Ciuccio et al. Functional, morphological, and biochemical effects of *Moringa oleifera* leaf extract in mdx mice. ABCS Health Sci. [Epub ahead of print]; DOI: 10.7322/abcshs.2025152.3205

ORIGINAL ARTICLE

Received: Nov 18, 2025

Revised: Jan 08, 2026

Approved: Jan 29, 2026

Functional, morphological, and biochemical effects of *Moringa oleifera* leaf extract in mdx mice

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Declaration of interests: nothing to declare

https://doi.org/10.7322/abcshs.2025152.3205

ABSTRACT

Introduction: Duchenne muscular dystrophy (DMD) is the most common hereditary myopathy in childhood. The dystrophin protein, encoded by the DMD gene, is essential for maintaining the integrity of the muscle fiber sarcolemma, and its absence leads to progressive muscle degeneration. The lack of a known cure underscores the importance of alternative therapies aimed at mitigating disease progression. *Moringa oleifera* (MO) has been studied for its anti-inflammatory and antioxidant properties, which may contribute to reduced inflammation and muscle degeneration. **Objective:** To evaluate the effects of MO leaf extract on functional, morphological, and biochemical parameters in mdx mice, a murine model of DMD. **Methods:** Male mdx mice and C57BL/10 controls, aged 2 months, were treated for 8 weeks with MO leaf extract (300 mg/kg/day, oral gavage) or saline. Muscle strength was assessed using the inverted screen test. Morphological analysis of the quadriceps, extensor digitorum longus, tibialis anterior, and diaphragm included measurement of minimum Feret diameter and percentage of fibers with internalized nuclei. Serum levels of creatine kinase, AST/TGO, ALT/TGP, and urea were evaluated. **Results:** Treated dystrophic animals showed reduced AST/TGO levels, increased muscle strength, and a higher proportion of fibers with internalized nuclei, indicating modulation of regeneration and reduced tissue damage. Decreased AST/TGO suggests reduced sarcolemmal disruption, while increased internalized nuclei is consistent with an active regenerative response. **Conclusion:** MO treatment promoted functional improvement and partial attenuation of tissue damage, suggesting a beneficial role in regeneration and reduced muscle injury in dystrophic muscle.

Keywords: Duchenne muscular dystrophy; *Moringa oleifera*; muscular dystrophies; inflammation; mdx mice

INTRODUCTION

Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD) is a severe and progressive neuromuscular disease and is considered the most common hereditary myopathy in childhood, with an incidence of approximately 1 in every 5,000 live male births[1]. The inheritance pattern is X-linked recessive and results from mutations in the dystrophin gene (*DMD*, #300377), whose encoded product—the dystrophin protein—is essential for sarcolemmal stability and maintenance of muscle fiber integrity[2,3]. The absence or deficiency of this protein leads to continuous cycles of necrosis and regeneration, causing inflammation, progressive fibrosis of the endomysial connective tissue, and gradual loss of muscle strength, which defines the clinical course of the disease[2].

Symptoms manifest in childhood as motor delays and progressive muscle weakness, leading to loss of ambulation in adolescence. Proximal weakness, calf pseudohypertrophy, and gait abnormalities are observed, along with respiratory and cardiac impairment in advanced stages. Elevated serum creatine kinase (CK) levels can aid in diagnosis[4-6].

Life expectancy has increased with advances in respiratory rehabilitation and the use of corticosteroids. More recently, the approval of the first gene therapy for DMD highlights the development of new therapeutic strategies[7,8].

Mdx mice

The mdx mouse is the animal model most commonly used to study the pathophysiology of DMD and to evaluate new therapeutic approaches that may benefit patients[9]. In this model, a point mutation occurs in exon 23 due to a premature termination

https://doi.org/10.7322/abcshs.2025152.3205

codon, resulting in loss of dystrophin protein function. The mice exhibit muscle weakness, increased CK levels, muscle necrosis, and respiratory problems[10,11]. The animals are physiologically and histologically similar to boys affected by the disease but have a shorter lifespan compared with other models, which facilitates analysis[8].

Mdx mice are widely used because of their reproducibility, accessibility, and pathophysiological similarity to human DMD[11]. Histologically, the persistent degeneration and regeneration of skeletal and cardiac muscle observed in affected patients is very similar to that seen in the mdx animal model[12].

Muscle morphology in mdx mice is initially preserved, with peak disease severity observed between 8 and 16 weeks of age. This phase is accompanied by necrosis, regenerated fibers with internalized nuclei, and elevated CK levels. The diaphragm muscle is an exception, showing progressive degeneration and respiratory impairment[13].

Moringa oleifera

Moringa oleifera (MO) is a plant of the Moringaceae family with nutritional and pharmacological properties, including anti-inflammatory, neuroprotective, and antioxidant effects.

Studies indicate that MO exerts anti-inflammatory effects by inhibiting activation of the NF-κB pathway, an important regulator of the inflammatory response[14-16]. Activation of the NF-κB pathway precedes the onset of the dystrophic process in mdx mice[17]. This pathway depends on signaling mediated by TNF-alpha, a pro-inflammatory cytokine[18], and MO inhibits the formation of pro-inflammatory mediators, including TNF-alpha itself[19].

In DMD, the sarcolemma becomes fragile and discontinuous. As a result, substances pass from the intracellular to the extracellular environment, such as the

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enzyme CK, as well as from the extracellular to the intracellular environment, as occurs with calcium ions[20]. This intracellular increase in calcium favors enhanced production of reactive oxygen species due to abnormal mitochondrial uptake of this ion[21].

An important antioxidant defense system exists in human cells and is essential for eliminating reactive oxygen species produced under normal conditions. This system is mainly composed of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase[22]. However, one of the pathophysiological characteristics of DMD is the persistence of oxidative stress due to exhaustion of the antioxidant enzyme system, resulting in a series of deleterious biological effects observed in the disease[23].

A previous *in vitro* study using MO leaf extract demonstrated improvement in the oxidative state of muscle cells through increased activity of enzymes such as superoxide dismutase, catalase, and glutathione peroxidase[24]. DMD is a progressive and fatal disease, and its standard treatment is associated with significant side effects and limited long-term efficacy.

The use of MO appears promising because of its antioxidant and anti-inflammatory activities. Its ability to inhibit NF-κB pathway activation, reduce the expression of pro-inflammatory cytokines, and improve antioxidant enzyme activity, as demonstrated in preclinical and *in vitro* studies, highlights its therapeutic potential in the pathophysiology of DMD, which is marked by oxidative stress and chronic inflammation[25,26].

Therefore, the objective of this study was to evaluate the effects of MO leaf extract administration on muscle strength, skeletal muscle morphological parameters (fiber diameter and internalized nuclei), and biochemical markers of muscle damage and hepatorenal toxicity in mdx mice.

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METHODS

Study of MO in mdx mice

Animals

Fifteen male mdx mice (2 months old) and 17 C57BL/10 mice were used. The animals were obtained from and maintained in the FMABC animal facility under controlled ventilation, with filtered water and feed provided *ad libitum*. The project was approved by the FMABC Animal Use Ethics Committee (protocol no. 5/2022).

Experimental study

The animals were divided into four groups: C57BL/10 control and mdx control (n=8/group), treated with saline solution; C57BL/10 treated with MO leaf extract (300 mg/kg; n=9); and mdx treated with MO leaf extract (300 mg/kg; n=7). Treatments were administered by gavage, in a volume of 0.2 mL, once daily.

Extract preparation

The MO leaf extract was prepared according to the methodology previously described[27,28] and stored in an alcoholic solution.

Analysis of muscle strength in the four limbs using the Kondziela inverted screen test

Muscle strength of the four limbs was assessed in all mice at weeks 0, 3, 5, and 7 using the Kondziela inverted screen test. The test was performed according to the standards and recommendations of the TREAT-NMD protocol, The use of four limb hanging tests to monitor muscle strength and condition over time. The holding impulse

https://doi.org/10.7322/abcshs.2025152.3205

was calculated using the following equation: holding impulse (gms) = body weight (g) × time the animal remained suspended (sec)[29].

Material collection

After 8 weeks of treatment, the mice were euthanized by anesthetic overdose with sodium thiopental (100 mg/kg), administered intraperitoneally. Blood samples were collected via caudal vena cava puncture for CK analysis. The quadriceps (QUA), extensor digitorum longus (EDL), tibialis anterior (TA), and diaphragm (DIAF) muscles were collected from both limbs. Hepatic and renal toxicity were evaluated by measuring aspartate aminotransferase/glutamic-oxaloacetic transaminase (AST/TGO), alanine aminotransferase/glutamic-pyruvic transaminase (ALT/TGP), creatinine, and urea levels.

Histological analysis of muscles

Muscles were embedded in Tissue-Tek O.C.T., frozen in liquid nitrogen, and stored at −80°C. Sections 8 µm thick were obtained using a cryostat.

The sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope to assess the presence of fibers with internalized nuclei, fibers with peripheral nuclei, and fiber diameter. ImageJ software (http://rsb.info.nih.gov/ij/index.html) was used to measure the minimum Feret diameter of the fibers and the percentage of internalized nuclei. The fields were analyzed using a Nikon Eclipse E-800 light microscope.

Statistical analysis

Statistical analyses were performed using GraphPad Prism software (San Diego, CA). Data are presented as mean ± standard deviation and were statistically compared

https://doi.org/10.7322/abcshs.2025152.3205

using one-way and/or two-way analysis of variance followed by Bonferroni's test. Confidence levels above 95% ($p<0.05$) were considered statistically significant.

RESULTS

Histological analysis of muscles

The architecture of the collected muscles was analyzed by light microscopy using photomicrographs with a 50-µm scale bar (Figure 1). Dystrophic muscle from mdx mice showed wide variation in fiber caliber and a high percentage of fibers with internalized nuclei. In healthy animals (C57BL), muscle fibers were uniform and polygonal, with no variation in caliber, and nuclei were peripherally located. The diaphragm muscle of healthy animals is represented by panels A and B in figure 1, while dystrophic muscle is represented by panels C and D.

Comparison of Feret diameter between mdx and C57BL (control) mice at the end of 60 days of treatment

In the DIAF muscle (Figure 2A), healthy animals treated with MO leaf extract showed an increased mean Feret diameter compared with the saline-treated control group. Control C57BL mice had a mean fiber diameter of 29.8±4.01 µm, whereas C57BL mice treated with MO extract had a mean of 30.7±1.89 µm. In mdx mice, those treated with extract showed a lower mean diameter than the mdx control group. The mdx control group had a mean of 26.5±3.21 µm, while the treated mdx group had a mean of 24.5±2.53 µm, with no statistically significant difference between groups ($p>0.05$).

In the QUA muscle (Figure 2B), the mean fiber diameter was 52.4±6.01 µm in control C57BL animals and 57.4±5.59 µm in MO-treated C57BL animals. In mdx mice,

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the mean values were 50.5±11.20 µm in the control group and 50.4±4.50 µm in the treated group, with no statistically significant differences observed ($p>0.05$).

The healthy C57BL groups treated with extract showed reduced mean diameters compared with the group treated with saline solution. The same pattern was observed in the mdx group treated with extract, with a reduction in the mean compared with the mdx control group, bringing values closer to those of the healthy control group. In the EDL muscle (Figure 2C), control C57BL animals had a mean diameter of 41.3±9.84 µm, while those treated with MO had a mean of 40.8±6.51 µm. In mdx animals, the control group had a mean of 50.2±13.64 µm, while the treated group had a mean of 42.3±4.72 µm, approaching values observed in healthy controls, without statistical significance ($p>0.05$).

Analysis of the TA muscle was the only Feret diameter assessment that showed statistical significance. There was a statistically significant increase in fiber diameter in control animals treated with MO leaf extract compared with untreated controls ($p=0.0087$), which may reflect a slight improvement in muscle integrity. In mdx mice, fiber diameter decreased in the treated group compared with the mdx control group, without statistical significance. In the TA muscle, control C57BL animals had a mean diameter of 44.63±3.84 µm, while MO-treated animals had a mean of 49.35±5.57 µm. In mdx animals, the control group had a mean of 48.04±13.64 µm, while the treated group had a mean of 47.02±4.05 µm. TA data are shown in figure 2D.

Comparison of the percentage of fibers with internalized nuclei in mdx and C57BL groups after 60 days of treatment

The ratio between fibers with internalized nuclei and total fibers was calculated, and the results are shown in figure 3. The average percentage of fibers with internalized nuclei was higher in mdx animals than in healthy control animals in all groups analyzed,

https://doi.org/10.7322/abcshs.2025152.3205

which is a characteristic of the model. Healthy groups showed nearly zero internalized nuclei, as expected. In all groups analyzed, MO leaf extract increased the percentage of internalized nuclei in both dystrophic and healthy animals.

The relevance of this increase was observed when comparing the mdx control group with the treated mdx group. In the EDL muscle, the percentage of fibers with internalized nuclei was 70.1%±2.09% in the mdx control group and 79.7%±5.03% in the treated mdx group, with a statistically significant difference between groups (p=0.0209). In the C57BL control group, the percentage was 0.36%±0.11%, while in the treated C57BL group it was 1.26% ±0.90%. The increase observed in the MO-treated mdx group suggests intensification of the active muscle regeneration process.

No statistically significant differences were observed in the other muscle groups analyzed. In the TA muscle, the percentage of fibers with internalized nuclei was 72.33%±7.8% in the mdx control group and 75.18%±4.8% in the treated mdx group. In the C57BL control group, the percentage was 1.2%±0.22%, and in the treated C57BL group it was 0.24%±0.47%. In the QUA muscle, the percentage was 67.84%±13.15% in the mdx control group and 68.15%±13.13% in the treated mdx group. In the C57BL control group, the percentage of fibers with centralized nuclei was 1.07%±0.28%, while in the treated C57BL group it was 2.03%±0.11%.

In the DIAF muscle, the percentage of fibers with internalized nuclei was 32.74%±6.04% in the mdx control group and 37.63% ± 9.36% in the treated mdx group. In the C57BL control group, the percentage was 0.57%±0.7%, and in the treated C57BL group it was 0.64%±0.62%.

https://doi.org/10.7322/abcshs.2025152.3205

Comparison of serum CK levels between mdx and C57BL groups after 60 days of treatment

With MO treatment, a slight reduction in CK levels was observed in mdx mice. CK values were 128.25±76.97 U/L in the C57BL control group, 107.7±123 U/L in the treated C57BL group, 3177.62±1432.45 U/L in the mdx control group, and 2219.62±774.35 U/L in the treated mdx group. Although CK levels were reduced in treated mdx animals, the difference was not statistically significant (Figure 4A).

Comparison of serum urea concentration in mdx and C57BL (control) mice after 60 days of treatment

Urea levels were evaluated to assess possible renal toxicity of the extract in both dystrophic and healthy animals. Mdx animals treated with MO extract showed improvement in their urea profile. No statistically significant variation was observed, suggesting absence of renal toxicity associated with MO treatment (Figure 4B). Urea levels were 63.16±5.72 mg/dL in the C57BL control group, 66±8.76 mg/dL in the treated C57BL group, 57.32±11.12 mg/dL in the mdx control group, and 47.88±9.44 mg/dL in the treated mdx group.

Comparison of liver transaminase levels in mdx and C57BL mice after the experiment

Serum ALT levels showed a slight reduction in the healthy group treated with MO compared with the control group, suggesting absence of liver toxicity. In treated mdx mice, ALT levels also showed a slight decrease without statistical significance, with values of 157.23±44.56 U/L in the mdx control group and 129.27±38.07 U/L in the treated mdx group. Regarding AST, a statistically significant reduction was observed in

https://doi.org/10.7322/abcshs.2025152.3205

dystrophic animals treated with MO (p=0.0209), with values of 764±189.09 U/L in the mdx control group and 551±132.46 U/L in the treated mdx group. This finding suggests attenuation of muscle damage and reduced sarcolemmal permeability, likely related to the antioxidant effect of MO.

Comparison of grip strength (g·s) in mdx and C57BL mice after the study

Figure 5 shows grip strength results throughout the experimental protocol (weeks 0, 3, 5, and 7). The mdx group treated with MO extract showed increased strength compared with the mdx saline-treated group at all time points except test number 3. The healthy group treated with extract showed increased muscle strength from the first test onward, which persisted until the end of treatment, although without statistical significance.

The MDX control group exhibited the lowest muscle strength values at nearly all experimental time points. The MDX + MO group showed progressive improvement over time, reaching significantly higher values at the end of treatment. Grip strength was 1370±360 g·s in the MDX control group and 1593±343 g·s in the MDX + MO group (p=0.0116).

The C57BL/10 control and C57BL/10 + MO groups maintained stable performance without functional impairment, indicating that the extract did not compromise muscle performance. Final values were higher than those observed in dystrophic animals, with grip strength of 2132±495.21 g·s in the C57BL control group and 2674±457.56 g·s in the C57BL + MO group.

https://doi.org/10.7322/abcshs.2025152.3205

DISCUSSION

When muscle architecture is considered, dystrophic muscle from mdx mice exhibits marked variation in fiber caliber and a high proportion of fibers with internalized nuclei, resulting from mutation of the dystrophin gene. The absence of dystrophin renders muscle cells more susceptible to mechanical stress and stretching.

The presence of internalized nuclei is notable in conditions involving muscle disorder and is characteristic of diseases such as DMD[30]. This feature reflects ongoing processes of degeneration, regeneration, and muscle inflammation that define DMD[31,32].

In the pathophysiology of the disease, disruption of the connection between the cytoskeleton and the extracellular matrix favors the development of inflammatory and fibrotic areas[33]. In healthy animals (C57BL), the presence of dystrophin ensures uniform muscle fibers without disproportion in caliber and maintains nuclei in a peripheral position.

One of the main parameters evaluated in this study was serum CK because elevated CK levels are a hallmark of DMD. CK is an intracellular enzyme that leaks into the extracellular environment due to loss of cell membrane integrity[20]. A tendency toward reduced serum CK levels was observed in mdx mice treated with MO extract compared with mdx control animals. This was reflected by lower mean CK values in treated animals (≈2219 U/L) compared with mdx controls (≈3177 U/L), suggesting reduced cell fragility and muscle damage, although without statistical significance. This trend is consistent with the antioxidant and anti-inflammatory effects described for MO[34], indicating a potential protective effect on muscle tissue. These findings are consistent with a recent study[35] using oral fast skeletal myosin inhibitor therapy in patients with dystrophinopathies, which demonstrated CK reduction associated with modulation of muscle contraction and decreased damage to fibers weakened by dystrophin deficiency.

https://doi.org/10.7322/abcshs.2025152.3205

Muscle strength is expected to be reduced in dystrophic animals compared with healthy controls. In this study, treatment with MO extract resulted in increased muscle strength in both healthy and dystrophic animals. In functional testing, mdx mice treated with MO showed a significant increase in grip strength at the end of the experimental protocol (1593±343 g·s) compared with mdx control animals (1370±361 g·s; p=0.0116), indicating functional improvement associated with treatment.

This result aligns with previous studies reporting improved muscle strength in rats with corticosteroid-induced muscle atrophy following treatment with MO extract combined with physical exercise[36]. These findings reinforce the potential of MO to improve muscle strength and endurance parameters.

Regarding Feret diameter, comparative analyses were performed among groups because mdx animals treated with MO tended to show mean values closer to those observed in healthy controls, suggesting possible muscle preservation. In healthy C57BL animals, TA muscle fiber diameter increased significantly in the MO-treated group (49.35 µm) compared with untreated controls (44.63 µm; p=0.0087). In mdx animals, fiber diameters in the TA (47.02 vs. 48.04 µm) and EDL (42.3 vs. 50.2 µm) muscles were reduced in treated animals compared with mdx controls; however, these differences were not statistically significant. These reductions brought values closer to those observed in healthy control muscles. A statistically significant increase in the percentage of fibers with internalized nuclei was observed in the EDL muscle of mdx mice treated with MO extract (p=0.0209). Internalized nuclei are expected to be more frequent in dystrophic tissue due to continuous cycles of inflammation, regeneration, and fibrosis characteristic of DMD[31,32], which was confirmed in the present study.

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According to the literature[31], muscle regeneration in mdx mice stabilizes around 6 months of age. In this study, mice were 4 months old at the time of euthanasia, indicating that active regeneration and inflammation were ongoing.

Under physiological conditions, mature skeletal muscle fibers contain peripherally located nuclei; however, following injury, satellite cell activation leads to the formation of new fibers, with nuclei remaining internalized until complete maturation[37,38]. Thus, increased internalized nuclei may reflect an active regenerative stage. This interpretation is supported by the observed reduction in serum AST levels, which was statistically significant in treated mdx animals (≈551 U/L) compared with mdx controls (≈764 U/L; p=0.0209). Reduced AST suggests lower muscle damage and decreased sarcolemmal permeability. Together, these findings suggest that MO treatment promoted muscle tissue regeneration and remodeling, although full morphological stabilization had not yet occurred within the 8-week experimental period. Longer treatment durations may clarify whether this early regenerative response progresses toward fiber maturation and nuclear repositioning, indicating structural recovery.

Measurement of liver enzymes was performed to assess potential hepatic toxicity and muscle necrosis. Because dystrophin deficiency allows intracellular enzymes to leak into circulation, transaminase levels are often elevated in DMD and may lead to misdiagnosis of liver disease, as these enzymes are also produced by muscle cells[36]. In this study, mdx mice exhibited higher serum transaminase levels than C57BL mice. ALT levels did not differ significantly between groups, and elevated values in mdx animals likely reflect muscle involvement rather than liver toxicity. This pattern supports the conclusion that elevated transaminases in mdx mice are primarily associated with muscle damage caused by sarcolemmal fragility, consistent with literature describing MO effects on skeletal muscle integrity[36].

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In pioneering studies of muscle pathology, Dubowitz identified internalized nuclei as a characteristic feature of fibers undergoing repair, reflecting structural reorganization following necrosis[39]. This nuclear repositioning, in contrast to the peripheral arrangement of mature fibers, indicates recent myoblast fusion and formation of new myofibers derived from satellite cells. Internalized nuclei are therefore recognized as a morphological marker of muscle regeneration and a histological biomarker of regenerative activity in DMD[39,40].

Thus, the morphological framework proposed by Dubowitz[39] aligns with the contemporary interpretation by Guiraud[40], reinforcing that the increase in internalized nuclei observed in this study likely represents not only prior degeneration but also an adaptive regenerative response stimulated by MO, possibly mediated by its antioxidant and anti-inflammatory properties.

Conclusion

Treatment with MO demonstrated beneficial effects on functional, biochemical, and morphological parameters in mdx mice. Biochemical analysis revealed a significant reduction in AST/TGO levels in treated mdx animals, suggesting decreased muscle damage without evidence of liver toxicity. Functional testing showed significant improvement in muscle strength, indicating preservation of contractile performance and attenuation of disease-related impairment. The increased proportion of fibers with internalized nuclei in treated animals suggests an active regenerative response, potentially associated with the antioxidant and anti-inflammatory effects of MO extract.

These findings support the therapeutic potential of MO in preserving functional integrity in dystrophic muscle tissue. However, additional studies with longer treatment durations are required to confirm and extend these observations.

https://doi.org/10.7322/abcshs.2025152.3205

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Figure 1: Photomicrographs of diaphragm muscles treated with *Moringa oleifera* leaf extract. (A) Diaphragm muscle of a normal control mouse (C57BL/10) and (B) diaphragm muscle of a C57BL/10 mouse treated with extract. In both cases, fiber diameter and peripheral nuclei are similar, with no relevant morphological differences. (C) Diaphragm muscle of a control mdx mouse and (D) diaphragm muscle of an mdx mouse treated with leaf extract. Both show similar morphology, characterized by reduced fiber caliber, the presence of larger fibers, centralized nuclei, and an expanded interstitial region. No notable morphological differences were observed between control and treated mdx muscles. Scale bar = 50 µm.

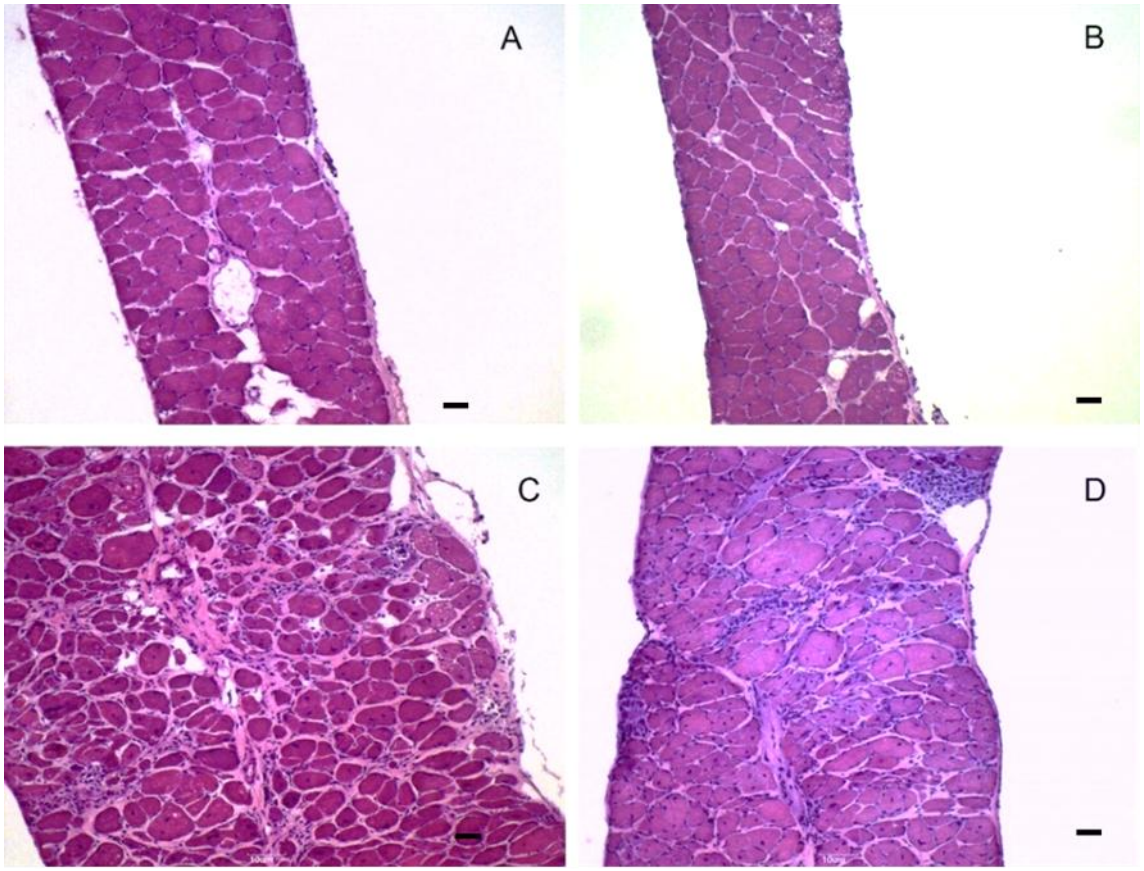

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Figure 2: Comparison of Feret diameters among analyzed muscles. (A) Mean diameters of diaphragm fibers, showing that C57BL/10 animals treated with *Moringa oleifera* extract had a slightly higher mean than did controls, while treated mdx animals had a lower mean than did dystrophic controls. No statistically significant differences were observed ($p>0.05$). (B) Quadriceps muscle, showing increased mean fiber diameter in treated C57BL/10 animals compared with healthy controls, while treated mdx animals showed reduced mean diameter compared with dystrophic controls, without statistical significance ($p>0.05$). (C) Extensor digitorum longus muscle, showing a slight reduction in mean fiber diameter in treated healthy animals compared with saline-treated controls, also without statistical significance. (D) Tibialis anterior muscle, showing a significant increase in mean fiber diameter in treated C57BL/10 mice compared with controls ($p=0.0087$), while treated mdx animals showed a reduction in mean diameter without statistical significance ($p>0.05$).

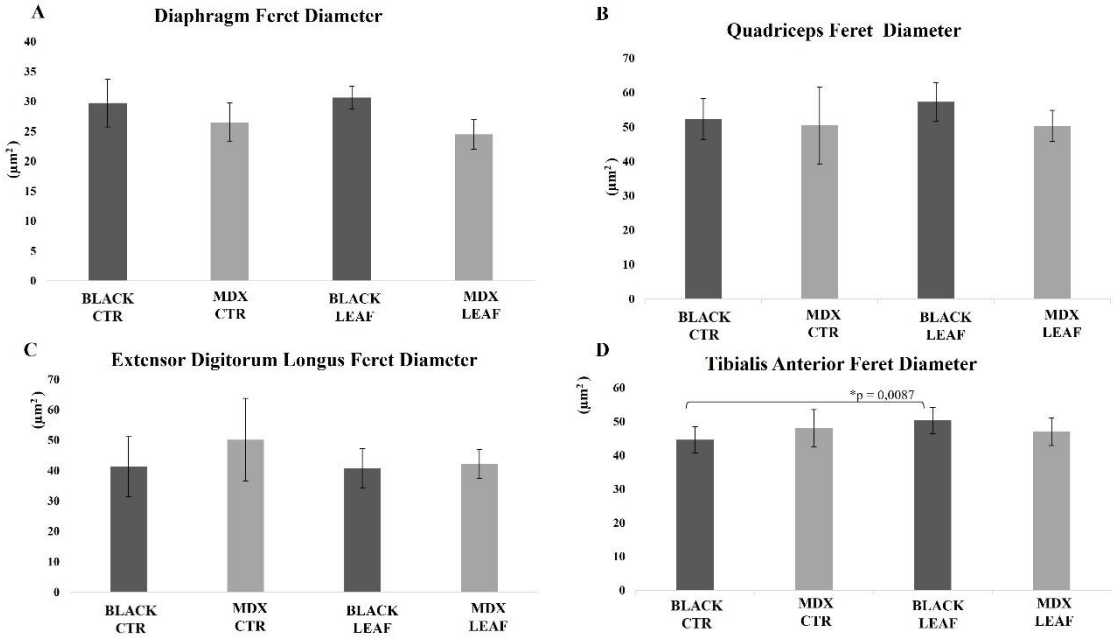

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Figure 3: Percentage of fibers with internalized nuclei in the analyzed muscles. Mdx animals exhibited a higher proportion of fibers with centralized nuclei in all muscles compared with healthy animals. (A) Diaphragm, (B) quadriceps, (C) extensor digitorum longus, and (D) tibialis anterior. A statistically significant difference was observed in the extensor digitorum longus muscle, where mdx animals treated with *Moringa oleifera* extract showed an increased percentage of internalized nuclei compared with mdx controls (p=0.0209).

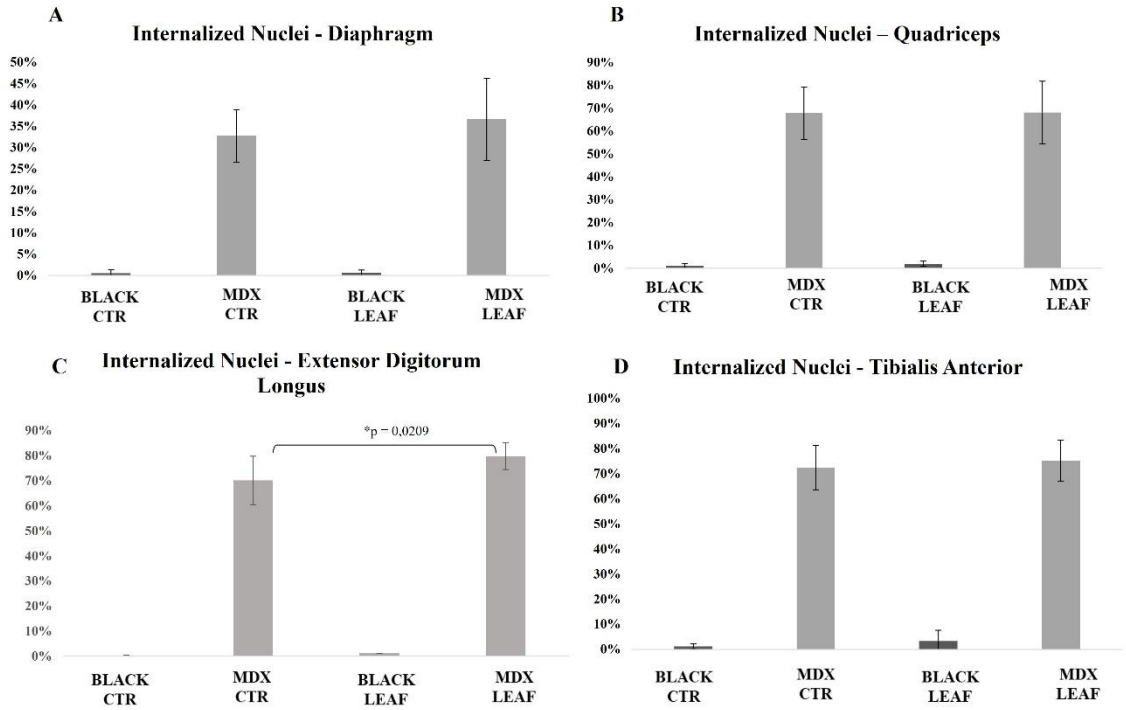

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Figure 4: Serum concentrations of biochemical markers in the experimental groups. (A) Serum creatine kinase levels, showing reduced values in mdx animals treated with *Moringa oleifera* extract compared with untreated mdx controls. (B) Serum urea levels, showing a slight increase in healthy animals treated with extract compared with saline-treated controls. (C) Alanine aminotransferase levels, showing a slight reduction in mean values in extract-treated control animals compared with saline controls. (D) Aspartate aminotransferase levels, showing a statistically significant reduction in treated mdx animals compared with mdx controls (p=0.0209).

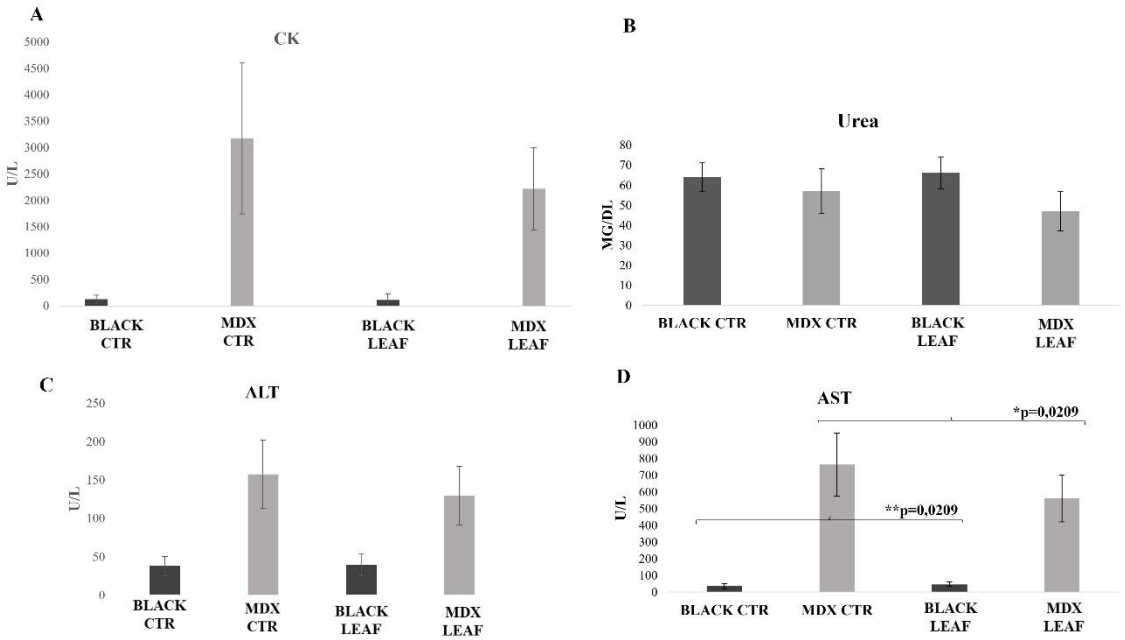

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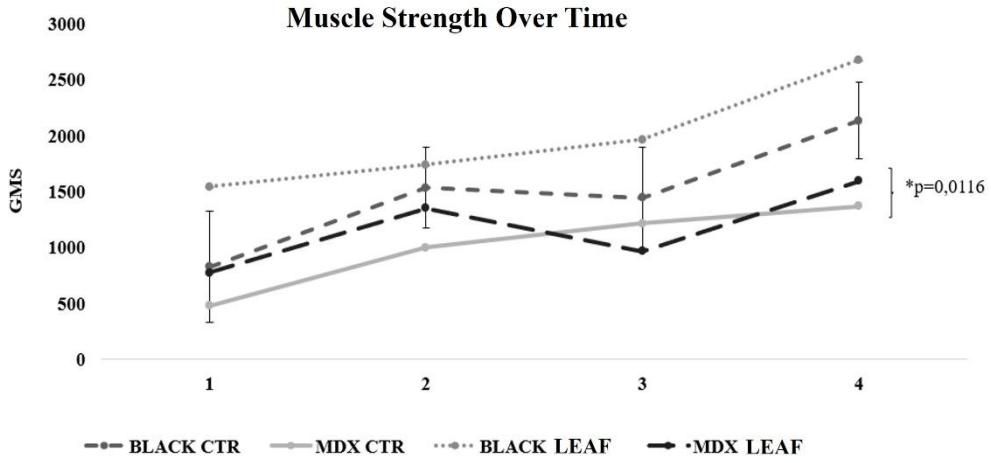
Figure 5: Measurement of grip strength over four time points (weeks 0, 3, 5, and 7). Healthy animals treated with *Moringa oleifera* extract showed a progressive increase in muscle strength from the first assessment, which was maintained through the end of the experimental period. Mdx animals treated with extract showed a significant increase in muscle strength compared with mdx controls at the end of the protocol (p=0.0116), indicating a potential beneficial effect of treatment on muscle function.



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