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Research Article

The Possible Protective Role of Coenzyme Q10 against Simvastatin Induced Gastrocnemius and Diaphragmatic muscles toxicity in Adult Male Albino Rats: Light and Electron microscopic Study

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Abstract

Background: Simvastatin is a powerful drug that has cholesterol concentration lowering impact. But it can cause skeletal muscles complaints that vary from mild myalgia to deadly rhabdomyolysis. The safety of statins on skeletal muscles including diaphragm as a respiratory muscle remains questionable. Coenzyme Q10 (CoQ10) is a chemical substance that is naturally present in most living cells. It serves as mitochondrial respiratory chain electron carrier. **Objective:** The current study set out to assess the possible protective impact of exogenous CoQ10 administration on the potential histological alterations in the rat gastrocnemius and diaphragmatic muscles induced by simvastatin. **Materials and Methods**: Forty mature male rats were split equally into four groups; control group, CoQ10 treated group, Simvastatin-treated rats, and Simvastatin accompanied with CoQ10 for a period of 4 weeks. At end of experiment rat animals were slaughtered, serum creatine kinase (CK) concentration was measured. Right gastrocnemius and diaphragmatic muscles samples were excised to be prepared for light and electron microscopic study. **Results:** Simvastatin treated rats showed significant rise in CK concentration and histopathological alterations, in the form of loss of transverse striations, separation of myofibril, damaged mitochondria and enlarged sarcoplasmic reticulum. CoQ10 co- administration could mitigate simvastatin-induced histological alterations and restore the muscle fiber architecture due to its anti-fibrotic and anti-apoptotic impacts. **Conclusion:** Exogenous CoQ10 administration mitigated degenerative alterations in both skeletal and diaphragmatic muscles triggered by simvastatin administration.

Key Words: Statins; CoQ10; skeletal muscles; diaphragm, Ultrastructure

Introduction

Hyperlipidemia is the leading cause of atherosclerosis, which has been linked with coronary arteries insufficiency, disability, even fatality. Modifying lifestyle is the first choice towards managing hyperlipidemia. In addition, one of the most commonly prescribed pharmaceuticals for the management of high lipid levels and its associated hazards including atherosclerosis is mainly statins [1].

Statins are a secure and well-tolerated lipid lowering medication. It operates primarily by diminishing serum cholesterol levels and

anchoring atherosclerotic plaques with subsequent minimization of the risk of acute cardiac attacks and strokes [2]. Statins additionally mitigate cellular oxidation and inflammatory conditions.

It acts by inhibiting 3-hydroxy-3-methyl glutaryl-coenzyme A (HMGCoA) reductase, an enzyme that promotes cholesterol formation via the mevalonate pathway [3].

Skeletal muscles are classified as a striated muscle that is responsible for movement of axial and appendicular skeletons, sustaining posture, metabolism, and nutrient storage. The thoracic diaphragm is an inner-stretched striated muscular sheet in human and other mammals separating the thoracic cavity from abdominal one. The thoracic diaphragm muscle is critical and essential skeletal muscle in the human body as it is the main muscle involved in the mechanism of respiration, In addition; diaphragmatic muscular contraction causes an increase in intra-abdominal pressure, which aids in the evacuation of wastes such as urine and feces [4]. The muscle mass of the human skeletal system accounted for 30 to 40% of the total human body. As a result, maintaining skeletal muscle mass is necessary for a satisfactory quality of life [5].

Since 40 to 75 percent of patients who take statins in a regular manner, stop taking them within a year or two as skeletal muscle issues brought on by statins therapy are the main reason why people stop taking them [6]. Skeletal muscle complains range from minor myalgia to catastrophic rhabdomyolysis. According to clinical trials, respiratory myopathy contributes to the myotoxic profile of statins. In earlier studies, statin-treated smokers have demonstrated diaphragmatic muscle malfunction. Furthermore, a patient with myopathic symptoms who used statins along with other concomitant medications was shown to have impaired respiratory functions, which ultimately resulted in increased mortality rate [7].

Statins exist in two varieties: hydrophilic (such as Pravastatin and Rosuvastatin) and lipophilic (such as Atorvastatin, Simvastatin, and Lovastatin). Hydrophilic statins are mostly found in hepatocytes while, lipophilic statins are found in various body tissues, easily penetrate cells, and possess an impact on muscles. As a result, lipophilic statins frequently trigger myopathy over time [8].

Coenzyme Q10 (CoQ10) is a benzoquinone ring with an isoprene side chain. Its major known form is the reduced active one, ubiquinol. Its molecular structure is remarkably similar to that of vitamin K but CoQ10 is naturally lipid-soluble antioxidant molecule created in body cells de novo while vitamins must be supplemented [9].

CoQ10 is a cofactor for mitochondrial enzyme complexes and it has a membrane stabilizer impact on both cell membrane and intracellular membranes. It is detected in both mitochondria and plasma membranes. Furthermore, it has antioxidant properties as it inhibits deoxyribonucleic acid (DNA) oxidation process [10].

In medicine, CoQ10 is used to deal with issues like aging process that are linked to mitochondrial malfunction. Furthermore, it lessens the detrimental impacts of heavy metals like lead and arsenic by reducing intracellular oxidation and inflammatory processes that contribute to cell death [11].

Statins work by inhibiting the mevalonate pathway, which lowers both cholesterol and CoQ10 levels [10]. Subsequently statins diminish the concentration of CoQ10 in blood and tissues. Multiple studies conducted on people and animals reported that exogenous CoQ10 supplementation can relieve statin-associated muscle symptoms (SAMS) [12].

Therefore, the purpose of this research is to assess the histopathological and ultrastructural changes in the gastrocnemius and diaphragmatic muscles that statin administration may induce in adult male albino rats, as well as the efficacy of exogenous CoQ10 administration in mitigating these changes to reduce hazards of stopping statins regimens because of SAMS.

Material and Methods

Chemicals:

1- Simvastatin: Simvastatin tablet 20 mg Merk and CO. Company of pharmacy, USA. The calculated therapeutic dosage was 7.2 mg /kg rat body weight given once per day via gastric tube after being dissolved in distilled water [13]. Each rat received one ml distilled water containing nearly 1.5 mg for four weeks.
2- Co enzyme Q10: CoQ10 gelatin capsule 30mg MEPACO-MEDIFOOD (Arab Company for Pharmaceutical and Medicinal plants), Egypt. Each rat received 0.5 ml soybean oil containing nearly 3 mg given once per day via gastric tube for four weeks. This dose is corresponding to 200 mg/day for humans [14].
3- Cytochrome C immunostaining: Rabbit pAb (Catalog No.: A0225, ABclonal, United States) at dilution of 1:150.

Animals:

From the animal shelter of the National Research Center (Cairo, Egypt), forty adult male albino rats weighing 200–240 grams were purchased. They were placed in hygienic plastic cages that provided a regular light/dark cycle. The animals were fed with an appropriate diet and free access to drink. The experiment methodology had been authorized by El Fayoum Faculty of Medicine's animal handling research ethical committee (Approval No R555/2024) in compliance with international guidelines (Act 1986).

After adapting for seven days, the research animals were grouped randomly into four equal groups. Each group is composed of 10 animal rats, as follows:

Group I (control group) fed with an appropriate diet and free access to drink equal to the amount introduced to other groups throughout period of experiment.

Group II (CoQ10 group), positive control was given the therapeutic dose which was nearly 3 mg/rat/day given via gastric tube for a period of four weeks [14].

Group III (Simvastatin group), was given the therapeutic dose which was approximately 1.5 mg /rat /day given via gastric tube for a period of four weeks [13].

Group IV. Simvastatin and CoQ10 group, received the aforementioned doses and routes of Co Q10 concomitantly with Simvastatin for four weeks

At end of four weeks duration of medications administration, all rat animals from all studied groups were weighted then anesthetized using sodium pentobarbital in a concentration of 40 mg/kg via intraperitoneal (IP) route. The gastrocnemius muscle from the right hind limb was dissected and weighed. Muscle / body weight ratio was calculated for each rat. The belly part of gastrocnemius muscle was dissected away from its related tendon of each rat animal then divided in a longitudinal direction into two pieces. Each part was processed separately to be examined under light microscope and electron microscope. In a similar manner, the diaphragmatic muscle was dissected and processed for further microscopic study.

Sampling:

Blood samples were drawn from tail vein of each rat animal just before anesthesia, to be utilized for determination of plasma level of CK, a biomarker for muscle injury, at first blood samples were subjected to centrifugation for 15 minutes at 3000 rounds per minute (rpm). The diagnostic kits (Sigma Aldrich Co., Cairo, Egypt) were utilized to measure the CK value (IU/L).

Histological study:

For each group, the gastrocnemius muscle was dissected in longitudinal manner, then fixed using neutral buffer formalin solution (10%), followed by dehydration step by subjecting the muscle specimens to series of ascending grades of alcohol then, cleaned, and finally embedded within blocks made of paraffin. For routine histological analysis, 4 to 5 µm-thick paraffin slices were cut and stained with H&E [15] and Mallory's trichrome stain to demons-trate collagen fiber[16]. The diaphragmatic muscles were processed similarly.

Immunohistochemical study:

Another set of paraffin made blocks were cut into 5µm sections to be stained immunohistochemically with cytochrome C antibody.

Sections were first subjected to boiling for ten minutes in 10 mM citrate buffer solution with pH adjusted at 6.0. These steps allow antigen retrieval under high pressure circumstances. Then these sections were exposed to hydrogen peroxide for 15 minutes duration to block endogenous peroxidase activities, after that, it was probed with a cytochrome C rabbit monoclonal antibody diluted in 3% bovine serum albumin-phosphate buffered saline (BSA-PBS) with a dilution adjusted at 1:150 for an hour at 37°C in a humidified room using. The cytoplasm showed a positive cytochrome C response. An Olympus digital camera was used to capture pictures of the slides. Adobe Photoshop 7 was employed to standardize brightness, contrast, and background color

Transmission electron microscopy (TEM) study:

Other portions of gastrocnemius muscles and diaphragmatic muscles were dissected into smaller pieces of about 1 mm then immersed in a mix of Glutaraldehyde (2.5%) and Paraformaldehyde (2%). Osmium tetroxide (1%) in 0.1 mol/l sodium cacodylate buffer (pH 7.4) was utilized to fix the tissue samples for one hour at 4 ℃. Then tissue samples were embedded in epoxy resin to be cut into semi-thin (1μm) sections stained with Toluidine blue. Uranyl acetate (2%) and lead citrate were the substances utilized to stain the ultrathin sections for electron microscopy at the Faculty of Science's electron microscope unit at Alexandria University in the Alexandria governorate, Egypt (JEM-100CXi, Jeol, Japan).

Morphometric studies:

The following variables were determined utilizing ten separate sectors from different slides of the studied groups:

- Diameter of longitudinal section (L.S.) of skeletal and diaphragmatic muscle fibers in H&E stained sections x100.
- Mean percent of degenerated muscle fibers of skeletal and diaphragmatic muscle
- Mean percent of collagen fibers stained with Masson's trichrome stained slides x100 of skeletal and diaphragmatic muscle
- Mean percent area of cytochrome C positive reaction of skeletal and diaphragmatic muscle x400.

Statistical analysis

The GraphPad Prism (version 9) was used to analyze quantitative variables. The data analyzed was rat muscle/body weight ratio, CK level, muscle fiber diameter, degenerated fibers percent, collagen fibers percent and percentage area of cytochrome C immune-reactivity; The results are reported as mean ± SEM. A p-value of less than 0.05 was considered statistically significant.

Results

Biochemical results:

Simvastatin therapy considerably raised serum CK levels ($P < 0.001$). Simultaneous Coq10 prescription led to significantly lower serum CK values ($P<0.001$) (Fig 1a).

Physiological Results:

The simvastatin-treated group had a signify-cantly less gastrocnemius muscle weight/ body weight ratio versus the control and Coq10 treated groups. The concurrent treatment of Coq10 with simvastatin showed no significant distinction with other groups (Fig 1b).

Results for the gastrocnemius muscle:

H&E-stained sections of gastrocnemius muscle:

Slices of longitudinal gastrocnemius muscle fibers from the control and Coq10 treatment groups revealed well-established transverse striations with acidophilic stained cytoplasm. Skeletal muscle fibers notably were long, cylindrical and parallel to each other with no-branching. Nuclei were peripheral in position directly under sarcolemma (Fig 2A, B).

Skeletal muscle fibers underwent treatment with simvastatin showed diminished typical structure in some fibers, which exhibited disorganization and fragmentation with vacuole formation, extravasated red blood cells, and infiltration with mononuclear cells in the CT. Nuclei were deeply stained and shifted centrally (Fig. 2C1, C2, C3).

Simvastatin with Coq10 administration improved the typical pattern of majority skeletal muscle fibers, which displayed elongated, cylindrical, parallel, and non-branching fibers with acidophilic sarcoplasm and the oval nuclei attain its peripheral position (Fig. 2D).

Mallory's trichrome-stained sections of gastricnemius muscle:

Longitudinal Mallory's trichrome-stained sections of gastrocnemius muscle fibers of the control and Coq10-treated groups exhibited positive expression of collagen amount (Fig. 3A,B). Simvastatin-treated group exhibited high positive expression of collagen (Fig. 3C). Simvastatin with Coq10 administration group demonstrated moderate reduction in collagen content (Fig. 3D).

Cytochrome C-stained sections of gastricnemius muscle:

Cytochrome C-stained sections of longitudinal gastrocnemius muscle fibers from the control and Coq10 treated groups revealed positive cytochrome C immunoreactivity in the sarcoplasm of muscle fiber (Fig. 4A, B).

Simvastatin-treated group revealed most fibers of skeletal muscle with a high positive sarcoplasmic immunoreaction (Fig. 4C).

Simvastatin with Coq10 administration revealed sporadic spots of positive sarcoplasmic immunoreaction in some muscle fibers (Fig. 4D).

Morphometric study of gastrocnemius muscles:

In the current work the average diameter of gastrocnemius muscle fiber in simvastatin group rats was considerably diminished. Conversely, concomitant administration of CoQ10 with simvastatin resulted in considerable increased in compare with simvastatin group (Fig. 5A).

In the current work the degenerated fibers percent in control and CoQ10 groups was minimal. While, in simvastatin group there was considerable rise. Simvastatin with Coq10 administration revealed considerable decline (Fig.5B).

In the current work the mean percent of collagen fibers in control and CoQ10 groups was low. While, in simvastatin group there was considerable rise. Simvastatin with Coq10 administration revealed considerable decline (Fig.5C).

In the current investigation, the percentage of the cytochrome C positive fibers in the control and CoQ10 groups was minimal. A higher percentage was observed in the simvastatin group. Simvastatin with CoQ10 treatment revealed a significant reduction (Fig.5D).

Toluidine blue stained sections of gastricnemius muscles:

Toluidine blue-stained semithin sections of longitudinal fibers of control and Coq10-treated groups revealed cylindrical regularly distributed muscle fibers with uniformly arranged transverse striations and peripherally located oval vesicular nuclei with noticeable nucleoli (Fig. 6A, B).

In simvastatin treated group there was disorganized myofibrils lacking transverse striations, sarcolemma with irregularity, and vacuolated cytoplasm. Nuclei were displaced, deformed, and pyknotic (Fig. 6C1 and C2).

Simvastatin with Coq10 administration revealed parallel muscle fibers with transverse striations remarkably similar to the control, with elongated peripherally located nuclei. However, blood capillaries are observable in-between muscle fibers (Fig.6D).

Electron microscopic examination of gastrocnemius muscles:

Ultrathin slices of longitudinal gastrocnemius muscle fibers from control and Coq10 treated groups revealed regularly arranged parallel muscle fibers with well-recognized sarcolemma. The nucleus is seen elongated and euchromatic. The characteristic striations consist of alternating bright (I) and dark bands (A). The more electron dense A band has a narrow lighter zone called H zone (H), which is bisected by the M line (M), while the light I band has a darker Z line. Mitochondria (Mi) were present near the Z line, glycogen particles is noted. Sarcomere is identified between two sequential Z lines (Figs. 7A, B).

In simvastatin treated group skeletal muscle fibers showed deteriorated myofibrils with disturbed or missing Z line with several areas of breaking down, damaged mitochondria within the myofibrils, and enlarged sarcoplasmic reticulum (Fig.8A, B and C).

Simvastatin with Coq10 administration revealed some myofibrils (Mf) with disorganized pattern while others appear well organized arranged almost in register across the width of a muscle fiber with some areas of myofibrillar breaking down and slightly

disfigured mitochondria between fibrils, degenerated portions of myofibrils with missing of Z line (blue arrow). Apparently normal form sarcoplasmic reticulum cisternae were noted (S) (Fig.9A, B).

Results of diaphragmatic muscles:

H&E-stained sections of diaphragmatic muscles:

Diaphragmatic muscle fibers from control and Coq10 treatment groups showed parallel arranged, elongated, cylindrical muscle fibers with multiple peripheral located flat nuclei and acidophilic stained cytoplasm (Fig.10A, B).

Diaphragmatic muscle underwent treatment with simvastatin revealed disorganized muscle fibers with aggregation of nuclei and cytoplasmic breakdown. In addition, congestion of blood vessel, extravasation of RBCs and inflammatory cellular infiltration were noted (Fig.10 C1, C2, and C3).

Simvastatin with Coq10 administration revealed near normal architectural pattern of some fibers as muscle fibers appeared parallel elongated cylindrical with multiple peripheral located long nuclei and acidophilic stained cytoplasm (Fig.10D).

Mallory's trichrome-stained sections of diaphragmatic muscles:

Longitudinal Mallory's trichrome -stained sections of diaphragmatic muscle fibers of control and Coq10 treated groups exhibited negligible collagen amount (Fig.11A, B).

Simvastatin-treated group exhibited increased collagen content (Fig.11C).

Simvastatin with Coq10 administration group demonstrated a level of moderate decrease in amount of collagen (Fig.11D).

Cytochrome C-stained sections of diaphragmatic muscles:

Longitudinal Cytochrome C-stained sections of diaphragmatic muscle fibers from control group and Coq10 treated groups revealed decreased brown coloration of cytochrome C immune-reactivity (Fig.12A, B).

Simvastatin-treated group revealed increased brown coloration of sarcoplasm of most diaphragmatic muscle fibers (Fig.12C).

Simvastatin with Coq10 administration revealed moderate brown coloration of sarcoplasm (Fig.12D)

Morphometric study of diaphragmatic muscles:

In the current work the average diameter of diaphragmatic muscle fiber in simvastatin group rats was considerably diminished. However, concomitant administration of CoQ10 with simvastatin resulted in considerable increased in compare with simvastatin group (Fig. 13A).

In the present work the percentage of degenerated fibers in control and CoQ10 groups was minimal. While, in simvastatin group there was considerable rise. Simvastatin with Coq10 administration revealed considerable decline (Fig.13B).

In the current work the mean percent of collagen fibers in control and CoQ10 groups was low. While, there was significant increase in simvastatin group. Simvastatin with Coq10 administration revealed considerable decrease compared to simvastatin group (Fig.13C).

In the current work the percent of cytochrome c positively stained fibers in control and CoQ10 groups was low. Higher percentage was noted in simvastatin group. Simvastatin with Coq10 administration revealed significant decrease compared to simvastatin group (Fig.13D).

Toluidine blue stained sections of diaphragmatic muscles:

Longitudinal toluidine blue stained semithin sections of diaphragmatic muscle fibers of control and Coq10 treated groups exhibited cylindrical regularly organized muscle fibers with uniform transverse striations across the entire thickness of the muscle fiber and peripheral oval vesicular nuclei with noticeable nucleoli (Fig.14A, B).

Diaphragmatic muscle fibers of simvastatin treated group showed disorganized myofibrils with lost transverse striations and cytoplasmic vacuolations. Nuclei were displaced, disfigured and pyknotic (Fig.14C1, C2).

Simvastatin with Coq10 administration revealed regularly arranged parallel muscle fibers with regularly arranged transverse striations almost comparable to the control with elongated peripherally located nuclei However, limited areas of sarcoplasmic dissolution were seen (Fig.14D1,D2).

Electron microscopic examination of diaphragmatic muscles:

Longitudinal ultrathin sections of diaphragmatic muscle fibers of control and Coq10 treated groups showed regularly arranged muscle fibers with parallel longitudinal myofibrils and well-defined sarcolemma. Nucleus is oval in shape, euchromatic and situated under sarcolemma. intermyofibrillar mitochondria and sarcoplasmic reticulum cisternae were noted. Sarcomeres were seen between 2 successive Z lines with characteristic striations of alternating light and dark bands (Fig.15 A, B, C, D).

Diaphragmatic muscle fibers of simvastatin treated group showed degenerated, disrupted or lost myofibrils with many areas of dissolution. Marked aggregation of mitochondria and giant mitochondria (Fig.16A, B, C, D).

Simvastatin with Coq10 administration revealed parallel myofibrils with clear regular striation with apparently normal sarcoplasmic reticulum cisternae nearly similar to control group (Fig.17A, B).

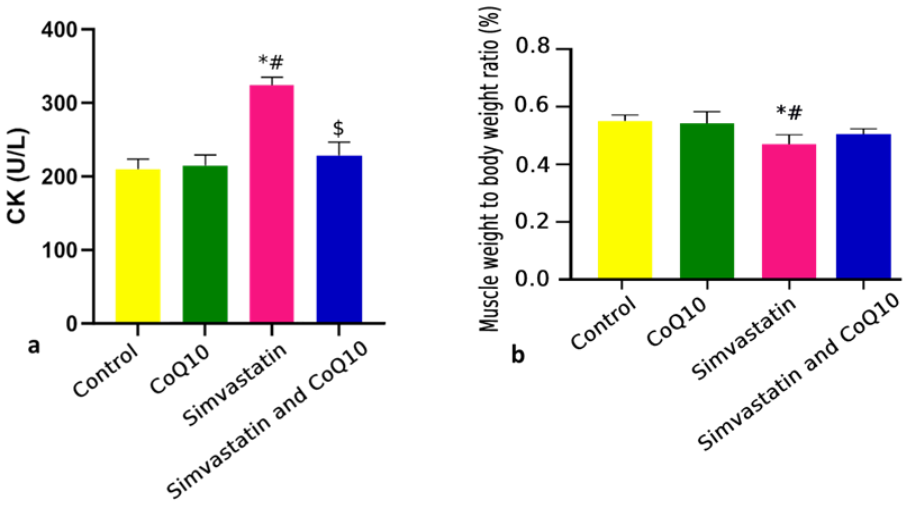

Fig.1a,b) Quantitative analysis of mean levels of CK muscle and weight to body ratio respectively, data are represented as mean ± SEM. *,#, and $ Significance differences from control, CoQ10, and simvastatin Groups respectively at P<0.05.

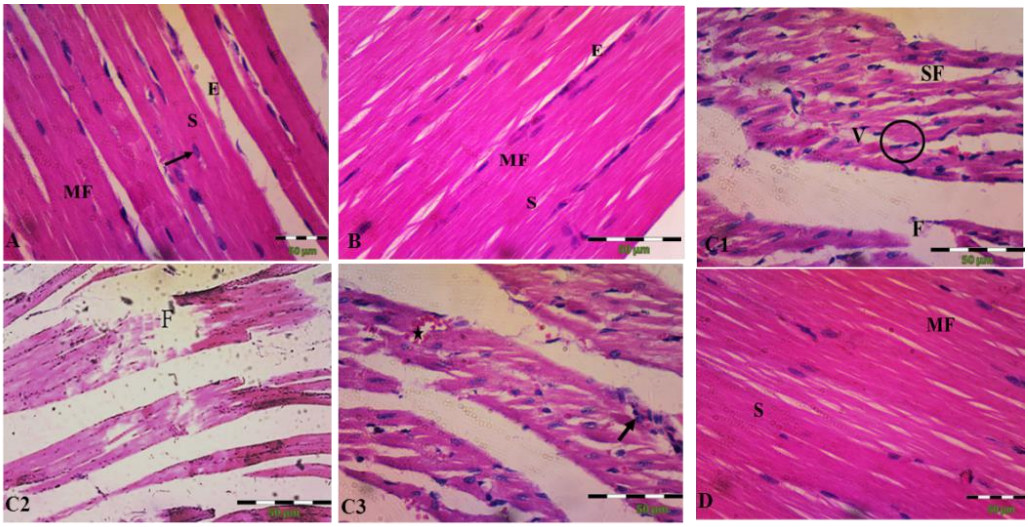

Fig. 2: photomicrograph of H&E stained longitudinal section of adult rat gastrocnemius muscle. (A) control group showing non branching parallel elongated cylindrical muscle fibers (MF). They were separated from each other by fine loose Connective tissue (E). Muscle fibers have acidophilic sarcoplasm (S), multiple peripheral oval nuclei (arrow).(B) Coq10 treated group showing Parallel elongated cylindrical muscle fibers (MF). They were separated from each other by fine loose Connective tissue (E). Muscle fibers have acidophilic sarcoplasm (S), multiple peripheral oval nuclei (arrow). (C1,C2,C3) rat skeletal muscle of simvastatin treated group showing splitting of muscle fiber (SF), disorganized fragmented and discontinued muscle fibers (F) with darkly stained nuclei (circle), vacuolization and fragmentation were seen(V), mononuclear cellular infiltration in CT appeared (black arrow) and extravasated RBCs (astresik). (D) Simvastatin + Coq10 treated group showing parallel non branching elongated cylindrical muscle fibers (MF). Muscle fibers have acidophilic sarcoplasm (S), multiple peripheral oval nuclei (arrow). (H&E X400)

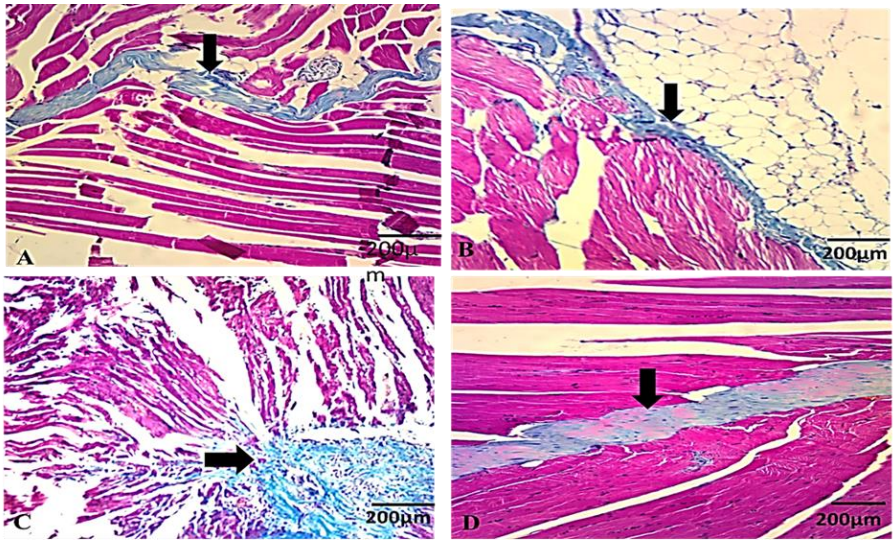

Fig. 3: Photomicrograph of Mallory's trichome- stained longitudinal section of adult rat gastrocnemius muscle. (A,B) control and Coq10 treated groups respectively showing minimal amount of collagen fibers (thick arrows) between muscle fibers. (C) Simvastatin treated group showing excessive amount of collagen fibers (thick arrows) between muscle fibers. (D) Simvastatin + Coq10 treated group showing little amount of collagen fibers (thick arrows between muscle fibers. (H&E x 100).

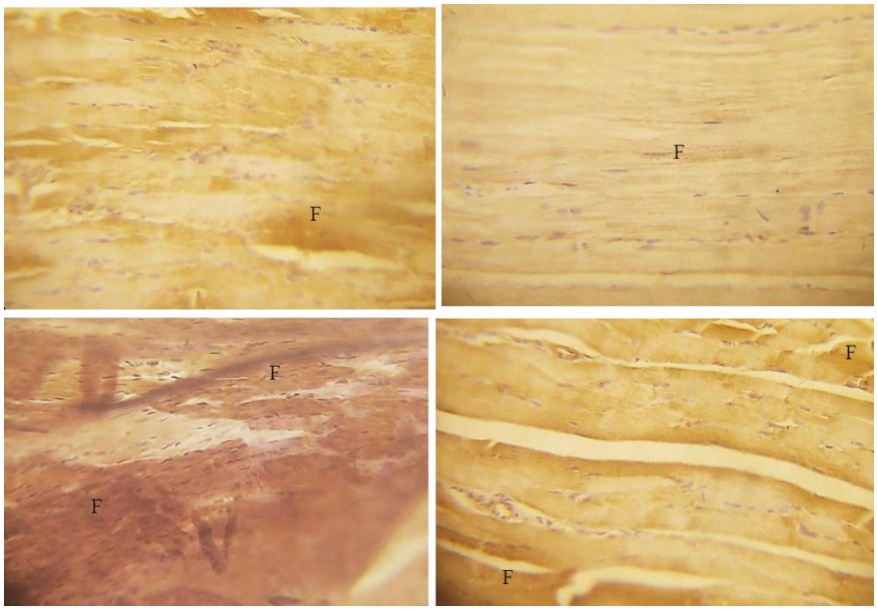

Fig. 4: photomicrograph of cytochrome C immunostaining longitudinal section of adult rat gastrocnemius muscle. (A, B) control and Coq10 treated groups respectively showing decreased brown coloration of sarcoplasm. (C) rat skeletal muscle of simvastatin treated group showing increased brown coloration of sarcoplasm. (D) simvastatin + Coq10 treated group showing moderate brown coloration of sarcoplasm.

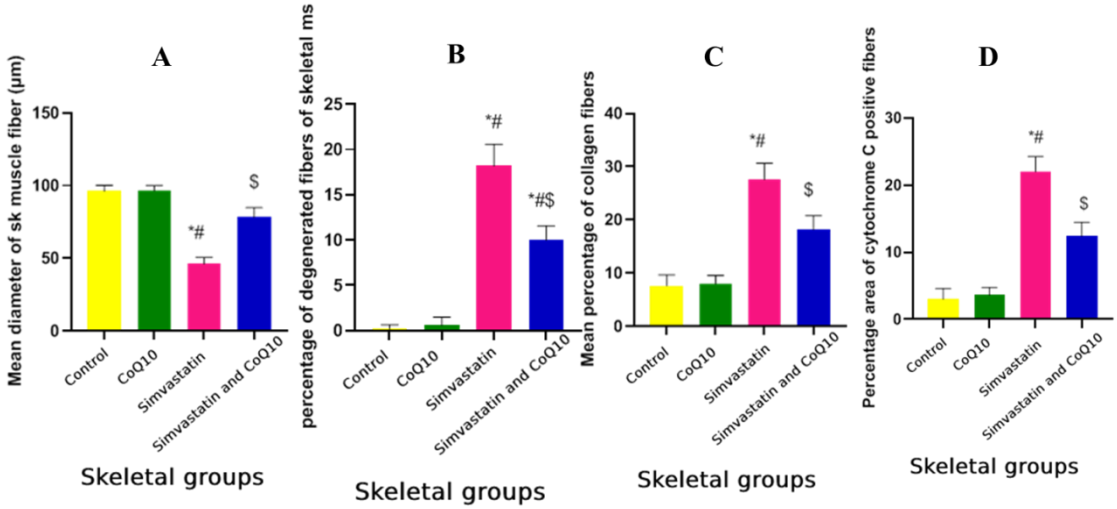

Fig. 5: Quantitative analysis of the mean diameter of L.S. of gastrocnemius muscle fibers (µm) X 400, mean percentage of collagen fibers and percentage area of cytochrome c positive fibers respectively between different groups (n=10). Data are presented as mean ± SD. *,#, and $ Significance differences from control, CoQ10, and simvastatin Groups respectively at P<0.05.

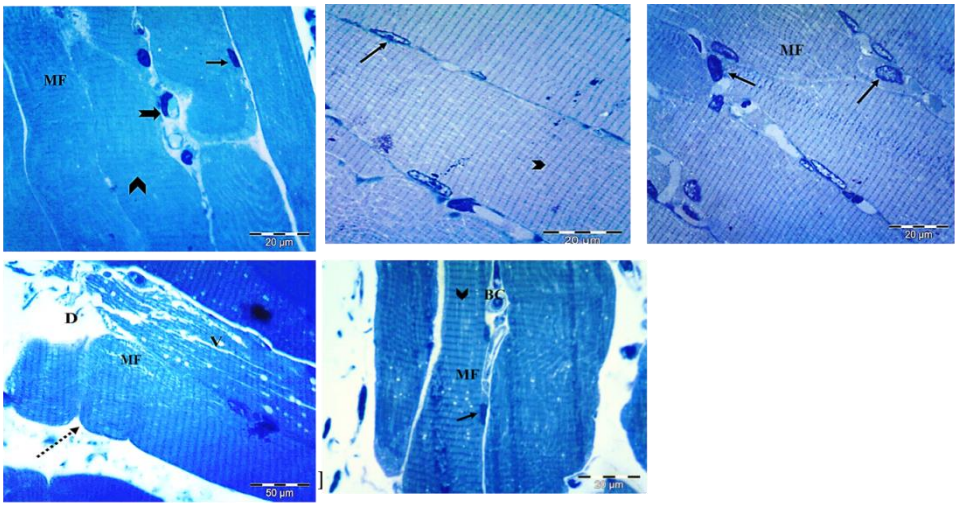

Fig. 6: Photomicrograph of gastrocnemius muscle semi thin sections stained with toluidine blue. (A) control group showing cylindrical regularly arranged muscle fibers (MF) with regular transverse striations (arrow head) across the whole thickness of the muscle fiber and peripheral flat vesicular nuclei with prominent nucleoli (arrow). (B) Coq10 treated group showing cylindrical regularly arranged muscle fibers with regular transverse striations (arrow head) across the whole thickness of the muscle fiber and peripheral oval vesicular nuclei with prominent nucleoli (arrow).(C1,C2) simvastatin treated group showing displaced disfigured pyknotic nuclei (arrow), irregular sarcolemma (dotted arrow), focal areas of sarcoplasmic dissolution (D) in a muscle fiber with disorganized myofibrils (mf) with lost transverse striations, cytoplasmic vacuole(V). (D) Simvastatin + Coq10 treated group regularly arranged parallel muscle fibers(MF) almost comparable to the control with peripheral elongated nuclei (arrow), with clear transverse striations (arrow head). However, Blood capillary is seen between muscle fibers (BC)

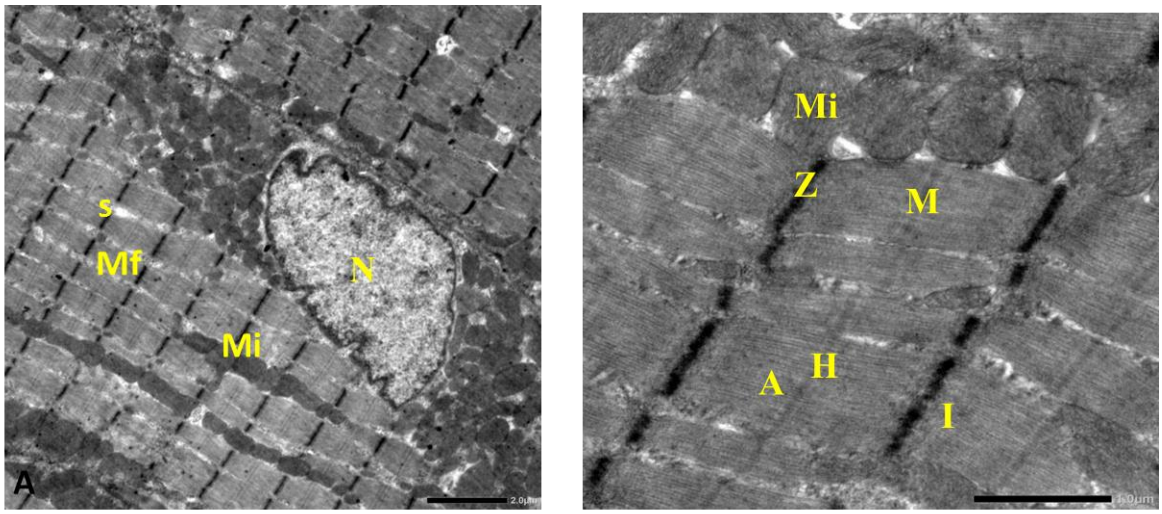

Fig. 7: Electron photomicrograph of rat gastrocnemius muscle from control and coq10 groups showing similar findings in the form of; A) Parallel regular arrangement skeletal muscle fibers with longitudinal parallel myofibrills(Mf), peripheral elongated euchromatic nucleus (N), intermyofibrillar sarcoplasmic reticulum cisternae(S) and mitochondria (Mi). B) Characteristic striations of alternating light (I) and dark bands (A). The more electron dense A band has narrow light zone, H zone (H) bisected by M line (M) and the light I band has dark Z line (Z).

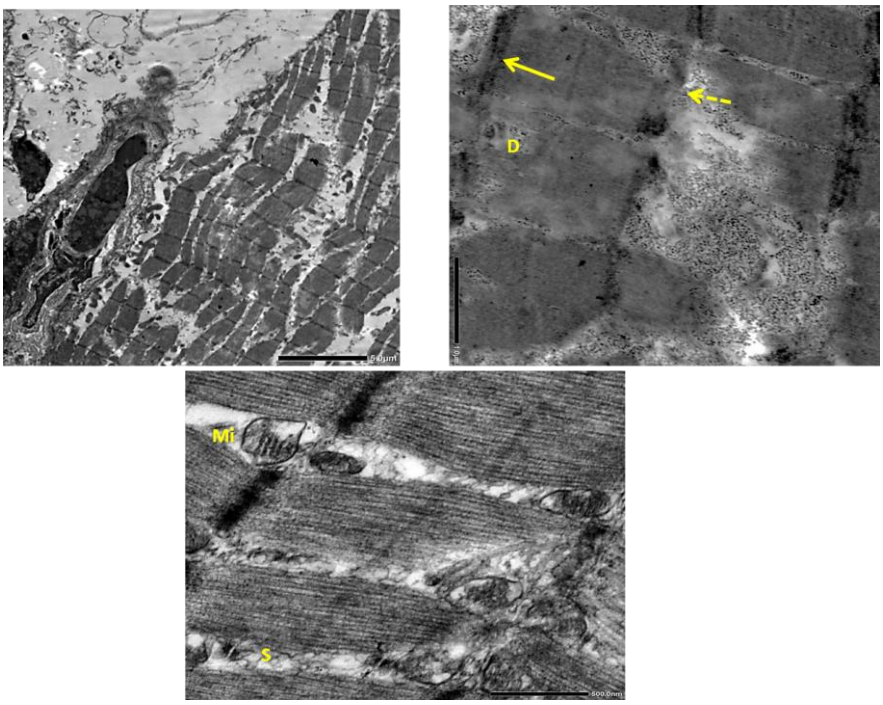

Fig. 8: Electron photomicrographs of simvastatin group of gastrocnemius muscle fibers showing: A,B) Degenerated myofibrils (Mf) with many areas of dissolution (D), myofibrils with disrupted (arrow) or lost (dotted arrow) Z line. C) disfigured mitochondria among the myofibrils (Mi), dilated sarcoplasmic reticulum(S)

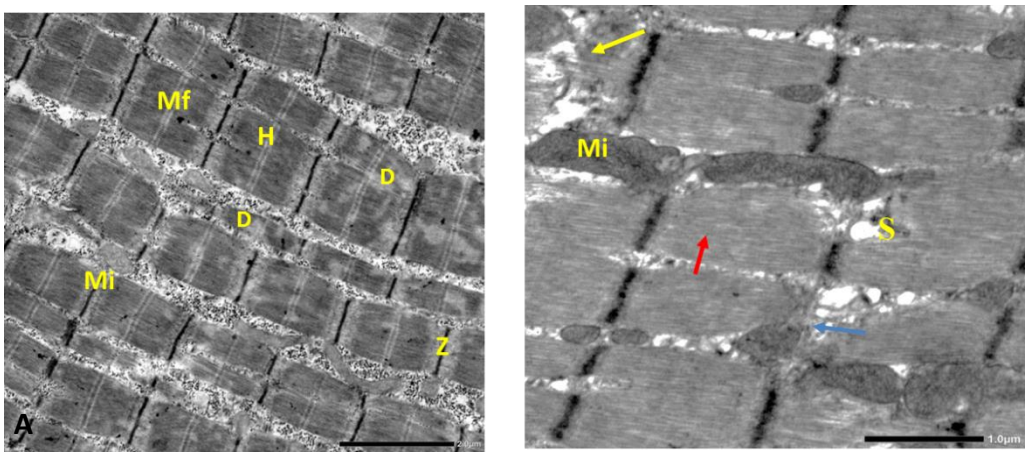

Fig. 9: Electron photomicrographs of simvastatin + Coq10 treated group of gastrocnemius muscle fibers showing A&B: Some myofibrils (Mf) appeared disorganized (yellow arrow) while other myofibrils appear normal (red arrow) arranged almost in register across the width of a muscle fiber with some areas of myofibrillar dissolution (D) and inter fibrillar slightly disfigured mitochondria (Mi), degenerated parts of myofibrils with loss of Z line (blue arrow). Additionally, apparently normal sarcoplasmic reticulum cisternae (S).

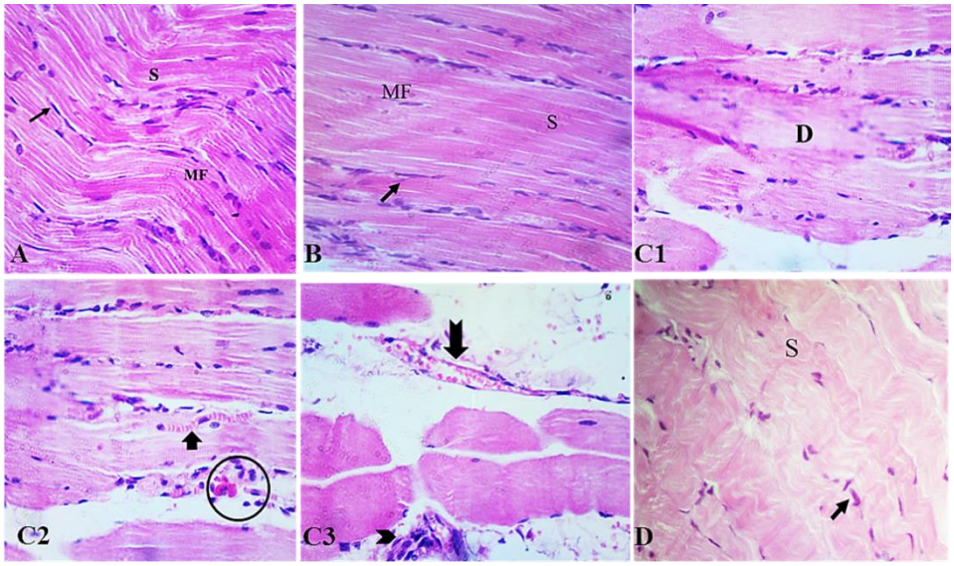

Fig.10: photomicrograph of longitudinal section of rat diaphragm H&E. (A)control group showing parallel elongated cylindrical muscle fibers (MF). Muscle fibers have acidophilic sarcoplasm (S) and multiple peripheral flat nuclei (arrow).(B) Coq10 group showing parallel elongated cylindrical muscle fibers (MF). Muscle fibers have acidophilic sarcoplasm (S) and multiple peripheral oval nuclei (arrow). (C1) statin treated group showing disorganized muscle fibers with an area of cytoplasmic dissolution (D). (C2,C3) simvastatin treated group showing aggregated nuclei (circle), extravasated RBCs (thick arrow), congested blood vessel (bifid arrow) and inflammatory cellular infiltration (arrow head). (D) simvastatin + Coq10 treated group showing parallel elongated cylindrical muscle fibers with acidophilic sarcoplasm (S) and multiple peripheral elongated nuclei (arrow)

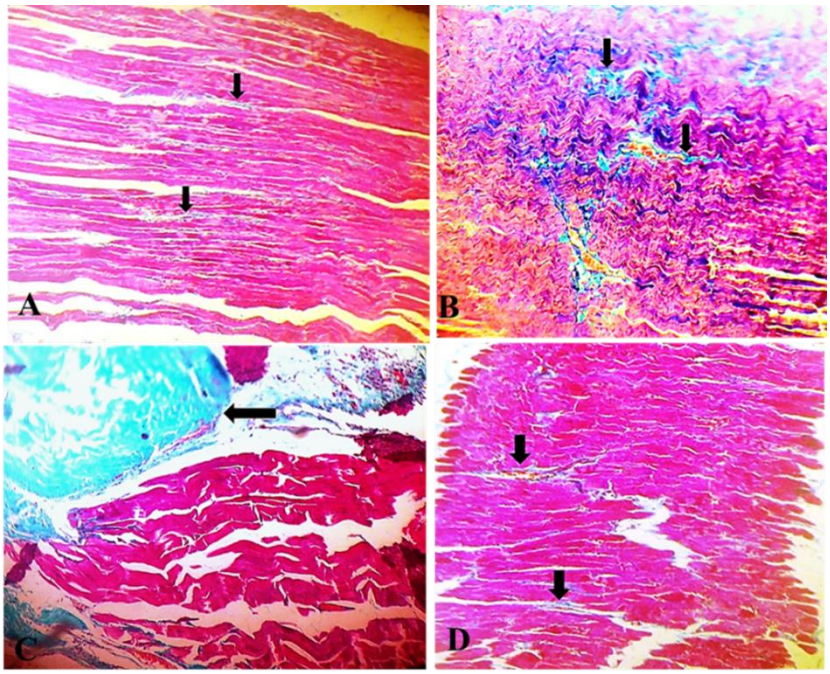

Fig. 11: A photomicrograph of Mallory's trichome- stained longitudinal section of adult rat diaphragm muscle. (A) Control group showing minimal amount of collagen fibers (thick arrows) between muscle fibers. (B) Coq10 group showing minimal amount of collagen fibers (thick arrows) between muscle fibers. (C) Simvastatin treated group showing excessive amount of collagen fibers (thick arrows) between muscle fibers. (D simvastatin + Coq10 treated group showing little amount of collagen fibers (thick arrows) between muscle fibers (H&E x 100)

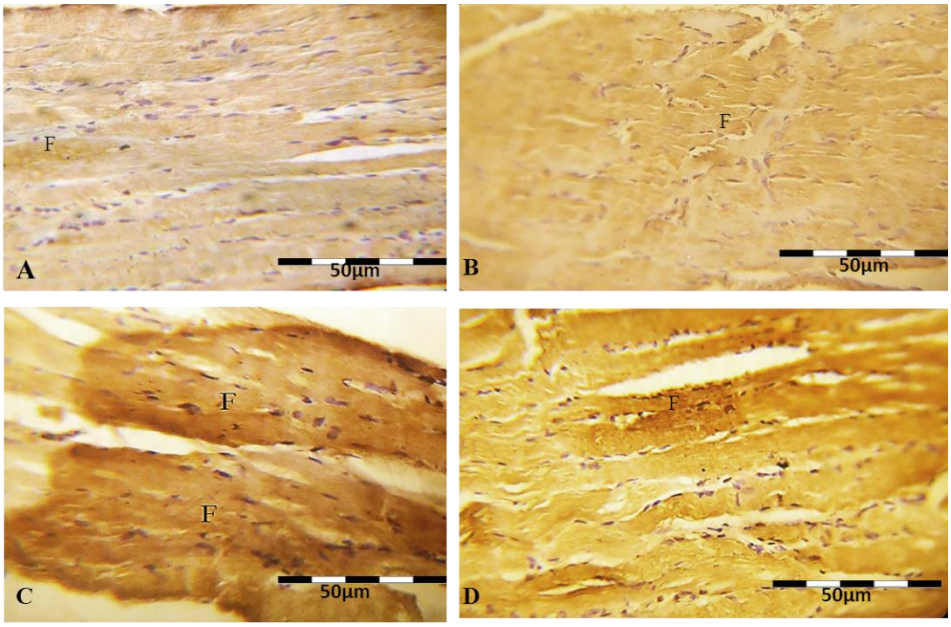

Fig.12: photomicrographs of cytochrome c immunostaining longitudinal section of rat diaphragm . (A,B) control and Coq10 group showing decreased brown coloration of sarcoplasm. (C) simvastatin treated group showing increased brown coloration of sarcoplasm. (D) simvastatin + Coq10 treated group showing moderate brown coloration of sarcoplasm.

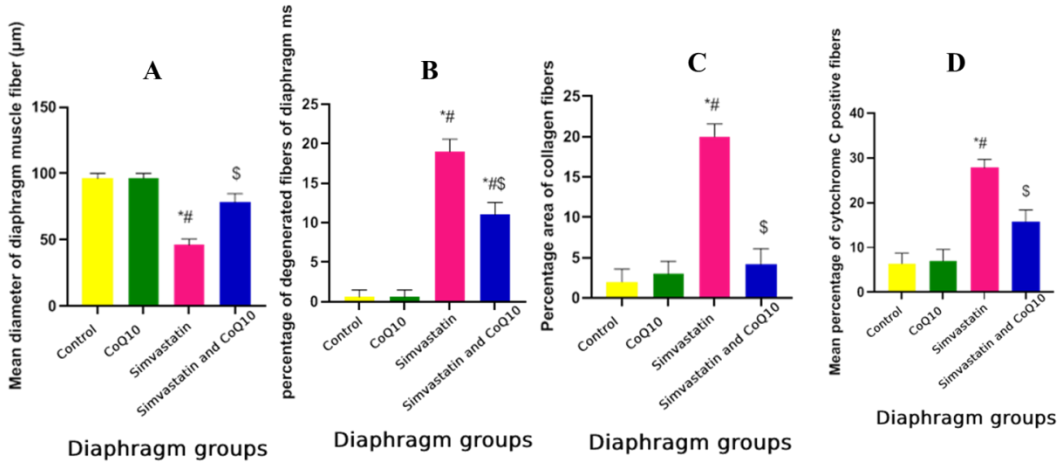

Fig. 13: Quantitative analysis of the mean diameter of L.S. of diaphragm muscle fibers (µm), percent of degenerated fibers, mean percentage of collagen fibers and percentage area of cytochrome c positive fibers respectively between different groups (n=10) X 400. Data are presented as mean ± SEM. *,#, and $ Significance differences from control, simvastatin, and statin + Coq10 groups respectively at P<0.05.

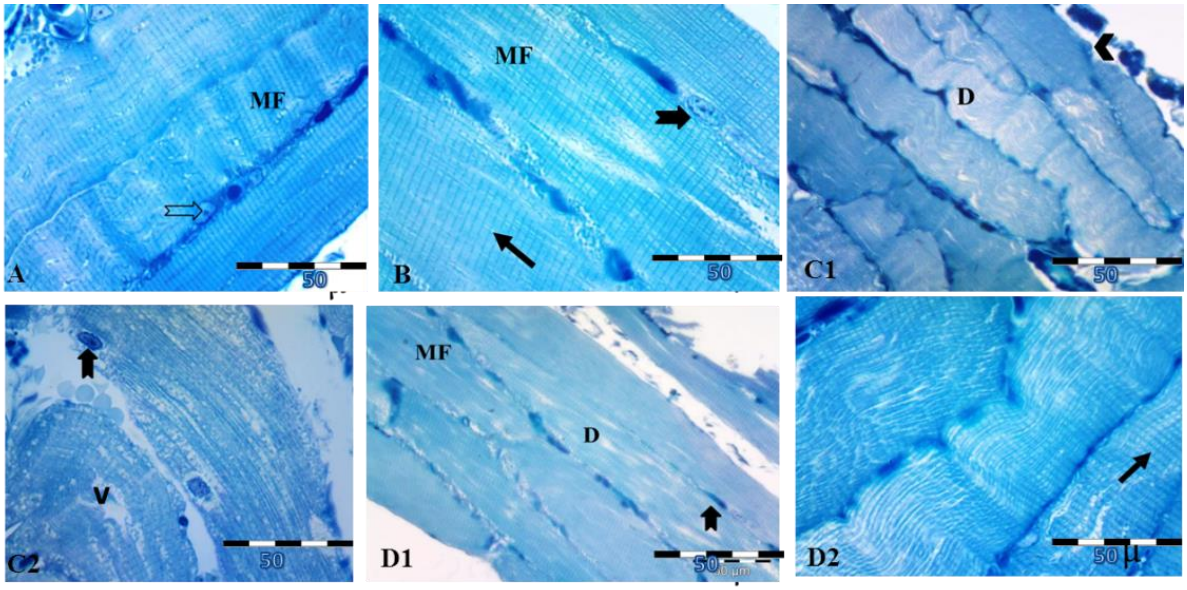

Fig. 14: A photomicrographs of diaphragm muscle section stained with toluidine blue. (A) Control semithin section showing cylindrical regularly arranged muscle fibers (MF) with regular transverse striations (arrow) across the whole thickness of the muscle fiber and peripheral oval vesicular nuclei with prominent nucleoli (bifid arrow). (B) Coq10 group showing cylindrical regularly arranged muscle fibers (MF) with regular transverse striations (arrow) across the whole thickness of the muscle fiber and peripheral oval vesicular nuclei with prominent nucleoli (bifid arrow). (C1,C2) simvastatin treated group showing irregular sarcolemma (arrow head), focal areas of sarcoplasmic dissolution (D) in a muscle fiber with disorganized myofibrils with lost transverse striations, cytoplasmic vacuole(V) and displaced pyknotic nuclei (bifid arrow).(D1,D2) simvastatin + Coq10 treated group showing regularly arranged parallel muscle fibers (MF) almost comparable to the control with peripheral elongated nuclei (bifid arrow), with clear transverse striations (arrow). However, few limited areas of sarcoplasmic dissolution (D)

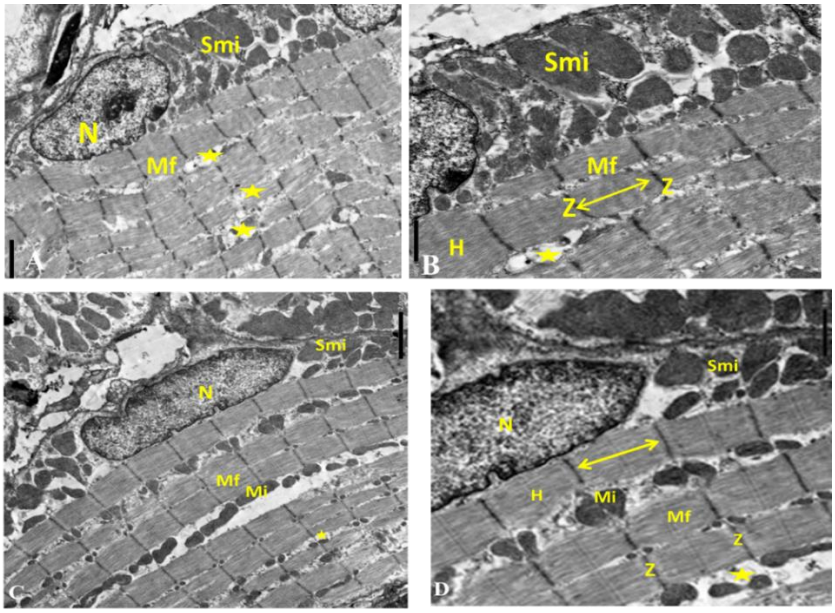

Fig. 15: An electron micrograph of longitudinal diaphragm sections from control and coq10 groups showing similar findings in the form of; A) parallel regular arrangement muscle fibers with longitudinal parallel myofibrils (Mf), sub sarcolemmal oval euochromatic (N), aggregated sub sarcolemmal mitochondria (Smi), as well as sarcoplasmic reticulum cisternae (astresik) is seen at A-I junction. B) Z lines (Z) appear in the middle of light band, H band (H) in the middle of A band, sarcomeres between 2 successive Z lines (double headed arrow). C) parallel regular arrangement muscle fibers with longitudinal parallel myofibrils (Mf), sub sarcolemmal oval euochromatic nucleus (N), aggregated sub sarcolemmal mitochondria (smi), intermyofibrillar mitochondria (Mi) as well as sarcoplasmic reticulum cisternae at A-I junction (asterisk). D) Z lines (Z) appear in the middle of light band, H band (H) appear in the middle of A band, sarcomeres between 2 successive z lines (double headed arrow).

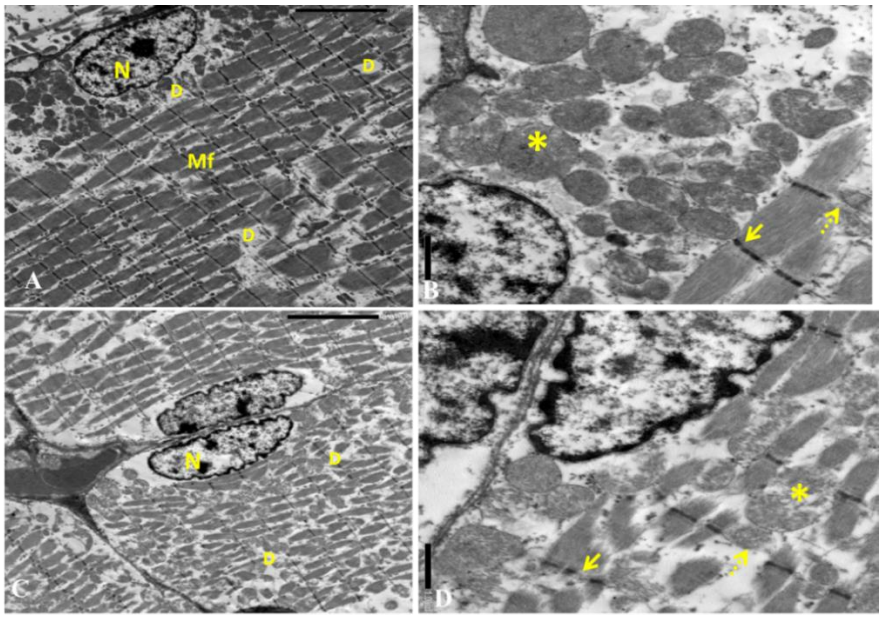

Fig. 16: (A,B,C,D) Electron photomicrographs of simvastatin group of diaphragm muscle showing: A,C) Degenerated myofibrils (Mf) with many areas of dissolution (D), subsarcolemmal pyknotic nucleus with clumped chromatin (N) and marked aggregation of mitochondria. B, D) myofibrils with disrupted (arrow) or lost (dotted arrow) Z line, giant mitochondria among the myofibrils (star).

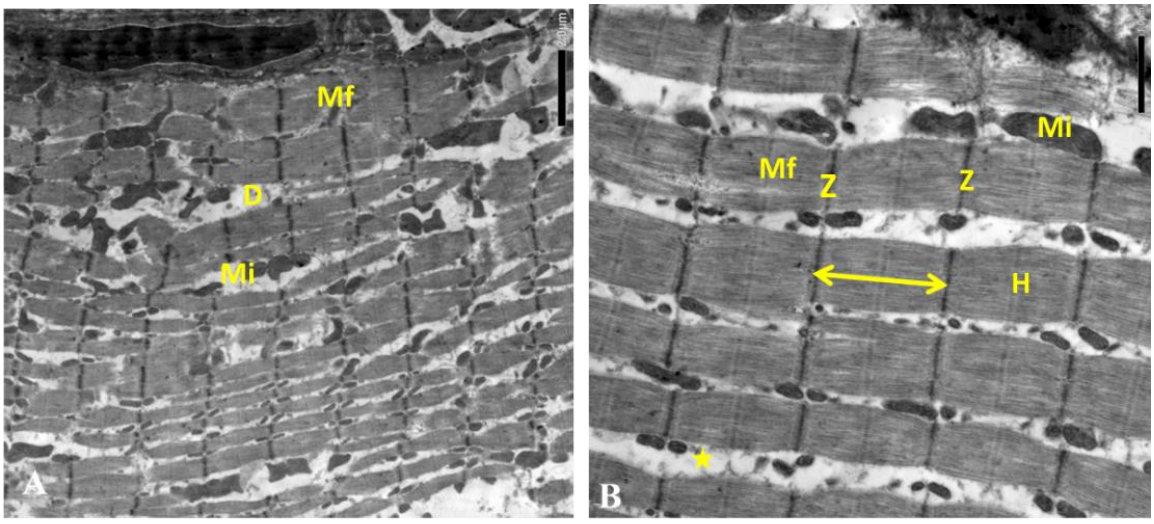

Fig. 17: Electron photomicrographs of simvastatin + Coq10 of diaphragm muscle showing: A) Parallel myofibrils (Mf) that are arranged almost in register across the width of a muscle fiber with few areas of myofibrillar dissolution (D) and inter fibrillar mitochondria (Mi). B) Clear regular striation nearly similar to that of the control group with sarcomere (double headed arrow), dark Z line in middle of I band (Z), H zone in the middle of A band (H). Additionally, apparently normal sarcoplasic reticulum cisternae (asterisk).

Discussion

Adult atherosclerosis-related disorders are among the most common leading causes of death. HMG-coenzyme reductase blockers are frequently recommended pharmaceuticals which lower LDL levels in the circulation, hence preventing the progression of atherosclerosis[9]. Pharmaceuticals called statins inhibit the enzyme known as HMG-CoA reductase. The most prevalent adverse reaction related to statin medication, is muscle problems, also referred to as statin-associated muscle symptoms (SAMS). Pains, weakness, and, in rare cases, rhabdomyolysis are the signs of mild to severe muscle issues. SAMS frequently results in discontinuation of statins [12]. For proper care, these side effects must be taken into account and diagnosed as soon as possible in order to lower drug-associated morbidity and mortality [17]. Discontinuation of statins has also been linked to increases the susceptibility to heart attacks and strokes [18].

In the current research, adult male albino rats were treated with simvastatin for four weeks. The physiological, biochemical, and histological alterations in the gastrocnemius and diaphragmatic muscles were examined.

The gastrocnemius muscle is formed mainly of type II white muscle fibers therefore it was chosen for the current investigation Since statins mainly alter the histopathology of type II white muscle fibers [19].

Since CK leaks from muscle cytoplasm when muscle cell membranes are damaged therefore, serum CK levels are used as an indicator of muscle injury. In the current research, the biochemical analysis revealed that the mean serum CK levels were considerably greater in the simvastatin-treated group than in control and CoQ10 treated group. When CK is eliminated from muscle tissues, both muscle structure and the stability of contracting muscular filaments are affected secondary to reduction in ATP synthesis with subsequent myopathy [20].

In the current research, the muscle weight/ body weight ratio was considerably lower in the simvastatin-treated group. Damaged muscle fibers could be the cause of this weight loss and decrease in muscle lipid reserve [21].

The increased-CK level could reflect the histological findings of myopathy that were

seen in gastrocnemius muscle and diaphragmmatic muscle as LM examination of simvastatin treated group in gastrocnemius muscles in the current research showed loss of normal architecture of some fibers as some muscle fiber appeared disorganized frag-mented with vacuolization, splitting of muscle fiber, extravasation of RBCs and sarcoplasmic mononuclear cellular infiltration. The nuclei were darkly stained and shifted near center. These findings are in accordance with [22] who reported that degenerated muscle fibers appear by microscopic examination either pale or dark. Degenerated myofibrils may exhibit variable microscopic changes, such as swollen cell, vacuolization, loss of striation, fragmentation, and fibers rupture. Also Mazroa and Asker [23] reported that the separation of muscle fibers could be owing to an inadequate oxygen supply.

Additionally morphometric study revealed a significant reduction in the mean diameter of muscle fibers with a significant increase in percent of degenerated fibers compared to control group. These findings indicate myofilaments degeneration. Similarly, Meregalli et al [24] reported that simvastatin administration result in significant morphological and structural damage of gastrocnemius muscle in rat animals.

Examination of different fields showed sarcoplasmic dissolution that might be due to loss of myofilament and destruction of mitochondria. Similar findings were reported by Abdel Hamid et al [25].

Increased collagen contents in-between skeletal muscle fibers in simvastatin treated group were noted in the present study. These findings were in close similarity to those recorded by Li and Huard [26] mentioned that muscle injury result in fibrotic changes with subsequent collagen deposition. Muscle injury triggers the release of growth factors including TGF-β1 and platelet-derived growth factor, causing extracellular matrix cells to produce and deposit collagen.

The immunohistochemical results of the present work confirmed the histological findings as there was a significant increase in the mean area percent of cytochrome C immunoreactivity in simvastatin treated group compared to both control and Co-Q10 groups. These results are in accordance with Mescher [27].

Cytochrome C is protein in nature part and involved in the chain of events for ATP production as it play an important role in the electron transport chain of mitochondria. When cell is exposed to stress, cytochrome C is released from the inter-membranous space of mitochondria into cell cytoplasm where it triggers proteolytic enzymes called caspases, which are essential mediators of apoptosis [27].

The ultrastructural examination of gastric-nemius muscle of simvastatin-treated group showed degenerated myofibrils with disrupted or lost Z line with many areas of dissolution, disfigured mitochondria among the myofibrils and dilated sarcoplasmic reticulum. Similar findings were reported by Ahmed et al [28] who stated that statin administration result in degeneration of skeletal muscle fibers and their membranous organelles as statins impair skeletal muscle mitochondria function resulted mitochondrial swelling and cytochrome C release into the muscle cytoplasm.

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Mitochondria and ER are connected in both structure and function through contact sites named mitochondria-associated ER membranes (MAMs) [29]. Mitochondria function as the main energy-producing organelles and the ER serves as the location of protein and lipid synthesis and plays a role in regulating calcium [30].

As statins inhibit the HMG-CoA reductase leading to suppression of mevalonate which is the precursor of ubiquinone. Ubiqunione is the main coenzymes for electron transport; in addition, it has antioxidant property for mitochondria and lipid membranes. Therefore, Statins administration affects mitochondrial ATP production and membrane characteristics with subsequent impairment of energy metabolism of muscle cells [31]. Moreover, the deficiency of prenylated protein, another important compound to HMGCoA reductase pathway, these factors change the intracellular messaging, inducing vacuolation of the myofibers, swelling of organelles and finally results in cell death[32]. These mechanisms

could explain the evident mitochondrial swelling and dilated cisternae of sarcoplasmic reticulum noted in the present work.

Statins, being HMG-CoA reductase inhibitors block mevalonate formation (precursor of cholesterol); therefore, the cholesterol component of sarcolemma membranes is decreased. Cholesterol is essential for proper structure and elasticity of cell membranes. The decreased cholesterol level affects these membranes stability with subsequent increased calcium influx. Therefore, the increased intracellular calcium activates apoptosis pathways [33]. This may in part explain the presence of darkly stained nuclei in some muscle fibers and the increased cytochrome C immunoreactivity in the present study.

Administration of CoQ10 in Simvastatin - treated rats showed remarkable preservation of the normal architecture of most muscle fibers. The improvement in this group may be explained by antioxidant properties of CoQ10.

The association between statins administration and decreased Co-Q10 in muscle cells mitochondria with subsequent myopathy may be secondary to decrease in mevalonate, a precursor for both cholesterol and Co-Q10. As statins inhibit (HMG-CoA) reductase; the enzyme that has a role in transformation of HMG-CoA to mevalonate with resultant reduction in Co-Q10 in muscle cells mito-chondria. The decreased Co-Q10 may decrease energy production by inhibiting ATP with subsequent myopathy [20].

CoQ1 is a natural lipid-soluble coenzyme that is being formed inside smooth endoplasmic reticulum by the mevalonate pathway[34]. CoQ10 plays critical role in bioenergetics of mitochondria as it has a powerful antioxidant property.

CoQ10 exerts its antioxidant properties via two mechanisms; direct one through Prevention the reactive oxygen species (ROS) generation and indirect through stimulation of essential natural antioxidants such as vitamin C and vitamin E. Oxidative stress can damage most cell components such as DNA, protein, lipid, and mitochondria so CoQ10 as a powerful antioxidant can be utilized as a potential treatment of many diseases such as neurodegenerative diseases, malignancy, diabetes mellitus, and cardiovascular diseases[35].

Therefore, CoQ10 was used in the present work to assess its effectiveness in alleviating the biochemical, physiological and histo-pathological alterations in skeletal muscles including diaphragmatic muscle that may occur after simvastatin administration.

In the current study, Simvastatin and CoQ10 treated group the CK serum concentration was significantly reduced when compared with Simvastatin treated group and non-significant difference versus control.

This is in accordance with Cirilli et al [36]. who reported that concomitant administration of Co-Q10 and Statin resulted in significant reduction in serum level of CK. these finding is in line with the results of present work and might explain the improvement in normal architecture of gastrocnemius muscle fibers. As in Simvastatin and CoQ10 treated group the skeletal muscle fibers appeared parallel, non-branching, elongated, and cylindrical with acidophilic sarcoplasm and multiple peripheral oval nuclei. This was confirmed in morphometric results by a non-significant difference in mean diameter of muscle fibers versus both control and CoQ10 groups.

The LM findings were also supported by immunohistochemical examination which showed considerable reduction in the mean area percent of cytochrome C immune-reactivity in simvastatin +CoQ10 versus simvastatin group.

Also, the EM findings revealed ultra-structural improvement of Co-Q10 and simvastatin group showed clear regular striation comparable to that of control one as myofibrils arranged parallel almost across the width of a muscle fiber with few areas of myofibrillar dissolution and inter fibrillar mitochondria, Clear regular striation within sarcomere, apparently normal sarcoplasmic reticulum cisternae

Respiration is mainly dependent on the function of diaphragmatic muscle, being of striated skeletal muscle type with special function.

Histological examination of the rat diaphragmatic muscle fibers demonstrated the basic structural organization of other mammalian striated muscle fibers. It characterized by presence of many mitochondria and capillaries due to its higher diaphragmatic muscle oxygen consumption than other skeletal muscle [37]. Similar to skeletal muscles, diaphragmatic muscles were affected by simvastatin administration in rat models. Also, concomitant administration of CoQ10 resulted in histopathological improvement.

Therefore, concomitant administration of a statin and CoQ_{10} is a commonly prescribed strategy and a generally accepted standard of medical practice [9].

Conclusion

Simvastatin induced obvious gastrocnemius and diaphragm muscle degeneration mainly through mitochondria affection with subsequent increase in muscle fibers fibrosis and apoptosis. The concomitant administration of CoQ10 preserved most muscle fibers normal structure with decreased cytochrome C immunoexpression and fibrosis. CoQ10may be recommended for patients on regular statins intake.

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