Original Article

Histological Study to Compare the Effect of Atomoxetine Versus Formetrol on Dexamethasone-Induced Skeletal Muscle Atrophy in Male Mice

Sarwat Lotfi Ahmed, Heba Essam Rashad, Noha Abd eLLatif Ibrahim, Marwa Omar Abd El All and Nehad Ahmed Sadek

Department of Histology and Cell Biology, Faculty of Medicine, Fayoum University, Egypt

ABSTRACT

Introduction: Atrophy of skeletal muscles is still a serious clinical problem. Formoterol, an agonist of the B2- adrenergic receptor, may prevent this atrophy. An FDA-approved inhibitor of reuptake of norepinephrine called atomoxetine was effective in the prevention of skeletal muscle atrophy.

Aim of Work: Compare the effect of atomoxetine versus formetrol on dexamethasone-induced skeletal muscle atrophy in male mice.

Material and Methods: Forty-eight adult male albino mice were divided into six groups (8 mice each): Group 1 (control group) animals were injected intraperitoneally with 0.5ml sterile saline daily for seven days. Group 2 (dexamethasone treated group) animals were injected intraperitoneally with 10mg/kg/day dexamethasone for seven days to induce muscle atrophy. Group 3 (atomoxetine only treated group): animals received atomoxetine at a dose of 6mg/kg/day orally using insulin syringe without needle for seven days. Group 4 (atomoxetine + dexamethasone treated group): animals received both dexamethasone and atomoxetine at same doses and routes of administrationin as groups 2 and 3 respectively. Group 5 (formertrol only treated group): animals were injected intraperitoneally with 0.6 mg/kg/day formetrol for seven days. Group 6 (formertrol + dexamethasone treated group): animals received both dexamethasone and formetrol at same doses and routes of administration as groups 2 and 5 respectively. Sections were stained with hematoxylin and eosin stain & Picro Sirius red (PSR) histochemical reaction. Immunohistochemical staining was done using nuclear factor kappa-B (NF-κB) and heat shock protein (Hsp70). Area percent of collagen fibers deposition, area percent of nuclear factor kappa-B immunoexpression, area percent of heat shock protein 70 immunoexpression and diameter of muscle fiber were measured.

Results: Group 4 (atomoxetine and dexamethasone treated group) and Group 6 (formertrol and dexamethasone treated group) showed increase in diameter of muscle fibers as compared to dexamethasone group.

Conclusion: Formetrol has a potential role in preventing skeletal muscle atrophy.

Received: 06 October 2023, **Accepted:** 20 November 2023

Key Words: Atomoxetine, dexamethasone, formetrol, skeletal muscle atrophy.

Corresponding Author: Nehad Ahmed Sadek, MD, Department of Histology and Cell Biology, Faculty of Medicine, Fayoum University, Egypt, **Tel.**: +20 10 1093 1970, **E-mail:** nas04@fayoum.edu.eg

ISSN: 1110-0559, Vol. 47, No. 4

INTRODUCTION

Skeletal muscle atrophy is characterized with a loss of the muscle mass that happens when the protein degradation exceeds the protein synthesis[1]. Catabolism is widely known to be induced by glucocorticoids. Increases in circulating glucocorticoids are linked to a variety of pathologic diseases that cause atrophy of muscle, such as diabetes, metabolic acidosis, sepsis, cachexia, chronic renal failure, etc. This shows that glucocorticoids may contribute to the onset of atrophy[2].

Clinically, significant muscular atrophy has been associated to higher rates of morbidity and mortality, particularly when it exists with other chronic disease conditions, high-dose of dexamethasone is considered to cause atrophy by indirect blocking of activation of muscle RING-finger protein-1 (MuRF1) and fork head box protein O3a (FoxO3a)[3,4].

Clenbuterol and formoterol are examples of beta 2 adrenergic receptors (2-AR) agonists, which are progrowth and anti-atrophic drugs. Skeletal muscle hypertrophy is induced by formoterol by increasing insulin growth factor (IGF-1) and peroxisomal proliferator-activated-γ-coactivator 1α4 (PGC- 1α4)[5].

An FDA-approved drug called atomoxetine, commonly known as (tomoxetine), treats attention deficit hyperactivity disorder (ADHD) by preventing neuronal reuptake of norepinephrine[6]. In a model of dexamethasone-induced muscle atrophy, it proved successful in preventing atrophy of skeletal muscles by activating protein kinase B (Akt), increasing p-FoxO3a, sustaining Peroxisome Proliferator–activated receptor-Y- coactivator-1 a 1 (PGC-1a1) expression, and blocking MuRF-1. Furthermore, it is potent inducer for mitochondrial biogenesis (MB)[7].

DOI: 10.21608/ejh.2023.240872.1958

AIM OF THE WORK

The aim of the study was to evaluate and compare the possible prophylactic effect of atomoxetine versus formoterol on dexamethasone induced muscle atrophy in male mice using histological, histochemical and morphometric study.

MATERIALS AND METHOD

The study used 48 mature male albino mice, six to eight weeks old and weighted 25 g body weight. The animals were raised in clean, well-ventilated cages made of sanitary stainless steel at the animal house of the Faculty of Science at Fayoum University. Tap water and typical lab food were accessible. The medical research ethics committee (10/1/2021, committee number 53, M352), Faculty of Medicine, Fayoum University, authorized the experimental design.

Experimental design

The male mice were divided into six groups (8 mice each):

1. Group1 (control group): animals were injected with 0.5ml sterile saline intraperitoneally daily for seven days.
2. Group 2 (dexamethasone treated group): animals were injected intraperitoneally with 10mg/kg/day dexamethasone for seven days for induction of muscle atrophy[8].
3. Group 3 (atomoxetine only treated group): animals received atomoxetine at a dose of 6mg/kg/day orally using insulin syringe without needle for seven days[9].
4. Group 4 (atomoxetine + dexamethasone treated group): animals received both dexamethasone and atomoxetine as the aforementioned doses and routes of administration in groups 2 and 3[9].
5. Group 5 (formertrol only treated group): animals were injected intraperitoneally with 0.6 mg/kg/day formetrol for seven days[10].
6. Group 6 (formertrol + dexamethasone treated group): animals received both dexamethasone and formetrol as the aforementioned doses and routes of administration in groups 2 and 5[10].

Methods

On the eighth day all mice were sacrificed under deep ether anesthesia. The gastrocnemius muscle was resected, then the specimens were fixed in 10% buffered formalin. After 72 hours the tissue blocks were dehydrated in increasing alcohol series, cleared through xylene, immersed in liquid paraffin, and embedded in paraffin blocks which were cut into 5 µm thin sections using a microtome and they were stained by:

1. Hematoxylin and Eosin staining for routine histological examination[11].
2. Picro Sirius red histochemical reaction to demonstrate collagen[12].
3. Immunohistochemical staining using heat shock protein (Hsp70) as marker for stress response and nuclear factor kappa-B (NF-κB) as marker for inflammatory and immune response[13].

Quantitative Morphometric Study

The data were obtained by using “Top view” image analyzer computer system (China), at Faculty of Medicine-Fayoum University. Images were captured from non-overlapping fields randomly chosen from the animal.

The following parameters were measured:

1) Area percent of collagen deposition:

Using objective lens of magnification 10.

2) Area percent of heat shock protein 70 immunoexpression:

Using objective lens of magnification 40.

3) Area percent of nuclear factor kappa-B immunoexpression:

Using objective lens of magnification 40.

4) Diameter of muscle fiber:

Using objective lens of magnification 40.

Statistical analysis

After applying the arithmetic mean, standard deviation (SD), one-way analysis of variance (ANOVA), and post-hoc test to compare each pair of groups, statistical analysis was carried out. The statistical program "SPSS for Windows" Version 19 was used for all computations on an IBM personal computer. According to Dawson *et al*.[14], results were deemed significant when the probability was 0.05.

RESULTS

Examination of H & E stained sections

Group 1 (control group) revealed elongated cylinderical fibers with acidophilic striated sarcoplasm, arranged parallel in bundles. Nuclei were flattened and peripherally placed (Figure 1a). There were narrow spaces between muscle fibers (Figure 1b). Group 2 (dexamethasone treated group) showed widening of spaces between muscle fibers and loss of striations. Some fibers displayed splitting and branching and occasionally central nuclei (Figure 2a). When observing the transverse sections, the muscle fibers were mostly fragmented and variable in the size and shape. Some fibers were apparently shrunken and some angulated and atrophic (Figure 2b). Group 3 (atomoxetine treated group) displayed a picture closely resembling control specimens as the acidophilic muscle fibers were, striated, cylinderical, parallel and arranged in bundles. Nuclei were multiple flattened and peripherally placed (Figures 3 a,b). Group 4 (atomoxetine with dexamethasone treated group) revealed cylinderical muscle fibers with restored size

arranged in bundles, most of them had regular striations. Some muscle fibers exhibited multiple peripheral nuclei while others exhibited central nuclei and splitting. The spaces between muscle fibers resembled the control with apparently restored size and few splitting of muscle fibers. Still some atrophic muscle fibers were noticed (Figures 4 a,b). Group 5 (formoterol treated group) displayed fibers appearing larger. Some showed splitting and central nuclei. Compared to the control group, transverse striations were less clear (Figures 5 a,b). Group 6 (formoterol with dexamethasone treated group) revealed splitting and branching hypertrophied muscle fibers, but few atrophic fibers were still observed. Some fibers showed striations, some had peripheral flattened nuclei while others had central nuclei (Figures 6 a,b).

Examination of Picro Sirius red (PSR) stained sections

Group 1 displayed a positive PSR reaction revealing thin delicate collagen fibers in the connective tissue sheath endomysium and the perimysium between the muscle bundles (Figures 7 a,b). Group 2 revealed thick extensive collagen fibers of the endomysium and perimysium (Figures 8 a,b). Group 3 showed thin delicate collagen fibers of the perimysium and the endomysium (Figures 9 a,b). Group 4 displayed thin delicate collagen fibers in the perimysium and the endomysium (Figures 10 a,b). Group 5 thin delicate collagen fibers in endomysium and perimysium (Figures 11 a,b). In Group 6 there was thin delicate collagen fibers in the endomysium and perimysium (Figures 12 a,b).

Examination of Heat shock protein (Hsp70) stained sections

Group 1 displayed negative immunoreaction (Figures 13 a,b). Group 2 showed mild positive immunoreaction with a brownish coloration of the sarcoplasm of muscle fibers (Figures 14 a,b). Group 3 revealed negative immunoreaction (Figures 15 a,b). Group 4 revealed intense positive brownish immunoreaction in the sarcoplasm (Figures 16 a,b). Group 5 revealed a mild positive reaction in skeletal muscle fibers (Figures 17 a,b). Group 6 revealed a mild positive reaction in the sarcoplasm of muscle fibers (Figures 18 a,b).

Examination of Nuclear factor kappa-B (NF-κB) stained sections

Group 1 displayed negative immunoreaction (Figures 19 a,b). Group 2 showed strong positive immunoreaction as a brown color in the sarcoplasm of muscle fibers (Figures 20 a,b). Group 3 revealed negative immunoreaction (Figures 21 a,b). Group 4 displayed mild positive brownish sarcoplasmic immunoreaction (Figures 22 a,b). Group 5 showed negative immunoreaction (Figures 23 a,b). Group 6 revealed moderate positive brownish immunoreaction (Figures 24 a,b).

Statistical Results

Mean area % of collagen fibers deposition

The Dexamethasone group (group2) showed an increase in area % of collagen fibers deposition. Compared to the control group and all other groups the difference was of statistical significance. No difference of statistical significance was found between the control (group1), atomoxetine (group 3) and formoterol (group5) groups respectively. In spite of an apparent histological improvement in group 4 and 6, there was still statistically significant difference between them and the control group respectively (Table 1).

Mean area % of heat shock protein (Hsp70) immunoreactivity

The dexamethasone + atomoxetine group (group4) displayed the strongest degree of Hsp70 immunoreactivity. Compared to all other study-groups the respective difference in area % of immunoreactivity was statistically significant. Each of the dexamethasone (group2), the formoterol (group5) and the dexamethasone + formoterol (group6) groups exhibited a less extensive but still increased degree of (Hsp70) immunoreactivity. The respective difference in area % between each group and the control group was statistically significant. No statistically significant difference was found between control and atomoxetine groups (group3) (Table 2).

Mean area % of nuclear factor kappa-B (NF-κB) immunoreactivity

The dexamethasone group (group 2) displayed the strongest degree of immunoreactivity to NF-κB. The difference between this group and all other study groups was of statistical significance. The dexamethasone + atomoxetine group (group4) and the dexamethasone + formoterol group (group6) possessed a statistically significant increase in the area % of NF-κB immunoreactivity compared to the control group respectively. No statistical significant difference was found between control (group1), atomoxetine group (group3) and formoterol group (group5) (Table 3).

Mean diameter of muscle fiber

The dexamethasone group (group2) revealed a decrease in the diameter of its muscle fibers. This decrease was of statistical significance compared to control group and the other groups. The formoterol (group5) and dexamethasone + formoterol (group6) groups on the other hand showed an increase in muscle fiber diameter. This finding was of statistical significance compared to the control and other groups. No difference of statistical significance was detected between the control, atomoxetine (group3) and dexamethasone + atomoxetine (group4) groups (Table 4).

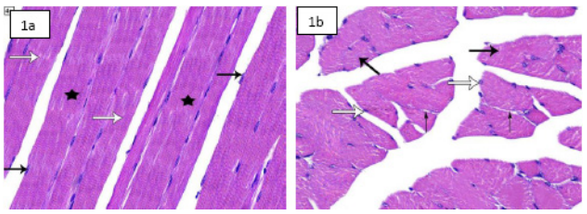

Fig. 1: Photomicrograph of skeletal muscle section from group 1 (control group) showing, 1a): Longitudinal section with elongated cylindrical muscle fibers (stars), multiple peripherally placed flattened nuclei (black arrows), striations (white arrows). 1b): Transverse section with polygonal muscle fibers (thick black arrows), peripherally placed flattened nuclei (white arrows). Note the narrow spaces between muscle fibers (thin black arrows) (H&E stain x400).

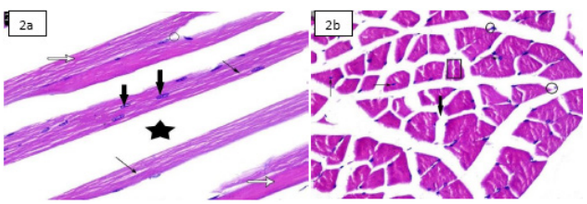

Fig. 2: A photomicrograph of skeletal muscle section from group 2 (dexamethasone treated group) showing, 2a): Longitudinal section with loss of striations (white arrows), widening of spaces between muscle fibers (star) central nuclei (thick black arrow), branching of muscle fiber (circle) and splitting of muscle fiber (thin black arrows). 2b): Transverse section with widening of spaces in between muscle fibers (thick black arrow), angulated atrophic fibers (circles), fragmented fibers (square), central nuclei (thin black arrows) (H&E stain x 400).

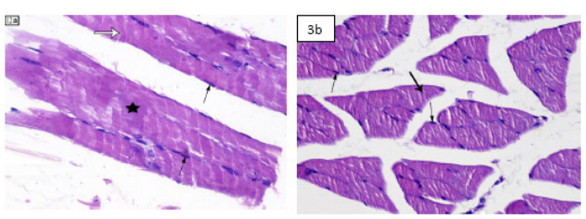

Fig. 3: A photomicrograph of skeletal muscle section from group 3 (atomoxetine treated group) showing, 3a): Longitudinal section with elongated cylindrical muscle fibers (star), multinucleated peripherally placed flattened nuclei (black arrows) and striations (white arrow). 3b): Transverse section with polygonal muscle fibers (thick black arrow), peripherally placed flattened nuclei (thin black arrows) (H & E stain x400).

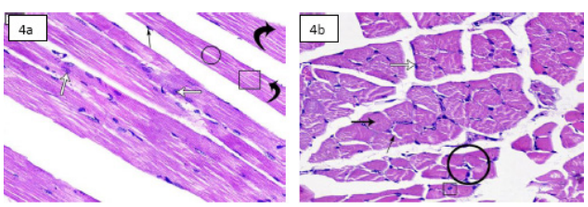

Fig. 4: A photomicrograph of skeletal muscle section from group 4 (atomoxetine with dexamethasone treated group) showing, 4a): Longitudinal section with apparently restored size of some muscle fibers (curved arrow). Some fibers show multiple peripherally placed flat nuclei (thin black arrow) while others show centrally located nuclei (white arrows), regular striations (square). Note few splitting of muscle fibers (circle). 4b): Transverse section with polygonal muscle fibers (thick black arrow), peripherally placed nuclei (white arrow). Note the narrow space between muscle fibers (thin black arrow), some fibers are atrophic (circle), while few have centrally located nuclei (square) (H&E stain x400).

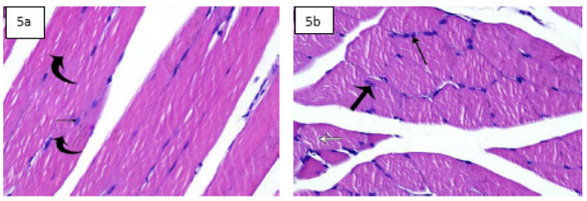

Fig. 5: A photomicrograph of skeletal muscle section from group 5 (formoterol treated group) showing, 5a): Longitudinal section with large hypertrophic fibers with less clear striations (curved arrow), central nuclei (thin black arrows). 5b): Transverse section with peripherally placed nuclei (thin black arrow), central nuclei (thick black arrow) and split muscle fibers (white arrow) (H&E stain x400).

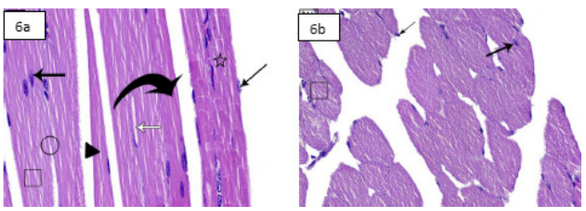

Fig. 6: A photomicrograph of skeletal muscle section from group 6 (formoterol with dexamethasone treated group) showing, 6a): Longitudinal sections with hypertrophic fibers (curved arrow), some fibers with central nuclei (thick black arrows), some fibers with peripheral flattened nuclei (thin black arrows), splitting of muscle fibers (white arrow), branching of some muscle fibers (circle). Note that some fibers show striations (star) while others show loss of striations (square). Few atrophic fibers are still observed (arrow head). 6b): Transverse section with splitting of muscle fibers (square), some fibers with central nuclei (thick black arrow) and others with peripheral nuclei (thin black arrow). (H & E stainx400).

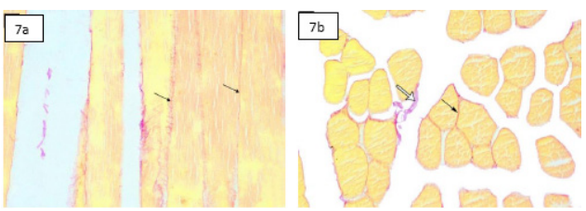

Fig. 7: A photomicrograph of skeletal muscle section from group 1 (control group) showing, 7a): Longitudinal section and 7b): Transverse section with thin delicate collagen fibers confined to endomysium (black arrows) and perimysium (white arrow) (PSR reaction x 400).

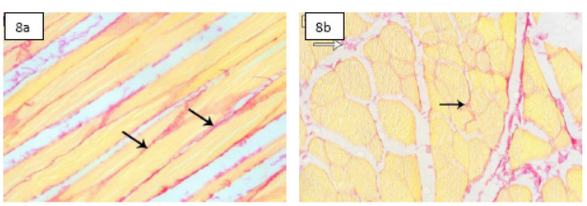

Fig. 8: A photomicrograph of skeletal muscle section from group 2 (dexamethasone treated group) showing, 8a): Longitudinal section and 8b): Transverse section with thick extensive collagen fibers confined to endomysium (thick black arrows) and perimysium (white arrow) (PSR reaction x 400).

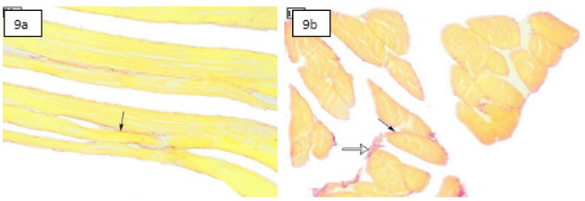

Fig. 9: A photomicrograph of skeletal muscle section from group 3 (atomoxetine treated group) showing, 9a): Longitudinal section and 9b): Transverse section with thin delicate collagen fibers that are confined to endomysium (black arrows) and perimysium (white arrow) (PSR reaction x 400).

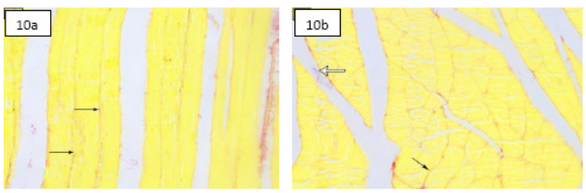

Fig. 10: A photomicrograph of skeletal muscle section from group 4 (atomoxetine with dexamethasone treated group) showing, 10a): Longitudinal section and 10b): Transverse section with thick extensive collagen fibers confined to endomysium (black arrows) and perimysium (white arrow) (PSR reactionx400).

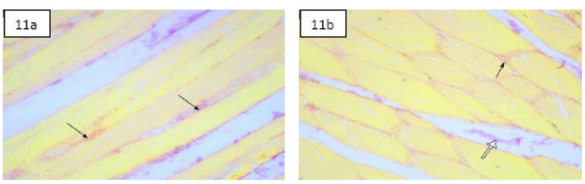

Fig. 11: A photomicrograph of skeletal muscle section from group 5 (formoterol treated group) showing, 11a): Longitudinal section and 11b): Transvers section with thin delicate collagen fibers confined to endomysium (thin black arrows) and perimysium (white arrow) (PSR reaction x400).

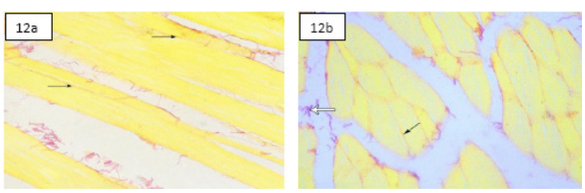

Fig. 12: A photomicrograph of skeletal muscle section from group 6 (formoterol with dexamethasone treated group) showing, 12a): Longitudinal section and 12b): Transverse section with thin delicate collagen fibers confined to endomysium (thin black arrows) and perimysium (white arrow) (PSR stainx400).

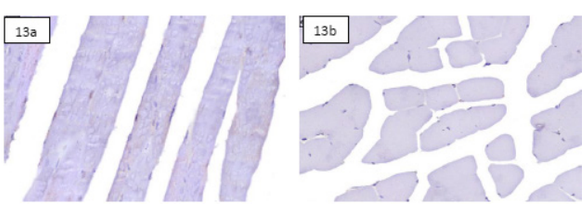

Fig. 13: A photomicrograph of a section from group 1 (control group) displaying, 13a): Longitudinal section and 13b): Transverse section with negative reaction in the sarcoplasm of the skeletal muscle fibers (Hsp70 stain x 400).

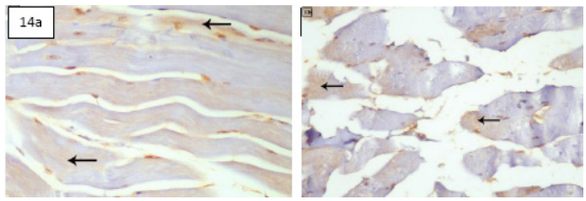

Fig. 14: A photomicrograph of skeletal muscle section from group 2 (dexamethasone treated group) revealing, 14a): Longitudinal section and 14b): Transverse section with mild positive immunoreaction in the form of brown color in the sarcoplasm of the skeletal muscle fibers (arrows) (Hsp70 stain x 400)

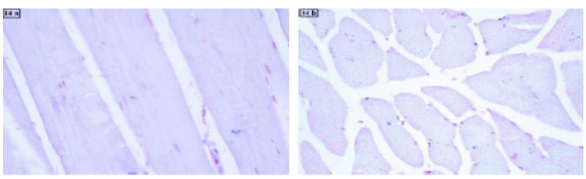

Fig. 15: A photomicrograph of skeletal muscle section from group 3 (atomoxetine treated group) showing, 15a): Longitudinal section and 15b): Transverse section with negative reaction in sarcoplasm of the skeletal muscle fibers (Hsp70 stain x 400).

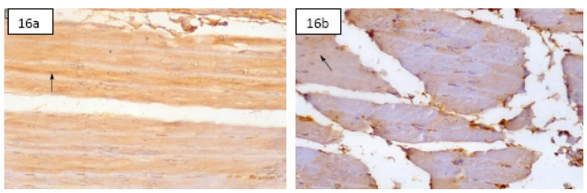

Fig. 16: A photomicrograph of skeletal muscle section from group 4 (atomoxetine with dexamethasone treated group) displaying, 16a): Longitudinal section and 16b): Transverse section with intense positive immunoreaction as a brown color in the sarcoplasm of the skeletal muscle fibers (arrow) (Hsp70stain x400).

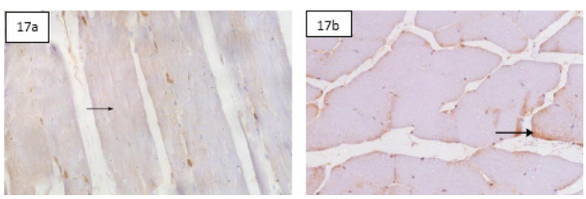

Fig. 17: A photomicrograph of skeletal muscle section from group 5 (formoterol treated group) revealing, 17a): Longitudinal section and 17b): Transverse section with mild positive immunoreaction as a brown color in the sarcoplasm of muscle fibers (arrows). (Hsp70stain x400)

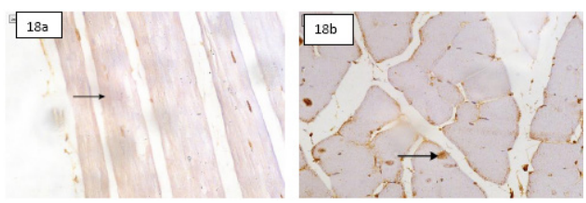

Fig. 18: A photomicrograph of skeletal muscle section from group 6 (formoterol with dexamethasone treated group) showing, 18a): Longitudinal section and 18b): Transverse section with mild positive reaction in the form of a brown color in the sarcoplasm (arrow). (Hsp70 stainx400)

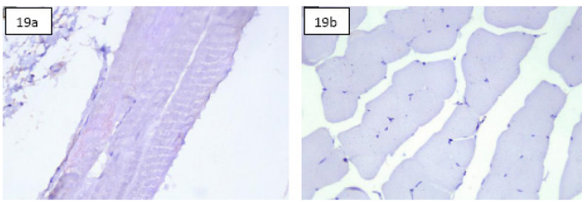

Fig. 19: A photomicrograph of skeletal muscle section from group 1 (control group) showing, 19a): Longitudinal section and 19b): Transverse section with negative immunoreaction in the sarcoplasm of skeletal muscle fibers (NF-κB stain x400)

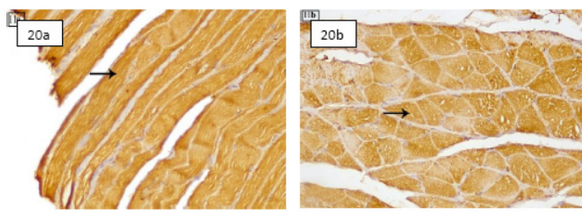

Fig. 20: A photomicrograph of skeletal muscle section from group 2 (dexamethasone treated group) revealing, 20a): Longitudinal section and 20b): Transverse section with intense positive immunoreaction as a brown color of the sarcoplasm of the muscle fibers (arrows). (NF-κB stain x 400)

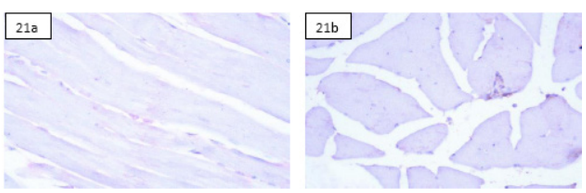

Fig. 21: A photomicrograph of skeletal muscle section from group 3 (atomoxetine treated group) displaying, 21a): Longitudinal section and 21b): Transverse skeletal muscle section with negative immunoreaction (NF-κB stain x 400)

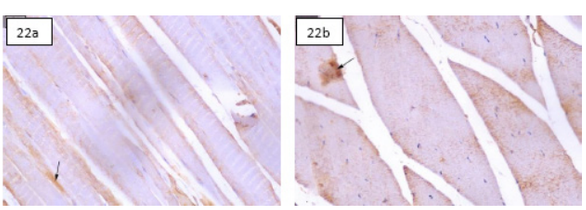

Fig. 22: A photomicrograph of skeletal muscle section from group 4 (atomoxetine with dexamethasone treated group) showing, 22a): Longitudinal section and 22b): Transverse section with mild positive immunoreaction as a brown color in the sarcoplasm of skeletal muscle fibers (arrow) (NF-κB stain x400)

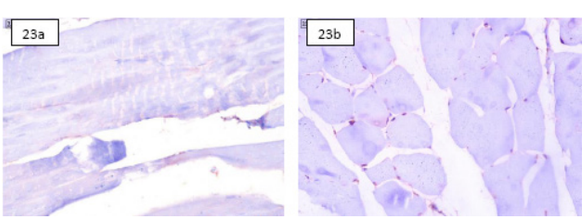

Fig. 23: A photomicrograph of skeletal muscle section from group 5 (formoterol treated group) displaying, 23a): Longitudinal section and 23b): Transverse section with negative immunoreaction (NF-κB stain x400)

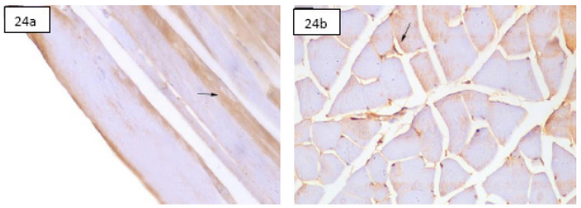

Fig. 24: A photomicrograph of skeletal muscle section from group 6 (formoterol with dexamethasone treated group) revealing, 24a): Longitudinal section and 24b): Transverse section with moderate positive immunoreaction as a brown color in the sarcoplasm of muscle fibers (arrows). (NF-κB stainx400)

Table 1: Comparisons of the area percentage of collagen fibers in the study groups.

Groups	Mean ± SD	
G 1 Control	0.53	0.10
G 2 Dexamethasone	2.85$^{\Delta}$	1.10
G 3 Atomoxetine	0.43	0.09
G 4 Dexamethasone & Atomoxetine	1.00*	0.06
G 5 Formoterol	0.42	0.07
G 6 Dexamethasone & Formoterol	1.07*	0.08

Data expressed as mean ± SD and significant difference was when *P value* ≤ 0.05.
Δ Statistically significant difference compared to rest of study groups respectively.
* Statistically significant difference compared with control group.

Table 2: Comparisons of area % of Hsp70 - immunoreactivity in the study groups.

Groups	Mean ± SD	
G 1 Control	0.19	0.08
G 2 Dexamethasone	1.54*	0.12
G 3 Atomoxetine	0.16	0.07
G 4 Dexamethasone &Atomoxetine	6.66$^{\Delta}$	0.29
G 5 Formoterol	1.81*	0.14
G 6 Dexamethasone & Formoterol	1.97*	0.17

Data expressed as mean ± SD and significant difference was when *P value* ≤ 0.05.
Δ Statistically significant difference compared to rest of study groups respectively.
* Statistically significant difference compared with control group.

Table 3: Comparisons of area % of NF-κB immunoreactivity in the study groups.

Groups	Mean ± SD	
G 1 Control	0.16	0.06
G 2 Dexamethasone	58.9$^{\Delta}$	6.7
G 3 Atomoxetine	0.28	0.07
G 4 Dexamethasone &Atomoxetine	6.83$^{\square *}$	0.99
G 5 Formetrol	0.35	0.08
G 6 Dexamethasone & Formetrol	11.7$^{\square *}$	0.95

Table 4: Comparisons of muscle fiber diameter in the study groups.

Groups	Mean(µm) ± SD	
G 1 Control	65.80µm	3.7
G 2 Dexamethasone	27.40µm*	2.05
G 3 Atomoxetine	65.59µm	3.8
G 4 Dexamethasone &Atomoxetine	59.90µm	4.3
G 5 Formetrol	81.90µmΔ	4.3
G 6 Dexamethasone & Formetrol	72.10µmΔ	2.2

Data expressed as mean ± SD and significant difference was when *P value* ≤ 0.05.

Δ Statistically significant difference compared to rest of study groups respectively.

* Statistically significant difference compared with control group.

DISCUSSION

There is a true condition called skeletal muscle atrophy. Many autoimmune and inflammatory illnesses are treated with dexamethasone, an immunosuppressive medication, although continuous use of large doses of dexamethasone results in muscular atrophy[15].

H&E stained slices of the dexamethasone-treated group showed a widening of the gaps between muscle fibers. Some muscle fibers exhibited striation loss. Others revealed the nuclear centralization and branching, These pathological changes were consistent with those described as characteristics of muscle atrophy by Hong *et al*.[16], who showed splitting of the muscle fibers with concentrated nuclei and total loss of normal striations. According to Jeong *et al*.[17], the high dose of dexamethasone that promotes muscle atrophy by a decrease and degradation of protein content, organelles, cytoplasm, fiber diameter, and fatigue resistance may be the reason of all the data found in the current study. They also discovered that catabolic signals, such as muscle-specific ring finger-1 (MuRF1), muscle atrophy f-box atrogin-1, and ubiquitin E3, were activated as result for muscle atrophy.

A sustained high dose of dexamethasone use was linked to myopathy and muscular atrophy, according to research by. Reactive oxygen species (ROS) are produced more often in muscle fibers after receiving a high dosage of dexamethasone, showing that ROS are crucial to the atrophy process. The function and structure of muscle tissue can be severely harmed by reactive oxygen species (ROS), which can reduce protein synthesis and increase proteolysis. Some of the atrophic fibers were spherical, while others were angulated. The gaps between muscle fibers appeared to be expanding. The majority of muscle fibers were broken up[18].

These histological alterations were in line with those reported by Hong *et al*.[16]. By measuring the diameter of the fibers, the shrinkage of muscle fibers was visible. Muscle fiber diameter was significantly lower than the control group. This difference between two groups was statistically significant, and it may have been brought about by an increase in the breakdown of protein or decrease in its synthesis, which ultimately led to significant reduction in the muscle fibers size. This was in line with the findings of Dumitru *et al*.[19], who hypothesized that the shrinkage of myofibers may be caused by reduction in number of sarcomeres and documented that activation of proteolytic systems results in the degradation of contraction-related proteins, which causes muscle atrophy, fragmented myofibers, shrunken atrophic fibers, and myofibers with central nuclei.

Collagen fibers exhibited a robust positive PSR reaction in Picro Sirius red reaction sections. In comparison to the control group, there was a considerable rise in collagen levels. This result was consistent with those of Peviani *et al*.[20]. According to research by Honda *et al*.[21], the increase in collagen fiber deposition that comes along with muscle atrophy is a reaction to the loss of myofibers, and fibroblasts restore the injured area by first forming collagen fibers.

Skeletal muscle analysis in the group treated with atomoxetine and dexamethasone revealed a clear reduction in the atrophic alterations brought on by dexamethasone. The muscle fibers showed signs of growth. They were similar in diameter to the controls'. This was validated by evaluating the muscle fibers' diameter, where there was no statistical significant difference between the two groups. Regular striations were present in most fibers. While some of the muscle fibers showed normal peripheral positioning of nuclei and splitting, others showed central nuclei. This was in line with the findings of Lim *et al*.[22], who demonstrated that atomoxetine reduces the muscular atrophy caused by dexamethasone.

Additionally, Jesinkey *et al*.[23] showed that ATX, when taken at a lower dose, maintained Peroxisome proliferator-activated receptor-- coactivator-1 (PGC1) expression, which prevented skeletal muscle atrophy. PGC1 controls mitochondrial biogenesis (MB) as a transcriptional co-activator. According to Lim *et al*.[22], who found that atomoxetine enhances mitochondrial biogenesis (MB) by directly acting on the 2-adrenergic receptor (2-AR), the action of atomoxetine may be caused by its norepinephrine reuptake inhibitor activity (NRI). Additionally, by increasing norepinephrine, atomoxetine may indirectly activate -adrenergic receptors.

Proliferator-activated receptor-coactivator-1 a (PGC-1a), according to Kitajima *et al*.[24], has a part in halting the loss of muscle. Transcription of genes that support mitochondrial biogenesis (MB) is regulated by the transcriptional coactivator PGC-1a. Induction of the PGC-1a1 isoform, which also stimulates MB, regulates the expression of the mitochondrial oxidative phosphorylation genes. Atomoxetine maintained PGC-1a1 expression as a result, preventing muscle atrophy. According to Yeo *et al*.[25], PGC-1a1 prevents nuclear FoxO3a from transcribing.

Collagen deposition was barely visible in sections dyed with Picro Sirius red. Comparing this drop to those that had received dexamethasone treatment, it was significant.

This result was in agreement with that of Dong *et al*.[26], who demonstrated that myostatin induces the growth of myofibroblasts. Expression of the extracellular matrix proteins and the growth of muscle fibroblasts are both directly influenced by myostatin. As PGC-1a1 expression is maintained by atomoxetine and PGC-1a4 isoform represses myostatin, fibroblast proliferation and collagen fiber synthesis are decreased[25].

Large hypertrophic fibers were visible in H&E stained sections for the formetrol-treated group. The muscle fibers' diameter was larger than the control's. Muscle fiber measurements showed a statistically significant increase in diameter. According to Gómez *et al*.[27], formoterol is a highly selective adrenergic receptor agonist that can induce skeletal muscle hypertrophy at microgram dosages. This study's findings corroborate those findings. Its hypertrophic impact is mediated by the skeletal muscle synthesis stimulation and a decrease in muscle proteolysis. Additionally, Formoterol is found to lower myostatin expression by Gómez *et al*.[27]. The growth and development of muscles are negatively regulated by myostatin. In the endomysium and perimysium, microscopic Sirius red sections showed trace levels of tiny collagen fibers.

Examining the skeletal muscles in the group that received formetrol and dexamethasone revealed a reduction in the atrophic alterations brought on by dexamethasone. The muscles' fibers seemed to have become larger. Compared to the control and dexamethasone groups, they were larger in diameter. Diameter of muscle fibers was measured in order to verify this. There was statistically significant increase in the diameter. According to Joassard *et al*.[28], formertrol's pro-growth and anti-atrophic activities may be the cause of the improvement that followed its administration.

Formoterol, according to Martinez *et al*.[5], is a potent inducer of skeletal muscle growth. It raises peroxisomal proliferator-activated coactivator (PGC-1) and insulin growth factor (IGF-1) levels. Comparatively to the dexamethasone-treated group, collagen deposition in Picro Sirius red sections was negligible. Despite this, the difference from the control group was still discernible. This result was consistent with Huang *et al*.[29], who showed that formetrol promoted a better organization of regenerating myofibers per fascicle as shown by a reduction in connective tissue, leading to the restoration of the structural regenerative capacity with no change in the number of regenerating myofibers.

Heat shock protein and nuclear factor kappa B immunohistochemistry staining were used to confirm the study's findings. In mice treated with dexamethasone, a weakly positive Hsp70 immunoreaction was found. In spite of this, the difference was statistically significant when compared to the control group. This was in line with the findings of Douglas *et al*.[30], who hypothesized that skeletal muscle atrophy may cause an increase in ATP concentration, which would then cause a decline in Hsc/Hsp70 interaction with the polysomes and a shift toward heavier polysomes, which might inhibit ribosome translation. As a result, protein synthesis is inhibited and the rate of elongation is decreased. Reduced HSP70 levels have been demonstrated to be harmful in a number of skeletal muscle atrophy models.

The possibility that Dex-induced skeletal muscle atrophy results from HSP70 being downregulated by MicroRNA1 (miR-1) is another reason for the moderate positive immunoreaction of hsp70. According to Kukreti *et al*.[31], HSP70 blockade boosted MuRF1 and Atrogin-1 levels and dephosphorylated forkhead box O3 (FOXO3). It is important to note that despite being greater than the control, immunoreactivity did not reach the target level, which may have aided in the recovery of the atrophied muscle fibers.

Heat shock protein 70 immunoreactivity in the atomoxetine plus dexamethasone-treated group was statistically significantly higher than in the control and dexamethasone-treated groups. According to Silvia *et al*.[32], increasing intracellular Hsp70 in skeletal muscle enhances the size of regenerated fibers, avoids muscle injury, and preserves the size of myofibers under atrophy-causing situations. The earlier discovery was in line with a report by De Oliveira *et al*.[33] that Hsp70's intracellular protective mechanisms were related to its capacity to support calcium homeostasis by modulating the activity of sarcoplasmic reticulum calcium-ATPase (SERCA) complex.

Nuclear factor kappa-B (NF-B) immunoreactivity was found strongly positive in the dexamethasone-treated group in the sarcoplasm of skeletal muscle fibers. The positive immunoraction was significantly higher than that of the control group. Statistics showed that the difference was significant. According to Khan *et al*.[34], dexamethasone caused NF-B to become activated, which serves as a pro-inflammatory signal for muscle tissue.

In contrast to the dexamethasone-treated group, nuclear factor kappa-B (NF-B) showed a statistically significant reduction in immunoreaction. The difference between the atomoxetine with dexamethasone treatment group and the control group was still quite substantial despite this improvement. This showed that atomoxetine had a partial ameliorative impact on dexamethasone-induced atrophy.

When compared to control group, the Heat shock protein (Hsp70) immunostaining in the formetrol-treated group exhibited a statistically significant increase. This result was consistent with research by Koya *et al*.[35], who showed that skeletal muscle hypertrophy causes an increase in heat shock protein 70 (HSP70). AKT, mTOR, and HSP70 have also been shown to be markers of muscle growth by Maeda & Nakamura[36]. While muscle fiber sarcoplasm displayed a negative immunoreaction to nuclear factor kappa-B immunostained sections. This result was consistent with Martin *et al*.[37] who showed that formoterol lowers nuclear factor kappa-B (NF-KB) activity. Formoterol is a selective adrenoreceptor agonist, according to Kim

et al.[38] and Hardy *et al*.[39]. On skeletal muscle, it exerts an anti-inflammatory and hypertrophic effect. In numerous inflammatory conditions, including bronchial asthma and chronic obstructive pulmonary disease, it has been shown to provide anti-inflammatory properties.

According to the current investigation, the formetrol with dexamethasone treated group had a statistically significant increase in heat shock protein (Hsp70) immunoreactivity when compared to control and dexamethasone treated groups. This result corroborated Koya *et al*.[35] discovery that skeletal muscle hypertrophy causes an increase in heat shock protein 70 (HSP70). However, when compared to the dexamethasone-treated group, nuclear factor kappa-B showed a statistically significant decrease in immunoreaction. The difference between the formametrol with dexamethasone treatment group and the control group was still substantial despite the improvement. This result suggested that formetrol had a partial ameliorative impact on dexamethasone-induced atrophy. This was consistent with Martin *et al*.[37] finding that formoterol decreases the activity of nuclear factor kappa-b (NF-KB). Additionally, Capellino *et al*.[40] demonstrated that formoterol has anti-inflammatory impact on skeletal muscles since it lowers the production of Cyclooxygenase-2 (COX-2) and tumor necrosis factor (TNF) as well as nuclear factor kappa-B phosphorylation.

CONCLUSION

In conclusion, Formetrol has a potential role in preventing skeletal muscle atrophy due to its ability to stimulate skeletal muscle growth better than atomoxetine.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Aversa, Zaira, *et al*. "The clinical impact and biological mechanisms of skeletal muscle aging." Bone 127 (2019): 26-36. doi.org/10.1016/j.bone.2019.05.021
2. Verzola, Daniela, *et al*. "Emerging role of myostatin and its inhibition in the setting of chronic kidney disease." Kidney international 95.3 (2019): 506-517. https://doi.org/10.1016/j.kint.2018.10.010
3. Shukla, Surendra K., *et al*. "Macrophages potentiate STAT3 signaling in skeletal muscles and regulate pancreatic cancer cachexia." Cancer letters 484 (2020): 29-39. https://doi.org/10.1016/j.canlet.2020.04.017
4. Sartori, Roberta, Vanina Romanello, and Marco Sandri. "Mechanisms of muscle atrophy and hypertrophy: implications in health and disease." Nature communications 12.1 (2021): 330. https://doi.org/10.1038/s41467-020-20123-1
5. Martinez-Redondo V, Jannig PR, Correia JC, Ferreira DM, Cervenka I, Lindvall JM, Sinha I, Izadi M, Pettersson-Klein AT, Agudelo LZ, Gimenez-Cassina A, Brum PC, Dahlman-Wright K, Ruas JL, (2016). Peroxisome proliferator-activated receptor gamma coactivator-1 alpha isoforms selectively regulate multiple splicing events on target genes. J. Biol. Chem 291, 15169–15184. https://doi.org/10.1074/jbc.M115.705822
6. Drechsler, R.; Brem, S.; Brandeis, D.; Grünblatt, E.; Berger, G.; Walitza, S. (2020). ADHD: Current Concepts and Treatments in Children and Adolescents. Neuropediatrics 2020, 51, 315–335. DOI: 10.1055/s-0040-1701658
7. Halling, Jens Frey, and Henriette Pilegaard. "PGC-1α-mediated regulation of mitochondrial function and physiological implications." Applied Physiology, Nutrition, and Metabolism 45.9 (2020): 927-936. https://doi.org/10.1139/apnm-2020-0005.
8. Schiaffino S, Dyar KA, Ciciliot S, Blaauw B, Sandri M. (2013). Mechanisms regulating skeletal muscle growth and atrophy. Proc Natl Acad Sci USA. 2013; 101: 6472–6477. doi: 10.1111/febs.12253.
9. Peterson YK, Cameron RB, Wills LP, Trager RE, Lindsey CC, Beeson CC, Schnellmann RG. (2013). b2-Adrenoceptor agonists in the regulation of mitochondrial biogenesis. Bioorg Med Chem Lett. 2013; 23: 5376–5381. https://doi.org/10.1016/j.bmcl.2013.07.052
10. Busquets S, Toledo M, Sirisi S, Orpi M, Serpe R, Coutinho J, Martinez R, Argiles JM,Lopez-Soriano FJ. (2011). Formoterol and cancer muscle wasting in rats: effects onmuscle force and total physical activity. Experimental and Therapeutic Medicine2:731–735 DOI 10.3892/etm.2011.260. doi.org/10.3892/etm.2011.260
11. Kiernan JA. (2008). Histological and Histochemical Methods: Theory and Practice. 4th ed. Bloxham, UK: Scion. 2008; 12: 154-167.
12. Dapson RW, Fagan C, Kiernan JA, Wickersham TW. (2011). Certification procedures for sirius red F3B (CI 35780, Direct red 80). Biotechnic & Histochemistry. 2011; 86(3): 133-9. https://doi.org/10.3109/10520295.2011.570277
13. Park HJ, Youn HS. (2013). Mercury induces the expression of cyclooxygenase-2 and inducible nitric oxide synthase. Toxicology and Industrial Health. 2013; 29(2): 169–74. https://doi.org/10.1177/0748233711427048
14. Dawson B, Trapp RG. (2004). Basic & Clinical Biostatistics. In: Basic & Clinical Biostatistics. 4th ed. Lange Medical Books / McGraw- Hill Medical Publishing Division; 2004: 162–89. Localization: BR1719.1; 610.1 LI, D269b, 4. ed. 2004 ex. 1. 011933
15. Renew, J. Ross, *et al*. "Neuromuscular blockade management in the critically Ill patient." Journal of Intensive Care 8 (2020): 1-15. https://doi.org/10.1186/s40560-020-00455-2

16. Hong Y, Lee JH, Jeong KW, Choi CS, Jun HS. (2019). Amelioration of muscle wasting by glucagon-like peptide-1 receptor agonist in muscle atrophy. Journal of cachexia, sarcopenia and muscle. 2019; 10(4): 903-18. https://doi.org/10.1002/jcsm.12434

17. Jeong, Hyeon-Ju, *et al*. "Ginsenoside Rg1 augments oxidative metabolism and anabolic response of skeletal muscle in mice." Journal of Ginseng Research 43.3 (2019): 475-481. https://doi.org/10.1016/j.jgr.2018.04.005

18. Drescher C, Konishi M, Ebner N, Springer J. (2015). Loss of muscle mass: current developments in cachexia and sarcopenia focused on biomarkers and treatment. J Cachexia Sarcopenia Muscle 2015;6:303–311. https://doi.org/10.1002/jcsm.12082

19. Dumitru A, Radu BM, Radu M, Cretoiu SM. (2018). Muscle Changes During Atrophy. Adv Exp Med Biol. 2018; 73-92. https://doi.org/10.1007/978-981-13-1435-3_4

20. Peviani SM, Guzzoni V, Pinheiro-Dardis CM, da Silva YP, Fioravante AC, Sagawa AH, Salvini TF. (2018). Regulation of extracellular matrix elements and sarcomerogenesis in response to different periods of passive stretching in the soleus muscle of rats. Scientific reports. 2018; 8(1): 9010-9019. https://doi.org/10.1038/s41598-018-27239-x

21. Honda, Yuichiro, *et al*. "Relationship between extensibility and collagen expression in immobilized rat skeletal muscle." Muscle & nerve 57.4 (2018): 672-678. https://doi.org/10.1002/mus.26011

22. Lim, Youngshin, *et al*. "β2-adrenergic receptor regulates ER-mitochondria contacts." Scientific Reports 11.1 (2021): 21477. https://doi.org/10.1038/s41598-021-00801-w

23. Jesinkey SR, Korrapati MC, Rasbach KA, Beeson CC, Schnellmann RG. (2014). Atomoxetine prevents dexamethasone-induced skeletal muscle atrophy in mice. J Pharmacol Exp Ther. 2014; 351: 663– 673. DOI: https://doi.org/10.1124/jpet.114.217380

24. Kitajima, Yasuo, Kiyoshi Yoshioka, and Naoki Suzuki. "The ubiquitin–proteasome system in regulation of the skeletal muscle homeostasis and atrophy: from basic science to disorders." The Journal of Physiological Sciences 70.1 (2020): 40. https://doi.org/10.1186/s12576-020-00768-9

25. Yeo, Dongwook, *et al*. "Intensified mitophagy in skeletal muscle with aging is downregulated by PGC-1alpha overexpression in *vivo*." Free Radical Biology and Medicine 130 (2019): 361-368. https://doi.org/10.1016/j.freeradbiomed.2018.10.456

26. Dong J, Dong Y, Chen Z, Mitch WE, Zhang L. (2017). The pathway to muscle fibrosis depends on myostatin stimulating the differentiation of fibro/adipogenic progenitor cells in chronic kidney disease. Kidney Int. 2017 Jan; 91(1):119-128. doi: 10.1016/j.kint.2016.07.029. https://doi.org/10.1016/j.kint.2016.07.029

27. Gómez-SanMiguel AB, Gomez-Moreira C, Nieto-Bona MP, Fernández-Galaz C, Villanúa MA, Martín AI, López-Calderón A. (2016). Formoterol decreases muscle wasting as well as inflammation in the rat model of rheumatoid arthritis. Am J Physiol Endocrinol Metab 310: E925–E937, 2016.atrophy. https://doi.org/10.1152/ajpendo.00503.2015

28. Joassard OR, Amirouche A, Gallot YS, Desgeorges MM, Castells J, Durieux AC, Berthon P, and Freyssenet DG. (2013). Regulation of Akt-mTOR, ubiquitinproteasome and autophagy-lysosome pathways in response to formoterol administration in rat skeletal muscle. Int J Biochem Cell Biol. 2013; 45: 2444–245. https://doi.org/10.1016/j.biocel.2013.07.019

29. Huang X, Zhang Q, Kang X, Song Y, Zhao W. (2010). Factors associated with cancer-related fatigue in breast cancer patients undergoing endocrine therapy in an urban setting: a cross-sectional study. BMC Cancer. 2010; 10(1): 1-7. https://doi.org/10.1186/1471-2407-10-453

30. Douglas W. Van Pelt, Amy L. Confides, Sarah M. Abshire, Emily R. Hunt, Esther E. Dupont-Versteegden, and Timothy A. Butterfield.(2019). Age-related responses to a bout of mechanotherapy in skeletal muscle of rats. 2019 Dec 1; 127(6): 1782–1791. https://doi.org/10.1152/japplphysiol.00641.2019

31. Kukreti, H., Amuthavalli, K., Harikumar, A., Sathiyamoorthy, S., Feng, P. Z., Anantharaj, R., *et al*. (2013). Muscle-specific microRNA1 (miR1) targets heat shock protein 70 (HSP70) during dexamethasone-mediated atrophy. J. Biol. Chem. 288, 6663–6678. doi: 10.1074/jbc.M112.390369.

32. Silvia Pomella , Matteo Cassandri ,Francesco Antoniani ,Samuele Crotti, Laura Mediani , Beatrice Silvestri, Margherita Medici, Rossella Rota ORCID, Alessandro Rosa ORCID and Serena Carra ORCID. (2023). Heat Shock Proteins: Important Helpers for the Development, Maintenance and Regeneration of Skeletal Muscles.2023, 2(2), 187-203; doi.org/10.3390/muscles2020014.

33. De Oliveira AA, Priviero F, Tostes RC, Webb RC, Nunes KP. (2021). Dissecting the interaction between HSP70 and vascular contraction: role of [Formula: see text] handling mechanisms. Sci Rep. 2021 Jan 14;11(1):1420. doi: 10.1038/s41598-021-80966-6. doi.org/10.1038/s41598-021-80966-6

34. Khan, Zhu S, Nagashima M, MA, Yasuhara, S Kaneki, M and Martyn, JA: (2013). Lack of caspase-3 attenuates immobilization-induced muscle atrophy and loss of tension generation along with mitigation of apoptosis and inflammation. Muscle Nerve.2013 47: 711-721. https://doi.org/10.1002/mus.23642

35. Koya T., Nishizawa S., Onho Y., Goto A., Ikuta A., Suzuki M., *et al*.. (2013). Heat shock transcription factor 1-deficiency attenuates overloading-associated hypertrophy of mouse soleus muscle. PLoS One 8:e77788. 10.1371/journal.pone.0077788. https://doi.org/10.1371/journal.pone.0077788

36. Maeda M., Nakamura H. (2020). Effects of bathing of Yunohama hot spring (chloride spring) at Yamagata prefecture in Japan. J. Hot Spring Sci. 2020;70:27–34.

37. Martín, A. I., Gómez-Sanmiguel, A. B., Priego, T. & López-Calderón, (2018). A.Formoterol treatment prevents the effects of endotoxin on muscle TNF/NFkB, Akt/mTOR, and proteolytic pathways in a rat model. Role of IGF-I and miRNA 29b. Am. J. Physiol. - Endocrinol. Metab. 315, E705–E714 (2018. https://doi.org/10.1152/ajpendo.00043.2018

38. Kim, J., Grotegut, C. A., Wisler, J. W., Li, T., Mao, L., Chen, M., Chen, W., Rosenberg, P. B., Rockman, H. A., & Lefkowitz, R. J. (2018). β-arrestin 1 regulates β2-adrenergic receptor-mediated skeletalRomano, *et al*. / Beta (2)-adrenergic receptors activation Vmuscle hypertrophy and contractility. Skeletal Muscle, 8. https://doi.org/10.1186/s13395-018-0184.

39. Hardy J, Baggott C, Fingleton J, Reddel HK, Hancox RJ, Harwood M, Corin A, Sparks J, Hall D, Sabbagh D, Mane S, Vohlidkova A, Martindale J, Williams M, Shirtcliffe P, Holliday M, (2019).Weatherall M, Beasley R;PRACTICAL study team. Budesonide-formoterol reliever therapy versus maintenance budesonide plus terbutaline reliever therapy in adults with mild to moderate asthma (PRACTICAL): a 52-week, open-label, multicentre, superiority, randomised controlled trial. Lancet 2019;394:919-28. https://doi.org/10.1016/S0140-6736(19)31948-8

40. Capellino S, Cosentino M, Wolff C, Schmidt M, Grifka J, Straub RH. (2010).Catecholamine-producing cells in the synovial tissue during arthritis:modulation of sympathetic neurotransmitters as new therapeutic target.Ann Rheum Dis. 2010; 69: 853–860. https://ard.bmj.com/content/69/10/1853.

الملخص العربى

دراسة نسيجية لمقارنة تأثير أتوموكسيتين مقابل فورمترول على ضمور العضلات الهيكلية المحدث بالديكساميثازون في ذكور الفئران

ثروت لطفى أحمد، هبه عصام رشاد، نهى عبداللطيف ابراهيم، مروه عمر عبد العال، نهاد احمد صادق

قسم الهستولوجى، كلية الطب، جامعة الفيوم

المقدمه: يعد ضمور العضلات مشكلة صحية كبيره. وقد يمنع الفورميترول (محفز مستقبلات بيتا) ضمور العضلات. بينما الاتوميكستين (مثبط نور ادرينالين) يلعب ايضا دور فى منع ضمور العضلات.

الهدف من الدراسة: مقارنة تأثير الأتوموكسيتين مقابل الفورميترول على ضمور العضلات الهيكلية الناجم عن الديكساميثازون في ذكور الفئران.

المواد والطرق: تم تقسيم ثمانية وأربعين من ذكور الفئران البيضاء البالغة إلى ست مجموعات (٨ فئران لكل مجموعة): تم حقن حيوانات المجموعة الأولى (المجموعة الضابطة) بمحلول ملحي معقم ٠,٥ مل يوميًا لمدة سبعة أيام. تم حقن حيوانات المجموعة الثانية (المجموعة المعالجة بالديكساميثازون) بجرعة ١٠ ملغم/كغم/يوم من ديكساميثازون لمدة سبعة أيام للحث على ضمور العضلات المجموعة ٣ (المجموعة المعالجة بالأتوموكسيتين فقط): تلقت الحيوانات الأتوموكسيتين بجرعة ٦ ملغم/كغم/يوم عن طريق الفم باستخدام حقنة الأنسولين بدون إبرة لمدة سبعة أيام. المجموعة ٤ (المجموعة المعالجة بالآتوموكسيتين + الديكساميثازون): تلقت الحيوانات كلاً من الديكساميثازون والأتوموكسيتين بنفس الجرعات وطرق الإعطاء كمجموعتين ٢ و ٣ على التوالي. المجموعة ٥ (المجموعة المعالجة بالفورميترول فقط): تم حقن الحيوانات داخل الصفاق بـ ٠,٦ ملغم / كغم / يوم من فورميترول لمدة سبعة أيام. المجموعة ٦ (المجموعة المعالجة بالفورميرترول + الديكساميثازون): تلقت الحيوانات كلاً من الديكساميثازون والفورميترول بنفس الجرعات وطرق الإعطاء مثل المجموعتين ٢ و٥ على التوالي. في نهاية التجربه (٧) ايام تم التضحسة بالفئران و إستأصال عضلة الساق وإخضاعها لصبغة الهيماتوكسيلين و الايوسين و صبغة بيكروسيريس الاحمر وصبغة هستوكيميائية مناعية مضادة لبروتين الصدمة الحرارية ٧٠ والعامل النووي كابا بي. تم قياس النسبة المئوية لمساحة ترسب ألياف الكولاجين، والنسبة المئوية لمساحة التعبير المناعي للعامل النووي، ونسبة مساحة التعبير المناعي لبروتين الصدمة الحرارية ٧٠ وقطر الألياف العضلية.

النتائج: المجموعة ٤ (المجموعة المعالجة بالأتوموكسيتين والديكساميثازون) والمجموعة ٦ (المجموعة المعالجة بالفورميرترول والديكساميثازون) أظهرت زيادة في قطر الألياف العضلية مقارنة بمجموعة الديكساميثازون.

الاستنتاج: للفورميترول دور محتمل في الوقاية من ضمور العضلات الهيكلية.