



## Original Research Article

Effect of *Moringa oleifera* leaf extract on exercise and dexamethasone-induced functional impairment in skeletal muscles

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## ABSTRACT

**Background:** Chronic administration of steroids like dexamethasone produces symptoms including weight loss and skeletal muscle dysfunction. Similar events are reported in chronic or high-intensity exercises, that can lead to fatigue and muscle damage.

**Objective:** In the present study, the effect of *Moringa oleifera* leaf extract was evaluated against dexamethasone (Dex) and exercise (Exe)-induced muscle changes in rats.

**Materials and methods:** Six groups each containing 6 rats, namely normal, Dex control, Exe Control, Dex + *M. oleifera* leaf extract (300mg/kg p.o.), Dex + Exe, Dex + Exe + *M. oleifera* leaf extract were assessed in the study. Dex was administered at 0.6 mg/kg i.p. daily for 7 days. Exercise was given for a total of 10 days after 30 minutes of dosing using treadmill equipment for 900 seconds at speed 18 m/min. Animals were assessed for variation in body weight, muscular endurance using treadmill, locomotor activity using actophotometer, motor coordination using rotarod on day zero, and day seven. Hemidiaphragm of rats were isolated and used for evaluation of the glucose uptake. Gastrocnemius muscle was isolated and subjected to hematoxylin and eosin staining.

**Results:** Dex and Exe control animals showed a significant decrease in skeletal muscle activity when compared to normal control animals in the actophotometer test. Improvement in endurance were seen in Dex + *M. oleifera* leaf extract, and Dex + exercise + *M. oleifera* leaf extract groups compared to Dex control group. Improvement in locomotor activity was seen in Dex group subjected to exercise and was significant when treated with *M. oleifera* leaf extract. Histology reports were in accordance with the functional parameters.

**Conclusion:** *M. oleifera* leaf extract supplemented with exercise showed a reversal in the dexamethasone-induced functional impairment in skeletal muscles.

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## 1. Introduction

Muscle dysfunction is defined as impairment in the ability of the muscle to perform the task effectively, which is attributed to central as well as peripheral factors [1,2]. Central factors include central nervous system (CNS) control of skeletal muscle by its sensory and motor nerves. Peripheral factors include muscle contraction coupling mechanism components namely actin-myosin, energy,

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and other essential factors. These peripheral and central changes in muscle contraction are seen prominently in the ageing population due to denervation, in patients suffering from cardiac and pulmonary diseases [3–5]. Apart from them, general muscular dysfunction due to chronic exercise and sports are also quite common. Factors affecting exercise-induced muscle dystrophy include the duration of exercise, the intensity of force applied, degree of training, etc. These factors cause reduction in force-generating capacity of muscle due to the depletion of energy and reduction in any necessary substances for contraction [1,6].

During chronic exercise, there is a loss of energy and a large amount of water with salts (potassium, and magnesium) in nutritionally significant quantities through sweat. Iron loss in sweat may contribute to iron deficiency, which is evident in some endurance runners. However, in athletes, adaptive mechanisms protect the body against electrolyte depletion. For a long time, athletes and sports personalities also use herbal supplementation for improvement in sports performance. These supplements help them improve their performance and gain a competitive edge. It also helps the body prepare for exercise and reduce the chance of injury. Some of them also aid in the recovery process. However, only a few supplements have scientific evidence to prove their claim of aiding in the improvement of athletic performance. Products such as protein powders, energy boosters, creatinine, and many more have a large market share in the synthetic supplement category. Athletes use these supplements to get rapid benefits. However, herbal, or natural medicines are also being consumed for ages, and hence can be assumed as safe and reliable. Most herbal medicines contain flavonoids as their principal constituents. Among the flavonoids, quercetin is one of the major constituents, which has various biological activity and health benefits. The use of flavonoids like quercetin has proven to effectively improve muscular endurance. The last few decades have seen a significant increase in the use of herbals along with studies proving their potential to facilitate muscular endurance. They are rich in bioactive compounds such as polyphenols, terpenoids, alkaloids, and flavonoids, which exert their beneficial activities in the body. One of the extensively studied herbal drug believed to improve muscle activity is *Moringa oleifera*, which is commonly known as Moringa or drumstick tree is a drought-resistant tree belonging to the family of Moringaceae and reported to possess antioxidant and antifatigue effect [7].

*M. oleifera* is cultivated in various parts of the world but is believed to be native to the tropical and subtropical regions of South Asia [8]. The Indian subcontinent is believed to be the largest producer of *M. oleifera*. *M. oleifera* is a superfood because of the multifarious nutritive properties it possesses, including but not limited to proteins, antioxidants, anti-fatigue, vitamin B, Iron, Magnesium, Calcium and anti-inflammatory, [9,10]. These nutritive properties possess by whole plant of *M. oleifera* are used to protect and nourish skin and hair, protect the liver, treat stomach complaints, fight bacterial diseases, strengthen the bones, and a lot more [11]. The genus *Moringa* comprises of 13 species distributed globally. However, for this study *M. oleifera* species has been selected as it is commonly found in India, and it is being used traditionally for ages. Identified as a “Miracle Tree”, *M. oleifera* is known for its rich nutrient, phytochemical and pleiotropic medicinal value such as anti-diabetic, anti-inflammatory, anti-oxidant and, anti-microbial activities [12]. Furthermore, reports claim that *M. oleifera* extract can be used as a herbal supplementation for sports performance. However, the scientific evidence to these claims are limited [7].

In the present study, the aim is to provide scientific evidence of *M. oleifera* as a herbal supplementation for sports performance through improved glucose uptake, muscle coordination and muscle endurance properties.

## 2. Materials and methods

### 2.1. Preparation of extract

Aqueous extract of *M. oleifera* leaves was procured from Herbo Nutra, New Delhi, India (HN/MLE/110880). Certificate of analysis is provided in [supplementary data](#).

### 2.2. Qualitative analysis of aqueous extract of *M. oleifera*

A preliminary pharmacognostic screening of the aqueous extract of *M. oleifera* leaves was performed by the standard procedures for flavonoids, tannins, sterols, carbohydrates, saponins, proteins and alkaloids [13,14].

### 2.3. Quantitative analysis of aqueous extract of *M. oleifera*

Colorimetric estimation was performed for the total phenolic and total flavonoid content in the aqueous extract of *M. oleifera* leaves.

#### 2.3.1. Total phenolic content

The total phenolic content was determined taking gallic acid as standard. Different concentrations of gallic acid, ranging from 6.25 to 5000 µg/ml concentration of aqueous extract of *M. oleifera* leaves were prepared. 200 µl of standard samples/extract sample was added to 1.5 ml of Folin-Ciocalteu reagent and incubated in dark for about 5 min after which 1.5 ml of Sodium carbonate was added followed by incubation in dark for about 90 min and their absorbance was read at 725 nm. The standard graph was plotted and the total phenolic content present in the extract was determined and expressed in terms of Gallic acid equivalents [15].

#### 2.3.2. Total flavonoid content

The total flavonoid content was determined by aluminium chloride colorimetric assay using Quercetin as standard in a concentration range of 6.25–200 µg/ml. The concentration of drug extract was 200 µg/ml. For the assay 0.5 ml of sample, 1.5 ml of 95% ethanol, 0.1 ml of AlCl<sub>3</sub>, 0.1 ml of potassium acetate and water to adjust the volume to 5 ml with were incubated for 30 min. The absorbance was recorded at 415 nm. The standard graph was plotted and the total flavonoid content present in the extract was determined and expressed in terms of Quercetin equivalents [15].

#### 2.3.3. HPLC analysis

Aqueous extract of *M. oleifera* leaves was dissolved in HPLC grade methanol (1 mg/ml) and subjected to analysis. The HPLC system (Shimadzu Corporation, Kyoto, Japan. Model Number: SPD-M20A) was equipped with dual pump LC-20AD binary system, photodiode array (PDA) detector SPD-M20A and Inertsil ODS-3VC18 reversed-phase column (I.D. 4.6 mm × 250 mm, 5 µm). Separation was achieved with a two-pump linear gradient program for pump A (acetonitrile) and pump B (water containing 0.1% formic acid) with 35 min run-time. Initially, elution was run with a gradient of 90% B changing to 60% in 15 min and finally to 90% in 25 min. Flow rate and injection volume were 1.0 ml/min and 20 µl, respectively. The chromatographic peaks of the analytes were confirmed by comparing their retention time and UV spectra with corresponding reference standards. The results were obtained by comparison of peak areas (at 380 nm) of aqueous extract of *M. oleifera* leaves with those of reference standard, Quercetin.

### 2.4. Animals

Thirty-six female Wistar rats (140g–230g) were used for the study. These rats were obtained from the Central Animal Research

Facility (CARF) of Manipal Academy of Higher Education and housed at 24°C–26 °C. Sterilized cages were used to house these animals. For 10 days they were fed a standard diet and tap water *ad libitum*. The study was approved by the Institutional Ethical Committee, Kasturba Medical College, Manipal (IAEC/KMC/79/2019).

## 2.5. Experimental design

Thirty-six animals were divided into six groups with six animals/group. The groups were designated as follows: Group I: Normal control, Group II: Dexamethasone Control (0.6 mg/kg*i.p.*), Group III: Exercise Control, Group IV: Dexamethasone + *M. oleifera* leaf extract, Group V: Dexamethasone + Exercise, Group VI: Dexamethasone + Exercise + *M. oleifera* leaf extract. *M. oleifera* leaf extract 300 mg/kg was administered orally an hour prior to administration of dexamethasone 0.6 mg/kg *i.p.* Dexamethasone was administered for 7 days intraperitoneally to induce the degeneration of skeletal muscles. The animals of the normal control group were not given any treatment. Animals were assessed for variation in bodyweight, muscular endurance using a treadmill, locomotor activity using actophotometer, muscle integrity, and handgrip strength using rotarod on day zero and day seven. Hemidiaphragm of rats were isolated and used for evaluation of the glucose uptake.

The exercise group animals were subjected to exercise daily for 10 days after half an hour of dosing using treadmill equipment (IITC 805 treadmill:5 lanes, without inclination). The time (900 s) and speed (max:18 m/min) parameters for the treadmill were set using the digital panel and the rats were placed in the lanes. Five animals were placed at a time. There were two criteria for scoring. First, physical positioning and action of taking support or touching the walls and second, number of times the rats stopped moving. For the first one, 1 s was deducted and for the second one, 3 s were deducted from the total time to obtain the final time of active movement. During the 10-day timeline, the animals were first trained for 3 days followed by 7-day treatment exposure. On day 0, the readings were recorded for all the groups. Readings for the exercise groups were recorded during the dosing phase as well. On day 7, all the groups were tested again to observe the change in locomotor activity based on the time of active movement.

## 2.6. Glucose uptake study

Overnight fasted Wistar rats were sacrificed by cervical dislocation and their diaphragms were dissected out quickly and divided into two halves and weighed. The hemidiaphragm of each half was rinsed in the glucose-free Tyrode solution [containing NaCl (134 mM), KCl (2.68 mM), CaCl<sub>2</sub> (1.80 mM), MgCl<sub>2</sub> (1.05 mM), NaH<sub>2</sub>PO<sub>4</sub> (417 μM), NaHCO<sub>3</sub> (11.9 mM)] to remove any blood clots. Tyrode solution is isotonic to interstitial fluid in the body. The electrolytes in the solution are crucial for muscle function and hence, this is used in the present study. The tissues were then transferred to organ baths containing 5 ml Tyrode solution each (with a glucose concentration of 200 mg/dL) and incubated for 30 min, maintained at 32 °C with aeration. After 30-min incubation, the glucose concentration in the Tyrode was quantified using Glucose detection kit (Aspen Laboratories Pvt Ltd). This determines the extent of glucose uptake by the skeletal muscles. Glucose uptake was calculated as the difference in the initial and final concentration of glucose in Tyrode and was expressed as glucose uptake per gram of tissue per 30 min [16].

## 2.7. Muscle coordination using rotarod

Muscle coordination using Rotarod was evaluated before and after the 7-day treatment schedule. Each rat was placed

individually on the rotating rod for a maximum of 60 s at 25 rpm. Three replicates of the trial were performed on each rat irrespective of their group. Each animal was trained for 60s period once a day for three days prior to test phase. After the training, most rats attained a stable baseline performance of latency to fall for a 60s time period. Following training, On day zero, these rats were exposed to the same environmental condition for 300 s on the rotating rod with increasing rpm of 4–40 rpm, and the latency time was recorded. The event period of 300s is the duration during which the animals are measured for latency to maintain balance on the rotarod [17].

## 2.8. Locomotor activity using actophotometer

Locomotor activity was assessed using a digital actophotometer (INCO Lab, India). Animals were placed individually in the activity meter, for them to get acclimatized to the environment before performing the test. For the next 5 min, the ambulatory movements were recorded and expressed in terms of total photobeam count [17].

## 2.9. Histology of gastrocnemius muscle

The gastrocnemius muscle was extracted and subjected to Hematoxylin –Eosin stain to study the changes in the muscle fibers.

## 2.10. Statistical analysis

All the values were represented as Mean and SEM for all the animals. Data were analyzed by One-way ANOVA followed by Tukey's posthoc test using Graph pad prism 8 (GraphPad Software, La Jolla California USA, [www.graphpad.com](http://www.graphpad.com)), where  $p < 0.05$  (i.e., the probability of random chance occurrence is less than 5%) in comparison to dexamethasone control group is considered significant.

## 3. Results

### 3.1. Qualitative analysis

Extract showed presence of Alkaloids, carbohydrates, flavonoids, phytosterols, steroids, saponins and tannins (Table 1).

### 3.2. Quantitative analysis

#### 3.2.1. Total phenolic content

A standard graph was plotted for quantitative determination of total phenolic content using Gallic acid as standard and the amount of phenolic content present in the aqueous extract of *M. oleifera* leaves was quantified and expressed as gallic acid equivalents/mg of extract. Total phenolic content was  $14.594 \pm 0.023$  μg Gallic acid equivalent/5000 μg extract.

#### 3.2.2. Total flavonoid content

A standard graph was plotted for quantitative determination of total flavonoid content using Quercetin as standard and the amount of flavonoid content present in the aqueous extract of *M. oleifera* leaves was quantified and expressed as quercetin equivalents/mg of extract. Total Flavonoid content was  $7.65 \pm 0.048$  μg Quercetin equivalent/5000 μg extract.

#### 3.2.3. HPLC analysis of aqueous extract of *M. oleifera* leaves for quantification of quercetin

The leaf extract showed presence of  $1.7 \pm 0.0043$  μg of quercetin/mg of extract in comparison to the reference standard quercetin (Fig. 1, Table 2).

**Table 1**  
Preliminary phytochemical screening of aqueous extract of *Moringa oleifera* leaves.

SL.NO.	Test	Result
1	<b>Alkaloids:</b>	
	a. Dragend'roff's test	+
	b. Hager's test	+
	c. Wagner's test	+
2	<b>Reducing sugars:</b>	
	a. Molisch's test	+
	b. Benedict's test	+
	c. Fehling's test	+
3	<b>Flavonoids:</b>	
	d. Mayer's test	+
	Shinoda test	+
	<b>Phytosterols:</b>	
4	Liebermann Burchard's test	+
	<b>Steroids &amp; Triterpenoids:</b>	
5	Salkowski test	+
	<b>Proteins:</b>	
6	a. Biuret test	-
	<b>Amino acids:</b>	
7	b. Million's test	-
	<b>Saponins:</b>	
8.	Foam test	+
	<b>Tannins:</b>	
	Ferric chloride	+

3.3. Effect on locomotion

All the treatments showed a significant decrease in the locomotion ( $p < 0.05$ ) compared to normal control. Impairment in locomotion due to chronic exercise and dexamethasone confirmed skeletal muscle impairment [16]. Administration of *M. oleifera* leaf extract to Dex-induced animals that were conditioned to exercise showed significant ( $p < 0.05$ ) improvement in locomotor activity compared to dexamethasone control (Fig. 2).

3.4. Effect on motor coordination

Exercise showed a significant ( $p < 0.05$ ) impairment in motor coordination compared to normal control demonstrating that 10 days of exercise may have caused chronic impairment of muscle function. Dexamethasone treatment caused a significant ( $p < 0.05$ ) impairment of skeletal muscles compared to normal control. Similar results were obtained with 0.6 mg/kg dose of dexamethasone in the earlier study [16]. Administration of *M. oleifera* leaf extract along with exercise showed significant ( $p < 0.05$ ) improvement in motor coordination in animals with

**Table 2**  
HPLC analysis for retention time and relative concentration of Quercetin in aqueous extract of *Moringa oleifera* leaves. Results are expressed as Mean  $\pm$  SEM.

Compound name	Retention time ( $R_t$ ) min		$\mu\text{g}/\text{mg}$ of extract
	Reference standard	<i>Moringa oleifera</i> extract	
Quercetin	19.5 min	19.4 min	1.7 $\pm$ 0.003

dexamethasone-induced functional impairment when compared to dexamethasone control group (Fig. 2).

3.5. Effect on endurance

Dexamethasone control group showed significant ( $p < 0.05$ ) decrease in endurance compared to normal control. In comparison with dexamethasone control group, there was a significant ( $p < 0.05$ ) increase in endurance in Dex + *M. oleifera* leaf extract, Dex + *M. oleifera* leaf extract + Exercise groups (Fig. 3).

3.6. Effect on body weight

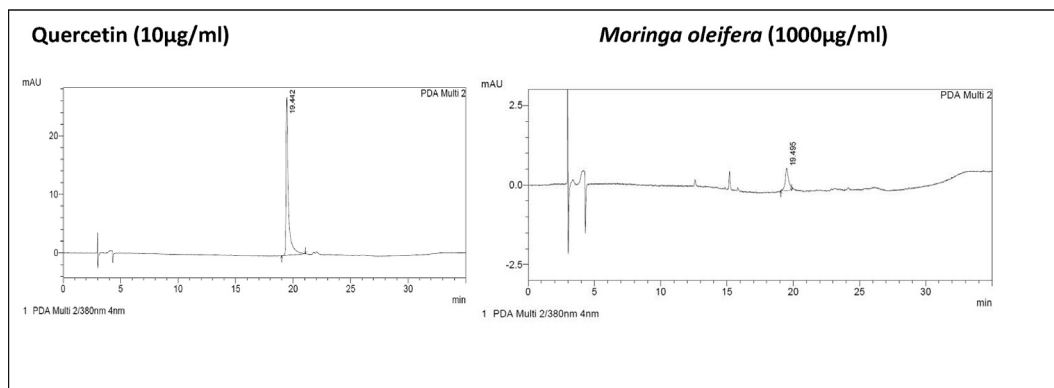
Body weight was measured on Day 0. Food was administered equally to all the groups. Dexamethasone control, Dex + *M. oleifera* leaf extract and Dex + *M. oleifera* leaf extract + Exercise showed a significant ( $p < 0.05$ ) reduction in body weight when compared to normal control group. However, the exercise control group did not show any change in the body weight in comparison with normal control group (Fig. 3).

3.7. Effect on glucose uptake

Dexamethasone treated animals exhibited a minimal decrease in glucose uptake compared to normal. Treatment groups showed increased glucose uptake at basal state. Although, the difference in the glucose uptake was not found to be statistically significant or something to the effect (Fig. 3).

3.8. Histology of gastrocnemius muscle

Marked reduction in the diameter of the muscle fibers was observed in the group treated with Dexamethasone and exercise control compared to normal control. Dexamethasone is known to induce toxicity on skeletal muscle. Conversely, a normal architecture of the gastrocnemius muscle was observed in Dexamethasone



**Fig. 1.** HPLC chromatogram of aqueous extract of *Moringa oleifera* leaves and reference standard – Quercetin.

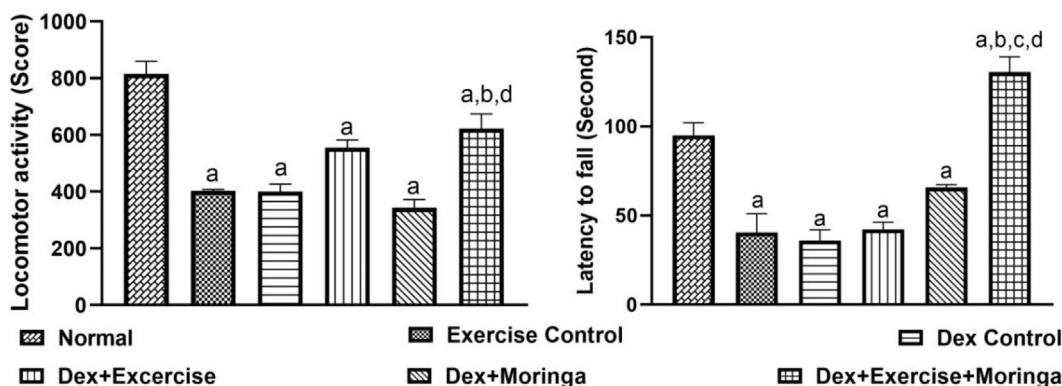


Fig. 2. Effect on motor coordination and locomotion. All the values are Mean ± SEM of six animals where <sup>a</sup>p<0.05 compared to normal control, <sup>b</sup>p < 0.05 compared to Dex control, <sup>c</sup>p < 0.05 compared to Dex + Exercise control, <sup>d</sup>p < 0.05 compared to Dex + *M. oleifera* leaf extract.

+ Exercise + *M. oleifera* leaf extract with no signs of inflammation, muscle atrophy, and fibrosis (Fig. 4).

4. Discussion

Dexamethasone is a known exogenous fluorinated glucocorticoid used in the treatment of multiple autoimmune and inflammatory disorders. Several reports claim that dexamethasone is known to produce skeletal muscle weakness and on prolonged use causes skeletal muscle atrophy, thereby causing functional impairment of limbs as a result of cascade of mechanisms. Dexamethasone induces muscle atrophy by mediating protein catabolism and the elevation of atrophic markers in the skeletal muscle cells, thus diminishing the muscle mass development [18,19]. The present study was designed to study the effect of standardized *M. oleifera* leaf extract against dexamethasone induced skeletal muscle impairment.

The extract was characterized by colorimetry assays and showed presence of Alkaloids, carbohydrates, flavonoids, phytosterols, steroids, saponins and tannins. Quantitative estimation showed presence of total phenol and total flavonoid were 14.59 ± 0.02 µg Gallic acid equivalent/5000 µg extract and 7.65 ± 0.05 µg Quercetin equivalent/5000 µg extract. The HPLC analysis of aqueous extract of *M. oleifera* leaves detected presence of Quercetin, (1.7 ± 0.003 µg/mg of extract). Report from Ngoc Hoan Le et al. (2014) purported that quercetin, which is an active constituent of *M. oleifera* leaf extract, reduced skeletal muscle atrophy in obesity-induced inflammation. It was described that the atrophic markers interceding protein catabolism are responsible for skeletal muscle impairment. The improvement in locomotion and muscle coordination may be attributed to *M. oleifera* leaf extract which could have played a similar role in amending the markers responsible for protein balance in the skeletal muscles [20]. Furthermore, continuous exercise thrusts muscle development and eliminates

age-induced deposition of intermuscular lipids that may hamper muscle movement and locomotion. Exercise stimulated changes may have minimal effect on body weight, however, improves muscle movement and coordination which was observed in Dex + *M. oleifera* leaf extract + Exercise treatment group [21]. Acute administration of dexamethasone impaired the body weight gain due to a reduction in linear growth [22]. In the present study, similar trend were observed. A remarkable weight loss was seen in dexamethasone treated group. However, no reversal was seen in body weight loss either by exercise alone or in combination with *M. oleifera* leaf extract. *M. oleifera* has potential to produce anti-obesity effect. The ability to induce weight loss may have contributed to the inconsequential weight gain in *M. oleifera* leaf extract alone and *M. oleifera* leaf extract + exercise treatment groups [23].

Histopathological data of gastrocnemius muscles of dexamethasone control group shows loss of muscle mass exhibited by reduced muscle cell diameter and cross-sectional area that denotes atrophy. No significant loss of muscle mass is seen in exercise control group, however, there is some amount of shrinkage in muscle diameter that could have occurred due to sarcomere length alteration so as to accommodate muscle mass development. Treatment with *M. oleifera* leaf extract alone and in association with exercise attenuated muscle atrophy and may have stimulated muscle mass development [24,26,27].

Skeletal muscle endurance activity uses Type-I (slow-twitching) muscle fibres, that can sustain longer contraction time and is dependent upon the mitochondrial health and ATP generation for greater endurance ability. Endurance aerobic activities such as treadmill improve mitochondrial biogenesis, and oxidative capacity in skeletal muscles. Administration of dexamethasone is claimed to induce mitochondrial content loss leading to ATP deprivation thus causing mitochondrial dysfunction. *M. oleifera* leaf extract alone and in combination with exercise may have enriched mitochondrial

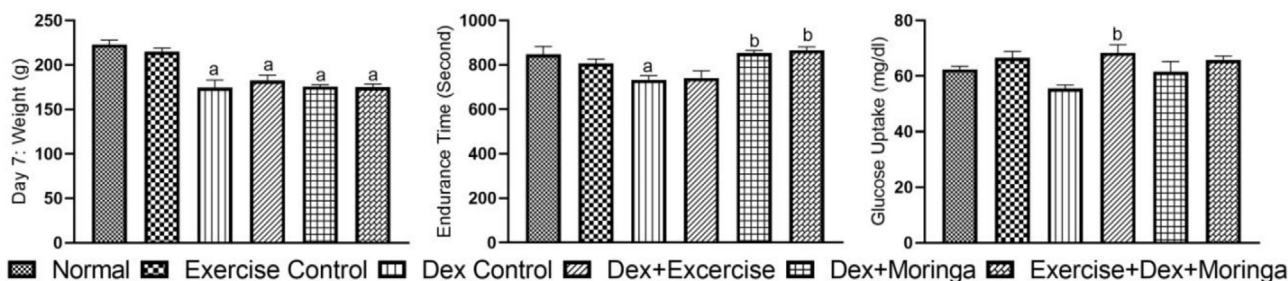
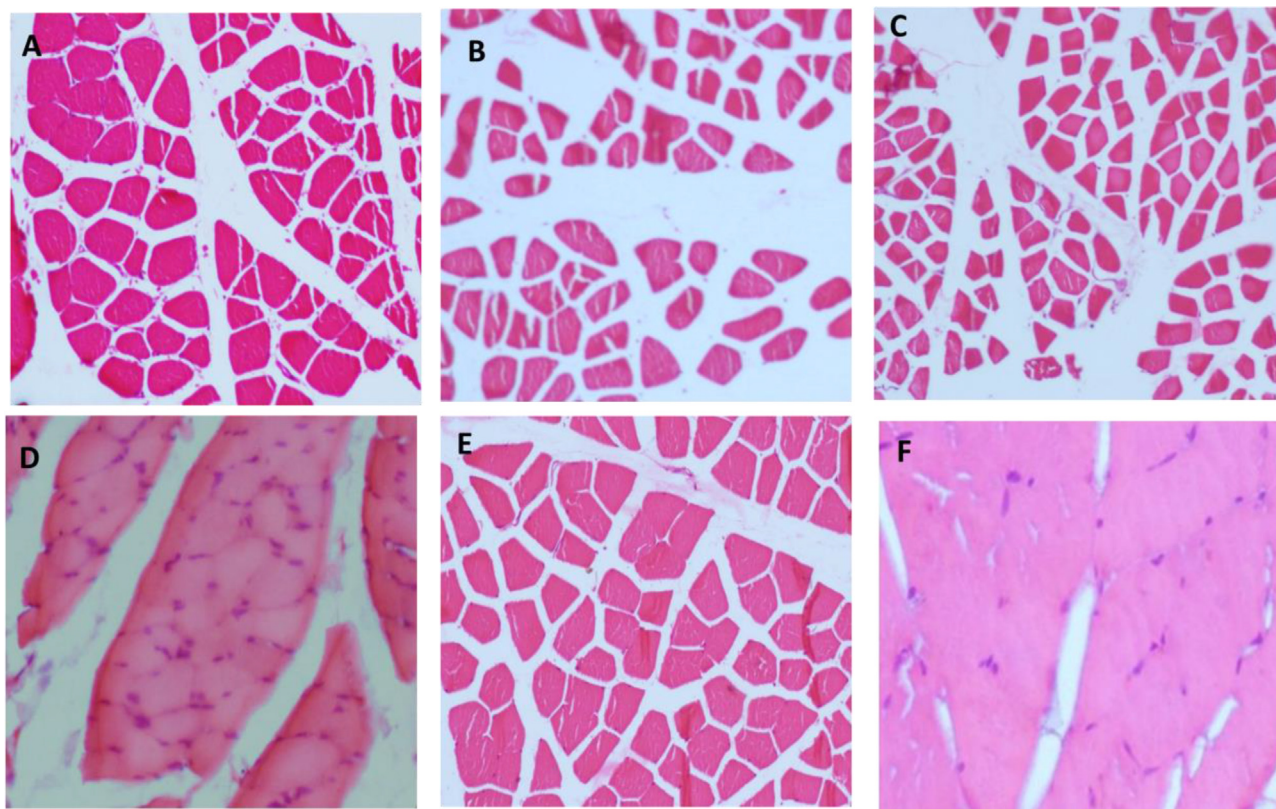


Fig. 3. Effect on body weight, endurance, and glucose uptake. All the values are Mean ± SEM of six animals where <sup>a</sup>p<0.05 compared to normal control, <sup>b</sup>p < 0.05 compared to Dex control.



**Fig. 4.** Histology of gastrocnemius muscle. Photomicrograph of H&E stained transverse section of gastrocnemius muscle: where A-Normal control; B-Dexamethasone Control; C-Exercise Control; D-Dexamethasone + *M. oleifera* leaf extract; E- Dexamethasone + Exercise; F- Dexamethasone + Exercise + *M. oleifera* leaf extract.

biogenesis through PGC-1 $\alpha$  upregulation in the skeletal muscles, thereby significantly improving endurance capacity. Furthermore, *M. oleifera* leaf extract is capable of reducing muscle fatigue and hence increasing the muscle endurance [7]. Likewise, regular exercise during dexamethasone injection might have attenuated muscle atrophy of the hind-limb muscles [18,28,29].

Skeletal muscle cells are responsible for about 80% of post-prandial glucose uptake. Chronic administration (>24h) of dexamethasone reportedly downregulates the expression of glucose transporter (GLUT4) thereby reducing the glucose uptake both at basal state and in the presence of insulin. On the contrary, chronic exercise increased the expression of GLUT4 stimulated by vigorous muscle contractions thereby improving the glucose uptake even in the absence of insulin. Increasing research evidence of *M. oleifera* suggests its anti-diabetic and anti-oxidative properties along with improvement in glucose uptake by the skeletal muscles. Furthermore, the anti-oxidative properties of *M. oleifera* leaf extract can further improve the nutritional status and body weight [30–33]. A similar result was seen in the study, where administration of *M. oleifera* leaf extract alone and in combination with exercise improved uptake of glucose at basal state remarkably. However, it is required to further evaluate the molecular mechanisms that determine the overall improvement of functional health of the muscles due to *M. oleifera* supplementation.

## 5. Conclusion

Treatment with *M. oleifera* leaf extract supplemented with exercise resulted in reversal of dexamethasone-induced functional impairment in skeletal muscles in test animals.

## Conflict of interest

None.

## Funding information

No funding was received for this work.

## Authors contributions

**Abhinav Kanwal, Yogendra Nayak, Nitesh Kumar:** Design of study; **Kalgi Barodia, Aayush Menon, Rutu Rajeevan, Aniket Rukade, Raghavendra Udaya. Kumar Shenoy, Chaitali Prabhu, Vaibhav Sharma, K.P. Divya:** Animal dosing and exercise training; **Sri Pragnya Cheruku, K.P. Divya, Nitesh Kumar:** Characterisation and standardisation of extract; **Suhani Sumalatha:** Histology of Muscle; **Kalgi Barodia, Aayush Menon, Rutu Rajeevan, Nitesh Kumar:** Result analysis and interpretation. All authors contributed equally to manuscript writing.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jaim.2021.07.019>.

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