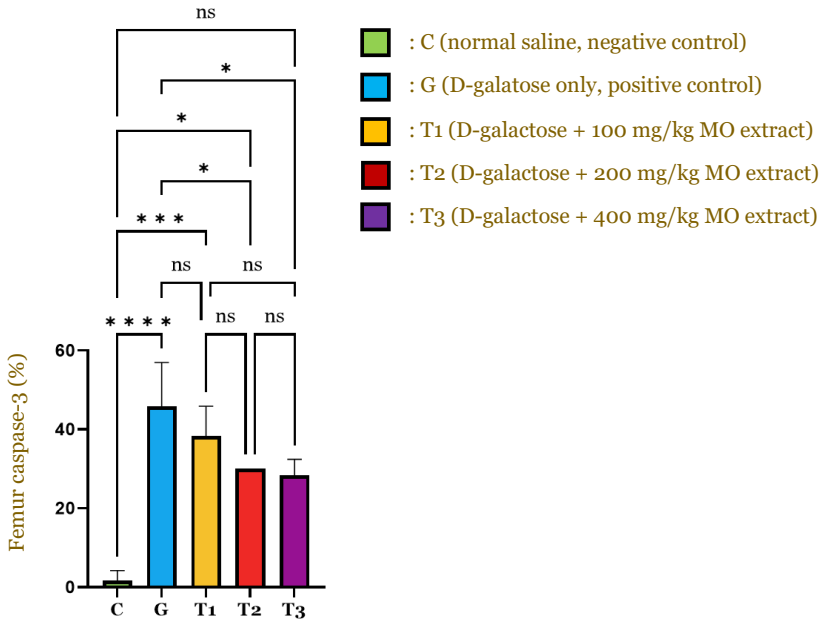

Figure 5. Comparison of femur caspase-3 expression between study groups. ns: not significant. Significant at $^{*}p<0.05$; $^{***}p<0.001$; $^{****}p<0.0001$.

Effect of *Moringa oleifera* extract on skeletal muscle mass fibrosis

The results of skeletal muscle (femur) mass fibrosis based on the treatments are presented in **Table 5**. The lowest skeletal muscle mass fibrosis was in group C and the highest skeletal muscle mass fibrosis was in group G. There was a significant difference in the skeletal muscle mass fibrosis between the treatments, $p<0.001$ (**Table 5**).

Table 5. Effect of *Moringa oleifera* extract on femur skeletal muscle mass fibrosis

Group	Skeletal muscle mass fibrosis (%)	*p*-value
	Mean±SD	
C (normal saline, negative control)	5.00±0.00	<0.001*
G (D-galactose only, positive control)	21.67±2.58	
T1 (D-galactose+100 mg/kg *Moringa oleifera* extract)	11.66±2.58	
T2 (D-galactose+200 mg/kg *Moringa oleifera* extract)	10.83±2.04	
T3 (D-galactose+400 mg/kg *Moringa oleifera* extract)	5.00±3.16	

Analyzed using Kruskal-Wallis test
*Statistically significant at $p=0.05$

Post hoc Dunn test was conducted and the results indicated that T2 and T3 treatment groups had lower value compared to group G, suggesting both treatments significantly decreased skeletal muscle mass fibrosis. MO 200 mg/kg and 400 mg/kg significantly decreased skeletal muscle mass fibrosis in the geriatric mice model, with the higher dose produced the best result (**Figure 6**).

Effect of *Moringa oleifera* extract on physical endurance

The results of skeletal physical endurance based on the treatments are presented in **Table 6**. Among the treatment groups, the physical endurance was highest in group T3, suggesting the treatment of MO 400 mg/kg had the best results. There was a significant difference in physical endurance between the treatments, $p<0.001$ (**Table 6**).

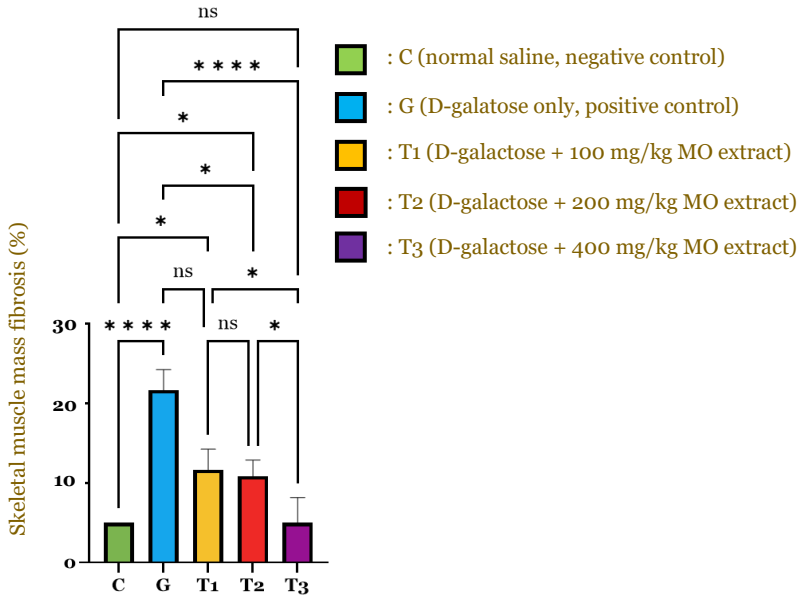

Figure 6. Comparison of femur skeletal muscle mass fibrosis between study groups. Statistical analysis was performed by post hoc Dunn test. ns: not significant. Significant at $^{*}p<0.05$; $^{****}p<0.0001$.

Table 6. Effect of *Moringa oleifera* extract on physical endurance

Group	Physical endurance (minutes) Mean±SD	*p*-value
C (normal saline, negative control)	50.55±0.59	<0.001*
G (D-galactose only, positive control)	29.91±0.38	
T1 (D-galactose+100 mg/kg *Moringa oleifera* extract)	35.39±0.31	
T2 (D-galactose+200 mg/kg *Moringa oleifera* extract)	36.15±1.19	
T3 (D-galactose+400 mg/kg *Moringa oleifera* extract)	39.73±0.79	

Analyzed using Kruskal-Wallis test
*Statistically significant at $p=0.05$

Post hoc Dunn test indicated that group G had lower physical endurance compared to the T2 and T3 treatment groups, suggesting both treatments significantly increased physical endurance. The MO 400 mg/kg treatment produced the best result in increasing physical endurance compared to the other groups (**Figure 7**).

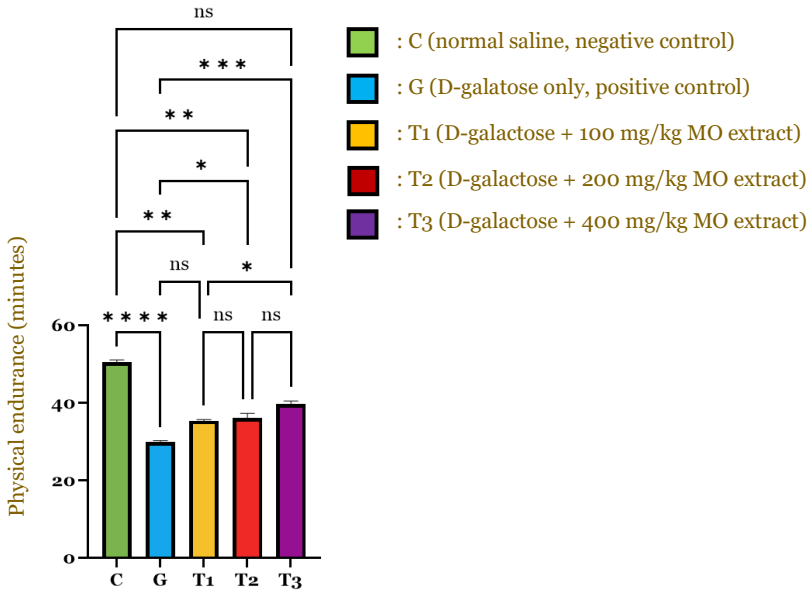

Figure 7. Comparison of physical endurance between study groups. Statistical analysis was performed by post hoc Dunn test. ns: not significant. Significant at $^{*}p<0.05$; $^{***}p<0.001$; $^{****}p<0.0001$.

Discussion

Based on the results, the age indicators increased when D-galactose was administered intraperitoneally. Excess D-galactose caused ROS levels to increase, which in turn led to oxidative stress, inflammation, cell death, and mitochondrial malfunction (**Figure 8**) [22]. MDA, TGF-β, and caspase-3 levels significantly decreased after eight weeks of administering D-galactose at 150 mg/kg/day, followed by five weeks of MO leaf extract administration at doses of 100, 200, and 400 mg/kg, compared to the positive and negative control groups. Furthermore, the IHC analysis showed that there was a substantial decrease in caspase-3 expression in the T1, T2, and T3 groups compared to the positive and negative control groups.

Anti-inflammatory medicines could be used as part of a treatment strategy to stop oxidative stress and fibrosis from developing in the heart and skeletal muscle. Quercetin and glycosinolate, which are two flavonoid-containing MO natural compounds, showed biological activity as antioxidants and anti-inflammatory agents. This study showed that administering MO extract to mice exposed to D-galactose reduced MDA expression. In another study, MO seed extract increased antioxidant levels such as T-SOD and GPx but decreased MDA, ROS, and NOX 4 in mice with diabetic nephropathy [23]. The treatment inhibited pro-inflammatory cytokines and inflammatory pathways caused by lipid peroxidation products, such as MDA, at a dose of 390 to 960 mg/kg BW at a human equivalent dose [24].

The results showed that D-galactose-induced geriatric model mice had increased blood levels of TGF-β. Tissue fibrosis increased with aging due to TGF-β, which played a significant part. According to previous studies, TGF-β was crucial for the process of vascular fibroproliferation [25]. TGF-β1 promotes cell differentiation, proliferation, apoptosis, autophagy, and the synthesis of extracellular matrix (ECM). One important element affecting renal fibrosis is the TGF-β1/Smad2/3 pathway [23].

Mice blood levels of caspase-3 significantly increased when D-galactose induction was administered. Apoptosis is induced by several genes alongside aging, with proapoptosis factors such as caspase-3, 9, and 8 increasing while p53 and Bcl-2 regulation decreased [26]. Based on the results, the administration of MO extract significantly decreased caspase-3 levels at all doses. Quercetin, a flavonoid component found in MO, functions as a proapoptotic agent by enhancing Bcl2 and decreasing Bax regulation, hence inhibiting the apoptotic process [20]. A previous study found that MO extract lowered caspase-3 activation in nephrotoxic model mice and the antioxidant inhibited caspase-3 signaling [27].

A significant difference was found in skeletal muscle mass fibrosis between different treatment formulations. Compared to other groups, MO 400 mg/kg treatment produced the fastest result in improving skeletal muscle mass fibrosis. One of the main causes of muscular weakening and decreased function is skeletal muscle fibrosis, a defining feature of aging. MO reduces the excessive buildup of ECM components, particularly collagen, potentially caused by changes in ECM-degrading activities, excessive ECM production, or a combination of the two, known as fibrosis [28]. In these pathologic circumstances, MO creates novel anti-fibrotic treatments to reverse fibrosis [29]. According to a previous study, cirrhosis of the liver caused an increase in collagen-1, α-SMA, and TIMP-1 all at the same time, worsening fibrosis [29]. By lowering the expression of all three indicators, ethanol extract of MO leaves inhibited the development of liver fibrosis [30].

The results showed that D-galactose-induced geriatric model mice had a significant increase in physical endurance. D-galactose enhances redox expression in the soleus and gastrocnemius muscles, according to a previous study [31]. People become physically weak at old age, due to increased redox reactions [32]. Poor skeletal muscular strength occurs due to mitochondrial dysfunction in muscle cells caused by D-galactose-induced aging [33]. Compared to the control group, the geriatric mice model induced with D-galactose had poorer swimming test capacity. However, a significant increase in physical endurance was observed with MO extract administration. Compared to dosages of 100 and 200 mg, the 400 mg/kg extract produced the best results regarding physical endurance. By promoting the mobilization and usage of body fat, reducing the breakdown of glycogen stores, and delaying the accumulation of blood lactate and urea nitrogen, MO could help mice swim better and combat tiredness [34]. Taken together, the proposed mechanism of actions of MO extract as potential anti-aging is presented in **Figure 8**.

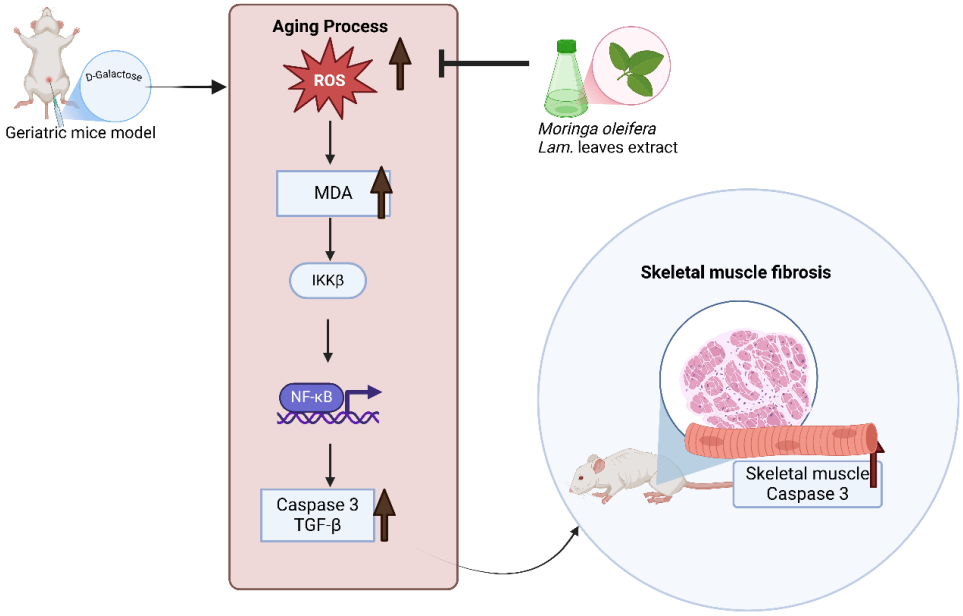

Figure 8. Proposed mechanism of action of *Moringa oleifera* leaves extract in anti-aging in geriatric mice model.

Conclusion

MO leaves extract reduced frailty in geriatric mice model induced by D-galactose by reducing inflammaging factors in the blood (MDA, TGF-β, and caspase-3), skeletal muscle tissue (caspase-3), and skeletal muscle fibrosis thereby improving physical endurance. These results offer the groundwork for future studies into approaches beyond the constraints of using geriatric model mice in lab settings. Phase 1 clinical trials with dose conversion for humans could be conducted using this preclinical report as a foundation.

Ethics approval

The study protocol received ethical approval from the Health Study Ethics Committee of Moewardi Hospital, Surakarta, Indonesia (approved number: 899/IX/HREC/2021).

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Competing interests

All the authors declared that there are no conflicts of interest.

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Underlying data

Derived data supporting the results of this study are available from the corresponding author on request.

Declaration of artificial intelligence use

We hereby confirm that no artificial intelligence (AI) tools or methodologies were utilized at any stage of this study, including during data collection, analysis, visualization, or manuscript preparation. All work presented in this study was conducted manually by the authors without the assistance of AI-based tools or systems.

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