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The Possible Protective Role of L-Carnitine versus Omega-3 Fatty Acids in Activation of Endogenous Stem Cells, for Attenuation of Simvastatin Induced Myopathy in Adult Male Albino Rats: Histological and Immunohistochemical and Electron Microscopic Study

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Abstract: Simvastatin is a drug prescribed to control dyslipidemia and lower serum cholesterol levels in order to lower the danger of heart conditions. Simvastatin is hydroxy methylglutaryl CoA (HMG-CoA) reductase inhibitor in both hepatic and extra hepatic tissues but it has a well-known side effect which is myopathy. L-carnitine is an amino acid that displays evidence of enhancing acute physical performance, maximum oxygen consumption, and post-exercise recovery and pain relief. Essential for good health, omega-3 fatty acids are known for their powerful anti-inflammatory effects. The present investigation sought to compare omega-3 fatty acids with L-carnitine in terms of their potential impact on activation of endogenous skeletal muscle satellite stem cells, for attenuation of simvastatin induced myopathy. The rat subjects were randomly assigned to one of four treatment groups orally for a period of 3 weeks. Group I (control group) was subdivided into three subgroups: Ia received distilled water, Ib received 100 mg/ kg/day of L-carnitine and Ic received 300 mg/kg/day of omega-3 fatty acids. Group II received 88 mg/kg/day of simvastatin. Group III received 88 mg/kg/day of simvastatin plus 100 mg/ kg/day of L-carnitine. Group IV received 88 mg/kg/day of simvastatin plus 100 mg/kg/day300 mg/kg/day of omega-3 fatty acids. Light microscopic studies with H&E, succinic dehydrogenase histochemistry and myogenin immunohistochemistry along with electron microscopic (EM) study were done. Morphometric and statistical studies were carried out. In group II, H&E showed muscle fiber disorganization, apoptosis, and displaced nuclei. Both succinic dehydrogenase activity and the number of satellite stem cells stained positively by myogenin were markedly decrease in comparison to group I. By EM, group II showed heterochromatic nuclei and disrupted sarcomeres. The combination of simvastatin and L-carnitine in group III showed less myopathic changes than group II by H&E as some fibers were regular while others are disorganized. In group III, there was increase in SDH activity and number of myogenin positive satellite stem cells in comparison to group II. By EM, some regular sarcomeres were present with some disrupted areas. concurrent administration of simvastatin and omega-3 fatty acids in group IV the majority of the muscle fibers appeared more or less like group I by H&E and EM. There was marked increase in SDH activity and number of myogenin positive satellite stem cells in comparison to both group II and group III. In conclusion, both L-carnitine and omega-3 fatty acids showed less pathological changes

in the simvastatin induced myopathy by microscopic examination. However, omega-3 fatty acids had better effect than L-carnitine in attenuation of simvastatin induced myopathy and more activation of endogenous muscle satellite stem cells.

Keywords: Simvastatin, L-carnitine, Omega-3 fatty acids, skeletal muscle, satellite stem cells, myogenin.

INTRODUCTION

To treat dyslipidemia and lower cardiovascular risk factors, doctors use statins. Inhibiting the cholesterol production pathway regulator 3-hydroxy-3-methyl glutaryl-coenzyme A (HMG CoA) reductase is their primary mode of action [1]. Reducing triglyceride and low-density lipoprotein cholesterol production follows [2]. However, they have a common side effect which is myopathy. Sometimes myopathy and other related muscular side effects can lead to poor compliance or even discontinuation of the drug regimen [3]. The spectrum of statin neuromuscular side effects includes myopathy, myalgia, rhabdomyolysis, mild creatine kinase elevation, and immune-mediated necrotizing myopathy[4].

An essential component of in the mitochondrial membrane, L-carnitine β-oxidizes long-chain fatty acids. Its building blocks are methionine and lysine amino acids or it could be taken as a supplement [5]. In addition, L-carnitine has a role in decreasing oxygen free radicals produced by mitochondria during respiration, this limits the process of lipid peroxidation and is known as free radical scavenging activity. It does away against mitochondrial swelling and promotes cytochrome C oxidase activity which is affected by statins. This action in particular improves skeletal muscle contractility and can reverse statin induced myopathy [6]. It is suggested that an athlete is fit or sick, L-carnitine is essential for enhancing endurance and fatigue recovery. Physical, psychological, and cognitive performance are all improved. As a result, athletes recover more quickly and oxygen and blood may more easily reach their muscles. Because it decreases free radical formation, L-carnitine also lessens signs of cellular damage and muscle damage [7]. L-carnitine protects the mitochondria from damage by warding off ischemia and hypoxia, two chemicals that disrupt mitochondrial activity. There are antioxidants and energy-boosting qualities in it as well. In addition, it can neutralize free radicals [8].

Naturally occurring omega-3 fatty acids cannot be manufactured by the human body, despite their importance due to the lack of needed enzymes. Among these, you can find fish-and algae-derived eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Plants also contain omega-3 fatty acids, one of which is alpha-linolenic acid (ALA) [9]. Tissue phospholipid membranes rely on omega-3 fatty acids for structural integrity and to maintain cell membrane fluidity. Because they are successfully decreases oxidative damage and inflammation and have a track record of managing dyslipidemia [10], Cardiovascular disorders (CVD) caused by atherosclerosis can be treated with omega-3 fatty acids. In metabolic syndrome, omega-3 fatty acids help with blood pressure and diabetes management [11]. The health of your muscles can benefit from omega-3 fatty acids [12]. Taking a supplement containing omega-3 fatty acids may help the elderly with things like walking speed, handgrip strength, and quadriceps strength, according to some reports. The synthesis of muscle protein, strength, athletic performance, and overall muscular mass are all positively impacted by omega-3 fatty acids [13].

Satellite cells are undifferentiated flat mononuclear stem cells representing about 2–7% of skeletal muscle cells. The process of repairing and regenerating injured skeletal muscles relies on it.[14]. The satellite stem cells differentiate into myotubes that fuse with the damaged muscle in the process of healing through expression of multiple myogenic regulatory proteins called myogenic regulatory factors (MRFs) as Myogenin, myoblast determination protein 1 (MyoD), Myogenic Factor 5 (Myf5), and Myogenic Regulatory Factor 4 (MRF4). These markers can be detected by immunohistochemical techniques [15].

This research set out to demonstrate the microscopic changes of simvastatin induced myopathy and to assess the possible protective role of L-carnitine vs Omega-3 fatty acids in myopathy attenuation and satellite stem cell activation.

MATERIALS AND METHODS:

Chemicals:

We crushed and dissolved Simvacor 40 mg pills, which contain simvastatin, which we purchased from the Sigma firm in Egypt. Carnitol 500 mg tablets containing L-carnitine were purchased from Global Napi pharmacutical company (GNP) in Egypt. The pills were crushed and dissolved in distilled water for preparation. The Omega-3 fatty acids were sourced from the SEDICO company in Egypt and were manufactured by dissolving them in oil and then

placed into 1000 mg gelatinous capsules.

Place of the study:

The current study followed all international protocols for the humane treatment of laboratory animals and was conducted in the Medical Histology and Cell Biology Department at Mansoura University. This study has the green light from the Institutional Review Board (IRB) at Mansoura University (MS.21.03.1422).

Duration of the experiment: 3 weeks

Experimental animals:

An average of sixty mature male albino rats weighing 200 grams were utilized for this study. The rats were three months old. Their breeding process included a regular light-dark cycle and temperature control (22-25°C). The animals were housed in an identical setting and provided with ample abundance of food and water at all times.

Animal groups:

There were four primary groups into which the animals were split at random. Thirty people made up Group I, which served as the control group. This group of rats was then randomly divided into three subgroups: During the three weeks, subjects in Subgroup Ia (n=10) were given 0.5 ml of distilled water orally, while subjects in Subgroup Ib (n=10) were given 100 mg/kg/day of L-carnitine orally [16] and for three weeks, members of Subgroup Ic (n=10) took 300 milligrams of omega-3 fatty acids orally per kilogram of body weight [17]. **Group II (simvastatin treated group) (n=10):** rats of this group received Simvacor at a dose of 88 mg/kg/day orally for three weeks [18]. **Group III (n=10):** Simvastatin and L-carnitine treated individuals The daily dosage of Simvacor for these rats was 88 mg/kg. [18] and L-carnitine at a dose of 100 mg/kg/day [16] orally for three weeks. **Group IV (simvastatin+omega-3 fatty acids treated group) (n=10):** A daily dosage of 300 mg/kg of omega-3 fatty acids and 88 mg/kg of Simvacor were given to these rats [17] orally for three weeks.

The rats were rendered unconscious at the conclusion of the experiment by injecting 40 mg/kg of sodium pentobarbital intraperitoneally (IP) [19]. Perfusion fixation was accomplished through intravascular (IV) perfusion using 4% paraformaldehyde [20]. Every rat's gastrocnemius muscle was sliced into smaller pieces after being dissected. The samples were ready for examination under the light and electron microscopes.

Light Microscopic Study

The specimens that were collected underwent routine histological examination by first being the specimen was first preserved in 10% neutral buffered formalin, dehydrated with progressively stronger concentrations of ethyl alcohol, rinsed in xylene, embedded in paraffin wax, sectioned at 4 µm intervals using a rotary microtome, placed on glass slides, and stained with H&E [21]. To further prove the presence of endogenous muscle satellite stem cells, an immunohistochemical research using Myogenin was also conducted [22].

The other parts of the specimens were put in cryostat for preparation of fresh frozen cryocut sections of 10 µm for histochemical staining with succinic dehydrogenase can be used to show mitochondrial activity and differentiate between oxidative and non-oxidative fibers in muscle [23].

Immunohistochemistry methodology of myogenin:

To prevent the sections from being displaced, the paraffin sections measuring 4-5 µm were treated with 10% neutral buffered formalin and then placed on super plus frost slides. After 30 minutes in xylene, they were deparaffinized. The sections were rehydrated using ethanol at progressively higher concentrations (100%, 90%, and 70%). Sections that had been embedded in paraffin and fixed with formalin were subjected to antigen retrieval by subjecting them to a citrate buffer pH 6.0 at 95°C for 20 minutes, allowing them to cool to room temperature for 20 minutes, and then rinsing them four times in PBS. To block the action of endogenous peroxidase, the pieces were immersed in hydrogen peroxide for 15 minutes at 37° C. Following that, they were immersed in PBS for five minutes twice, each time, to ensure a thorough washing. The main antibody for myogenin (5FD) was bought from Santa Cruz Biotechnology, Inc. in Dallas, Texas, USA (catalogue number: sc-52903). It is a mouse monoclonal antibody of the class IgG kind. The sections were treated with the main antibody, which was diluted 1:50 according to the manufacturer's instructions. To prevent the specimen from drying out due to evaporation, the slides were incubated in a humidity chamber at room temperature for 60 minutes. After three 5-minute rinses in PBS, the slides were dried using absorbent paper around the section borders [24].

The next step was to place the slides in a room-temperature humidity chamber and let them incubate for ten minutes after adding the secondary antibody, which was biotinylated goat anti-polyvalent. After three five-minute washes in PBS, the extra fluid was drained from the slides. After 100 µl of streptavidin peroxidase was applied to each section, the slides were placed in the humidity chamber and let to incubate for 10 minutes at room temperature. After being rinsed in PBS, the slides were allowed to dry. After incubating the sections for 5–15 minutes, two drops of the newly prepared substrate–chromogen mixture were applied

to each section. The parts were rinsed with distilled water until they were completely clean. This experiment made use of Mayer's hematoxylin solution as its counterstain. Increasing concentrations of ethanol were used to dehydrate the slides. The slides were mounted using Canada balsam after they were cleared in xylene. Then, they were cover-slipped [25].

For positive tissue control **(Figure 4 A)**, sections of rhabdomyosarcoma collected from the pathology department of Mansoura University's medical faculty in order to serve as a positive control for myogenin [26]. Positive control sections were used to determine whether tissue sections were positively stained in each staining run when placed incubated with the experimental slides. The positive reaction was in the form of brown cytoplasmic and/or nuclear reaction. The proper functioning of processed tissues and test reagents was ensured by their use. Positive reactivity was demonstrated when the desired reaction end product was present [24].

A negative tissue control was prepared using slices of normal skeletal muscle (**Figure 4 B**). After the primary antibody was removed, negative control sections were subjected to the identical staining procedures. In order to evaluate the background staining, the negative control slide was crucial. Antibody cross reactivity was proven to be nonexistent since the negative control slide did not exhibit any particular staining. [25].

Transmission Electron Microscopic Study

The gastrocnemius muscle specimens were cut into small pieces (1x1x1 mm), quickly preserved for 24 hours in a cacodylate buffer containing 2.5% glutaraldehyde and paraformaldehyde, and subsequently post-fixed for 2 hours at 4 °C in a fume closet with osmium tetroxide. Following a 30-minute clearing process in propylene oxide, the specimens were dehydrated using five different grades of ethyl alcohol. Specimens were subsequently placed in Beam capsules, which were filled to the brim with epoxy resin, and subjected to a 24-hour polymerization process at 60°C. Subsequently, the capsules were sawn into 1 µm sections, which were stained with 1% toluidine blue, and 60-70 nm sections, which were stained with uranyl acetate and lead citrate, respectively, to represent ultrathin sections [27]. The next step was a transmission electron microscope analysis and photography session at the University of Alexandria.

Histomorphometric Study:

Computer assisted digital image analysis was done for the percentage area of succinic dehydrogenase enzyme activity in SDH stained sections at magnification X 400 and the percentage area of intensity of myogenin positive satellite stem cells immunoreaction in myogenin stained sections at magnification X 1000. A 0.5 X photo adapter was used to photograph the microscope with an Olympus® digital camera attached to the slide. In order to estimate the morphometric data, 10 non-overlapping high-power fields were randomly selected and six slides from each experimental rat were analyzed. The resulting photos were examined on a computer based on the Intel® Core I3® architecture using the Video Test Morphology® program (Russia), which has a built-in, customized method for measuring area, calibrated distance, automatically analyzing objects, and color intensity.

Statistical Study

The histomorphometric data were coded, tabulated and then applied statistical analysis with the help of the Social Science Statistics Package (SPSS 25.0, IBM/SPSS Inc., Chicago, IL) We ran two separate statistical analyses. If there were more than two normally distributed groups, we utilized a one-way analysis of variance test (ANOVA or F test) on the continuous data to see if there was a substantial difference. We used the Shapiro-Wilk test to ensure that the groups were normally distributed, and we used Levine's test to ensure that the variances were homogeneous. After doing a substantial ANOVA test to determine whether groups differed substantially, a post hoc test called the Tukey-HSD test was utilized to account for multiple comparisons. The level of significance, denoted as P-value, was linked to test statistics and served to reject the null-hypothesis, or hypothesis of no difference. P-values<0.005 was considered substantial.

RESULTS:

I) Light Microscopic Results

(1) Hematoxylin and Eosin (H&E) Stain:

Group I (the control group) was examined under a light microscope using H&E dye, and three subgroups were identified: subgroup Ia (the negative control subgroup), subgroup Ib (L-carnitine treated subgroup) and subgroup Ic (omega-3 fatty acids treated subgroup) were more or less similar. The muscle fibers were long, cylindrical, parallel striated muscle fibers at the longitudinal sections. The sarcoplasm was acidophilic and the nuclei were multiple, elongated, vesicular and peripheral **(Figure 1 A, B)**. In transverse sections **(Figure 2 A, B)**, With acidophilic sarcoplasm and peripheral nuclei, the fibers had a polyhedral form. The satellite stem cells with dark nuclei and small amount of cytoplasm were seen in between the basal lamina and the sarcolemma in both Ls and TS sections. The connective tissue was formed of dense connective tissue epimysium surrounding the whole muscle, dense connective tissue perimysium dividing the muscle into bundles and loose connective tissue

endomysium in between the muscle fibers containing blood vessels and fibroblasts nuclei.

Scan of skeletal muscle fibers by light microscopy at group II (simvastatin treated group) **(Figure 1 C, D)** showed degeneration, splitting, vacuolation and loss of transverse striations in most of the skeletal muscle fibers. The fibers were variable in diameter with some displaced nuclei. There was fragmentation of the muscle fibers into blebs that had small amount of the sarcoplasm and nuclei. Congested blood vessels were found in the connective tissue between the muscle fibers. In the transverse sections **(Figure 2 C, D)**, the skeletal muscle fibers were variable in size and shape with sarcoplasmic fragmentation and vacuolation. Some fibers were rounded and shrunken. In both sections, the satellite stem cells were almost absent. The nuclei in some fibers were rounded and located in center of the muscle fiber. There was marked thickening of the connective tissue of the perimysium.

Scan of skeletal muscle fibers by light microscopy at Group III (simvastatin+L-carnitine treated group) stained by H&E showed less pathological changes than that observed at group II (simvastatin treated group). Many of the muscle fibers were cylindrical, parallel with regular transverse striations while some others showed splitting and disorganization. Most of nuclei were elongated, vesicular and peripherally located **(Figure 1 E, F)**. In the transverse sections **(Figure 2 E, F)**, group III showed some well-organized polyhedral acidophilic muscle fibers and some disorganized fragmented muscle fibers. The muscle fibers nuclei were mostly peripheral and vesicular with moderate thickening of the connective tissue of the perimysium. Many satellite stem cells were present across the sarcolemma and the basal lamina in both sections.

The bulk of the muscle fibers in group IV (the group treated with Simvastatin and omega-3 fatty acids) had similar characteristics to those in group I, including long, parallel fibers with regular transverse striations, as revealed by histological inspection by H&E of longitudinal sections (LS). According to Figure 1 G and H, the nuclei were elongated, vesicular, and situated on the periphery. By the transverse sections (TS) **(Figure 2 G, H)** the majority of the muscle fibers were well organized, polyhedral with acidophilic sarcoplasm and peripheral nuclei. In both sections, Intersecting the sarcolemma and basal lamina were many satellite stem cells.

(2) Succinic dehydrogenase enzyme (SDH) activity:

Research using histochemistry to identify enzyme activity in frozen meals containing succinic dehydrogenase skeletal muscle samples from the "control group" (group I) were rather consistent throughout three subgroups: subgroup Ia (negative control subgroup), subgroup Ib (L-carnitine treated subgroup) and subgroup Ic (omega-3 fatty acids treated subgroup). Group I (control group) **(Figure 3 A)** showed that the majority of the muscle fibers were type I (oxidative) muscle fibers that are small in diameter and deeply blue stained with strong SDH activity. Some type IIa (intermediate) muscle fibers with intermediate diameter and moderate SDH activity were found. Type IIb (glycolytic) muscle fibers with large diameter and weak SDH activity were also seen.

Group II (simvastatin treated group) **(Figure 3 B)** showed weak succinic dehydrogenase activity in the majority of muscle fibers and few type I (oxidative) muscle fibers show strong SDH activity were also present. Group III (simvastatin+L-carnitine treated group) **(Figure 3 C)** showed increase in SDH activity of the muscle fibers in comparison to group II with the presence of three muscle fibers types. Group IV (Simvastatin+Omega-3 fatty acids treated group) **(Figure 3 D)** showed increase in SDH activity in comparison to both group II and group III with the presence of three muscle fibers types.

(3) Myogenin immunohistochemical stain:

Group I (control group) immunohistochemical stained sections with myogenin were more or less similar in the three subgroups: subgroup Ia (negative control subgroup), subgroup Ib (L-carnitine treated subgroup) and subgroup Ic (omega-3 fatty acids treated subgroup). Group 1 longitudinal **(Figure 4 C)** and transverse **(Figure 4 D)** sections showed positive brown cytoplasmic myogenin immune reaction of the satellite stem cells between the sarcolemma and the basal lamina. Group II (simvastatin treated group) **(Figure 4 E, F)** showed positive immune reaction in the cytoplasm of few satellite stem cells. Group III (simvastatin+L-carnitine treated group) (**Figure 4 G, H)** showed positive immune reaction in the cytoplasm of many satellite stem cells. Group IV (Simvastatin+Omega-3 fatty acids treated group) showed positive immune reaction in the cytoplasm of multiple satellite stem cells. **(Figure 4 I, J).**

II) Electron Microscopic (EM) Results

Group I (control group) EM studies were more or less similar in the three subgroups: subgroup Ia (negative control subgroup), subgroup Ib (L-carnitine treated subgroup) and subgroup Ic (omega-3 fatty acids treated subgroup). Group 1 **(Figure 5 A, B)** showed regular euchromatic nuclei just under the regular sarcolemma.The sarcomeres showed regular arranged myofibrils in light bands and dark bands with pale H zone (bifid arrow) in the center and dark M line within it. Z lines were separating the adjacent

sarcomeres. The mitochondria were present beside the Z lines with triad tubules and few glycogen granules that were also seen.

Group II (simvastatin treated group) **(Figure 5 C, D)** by EM showed irregular heterochromatic nuclei with disrupted nuclear envelope & displaced away from the sarcolemma. The sarcolemma was irregular with collagen fibers deposition. The sarcomeres were disrupted with irregular shaped mitochondria and large amount of glycogen granules. The triad tubules were markedly wider than group I and Z lines were discontinuous at some points.

Group III (simvastatin+L-carnitine treated group) **(Figure 5 E, F)** by EM showed regular peripheral euchromatic nuclei with prominent nucleolus just under the sarcolemma. Some sarcomeres were regular with some disrupted areas. Some mitochondria were regular in shape and size, while others were not. Many Z lines were intact and triad tubules were not widely dilated.

Group IV (Simvastatin+Omega-3 fatty acids treated group) **(Figure 4 G, H)** by EM showed regular peripheral euchromatic nuclei with prominent nucleolus just under the sarcolemma. The majority of the sarcomeres were regular with few localized areas of disruption. Most of the mitochondria, Z lines and triad tubules were regular in size and shape.

III) Image Analysis Results and Statistical Analysis

(1) Percentage area of succinic dehydrogenase enzyme activity in the control and the experimental groups:

The results of the ANOVA test indicated that there was a substantial (P<0.001) change in the average percentage area of succinic dehydrogenase enzyme activity across the various experimental groups. Subgroup Ib (the L-carnitine treated subgroup) did not differ substantially from subgroup Ia (the negative control subgroup) with respect to the mean value of the percentage area of succinic dehydrogenase enzyme activity, according to post hoc Tukey analysis (P=0.996).The mean value of the percentage area of succinic dehydrogenase enzyme activity in subgroup Ic (omega-3 fatty acids treated subgroup) also showed insubstantial (P=0.999) difference as compared to subgroup Ia (negative control subgroup). On the other hand, the mean value of the percentage area of succinic dehydrogenase enzyme activity in group II (simvastatin treated group) showed high substantial (P<0.001) decrease as compared to that of subgroup Ia (negative control subgroup).

Group III (simvastatin+L-carnitine treated group) showed substantial (P=0.002) decrease in the mean value of the percentage area of succinic dehydrogenase enzyme activity as compared to subgroup Ia (negative control subgroup). However, group III (simvastatin+L-carnitine treated group) showed high substantial (P<0.001) increase in the mean value of the percentage area of succinic dehydrogenase enzyme activity as compared to group II (simvastatin treated group). Group IV (simvastatin+omega-3 fatty acids treated group) showed insubstantial (P=0.824) difference in the mean value of the percentage area of succinic dehydrogenase enzyme activity as compared to subgroup Ia (negative control subgroup). However, group IV (simvastatin+omega-3 fatty acids treated group) showed high substantial (P<0.001) increase in the mean value of the percentage area of succinic dehydrogenase enzyme activity as compared to group II (simvastatin treated group). The mean value of the percentage area of succinic dehydrogenase enzyme activity in group IV (simvastatin+omega-3 fatty acids treated group) showed substantial increase (P=0.039) as compared to group III (simvastatin+L-carnitine treated group) **(histogram 1).**

(2) Percentage area of the positive myogenin immune-stained area for satellite stem cells within the control and the experimental groups:

Using ANOVA test, a substantial (P<0.001) change was found in the mean value of percentage area of the positive myogenin immune-stained area for satellite stem cells among the different groups of the experiment. Post hoc Tukey showed that the mean value of the percentage area of the positive myogenin immune-stained area for satellite stem cells in subgroup Ib (L-carnitine treated subgroup) showed insubstantial (P=0.999) difference as compared to subgroup Ia (negative control subgroup). Also, the mean value of the percentage area of myogenin positive satellite stem cells in subgroup Ic (omega-3 fatty acids treated subgroup) showed insubstantial (P=0.999) difference as compared to subgroup Ia (negative control subgroup). The mean value of the percentage area of the positive myogenin immune-stained area for satellite stem cells in group II (simvastatin treated group) showed substantial (P=0.024) decrease as compared to that of subgroup Ia (negative control subgroup).

On the other hand, the mean value of the percentage area of the positive myogenin immune-stained area for satellite stem cells in group III (simvastatin+L-carnitine treated group) showed high substantial (P<0.001) increase as compared to subgroup Ia (negative control subgroup). Also, group III (simvastatin+L-carnitine treated group) showed high substantial (P<0.001) increase as compared to group

II (simvastatin treated group) in the mean value of the percentage area of the positive myogenin immune-stained area for satellite stem cells. The mean value of the percentage area of the positive myogenin immune-stained area for satellite stem cells in group IV (simvastatin+omega-3 fatty acids treated group) showed high substantial (P<0.001) increase as compared to subgroup Ia (negative control subgroup). Also, group IV (simvastatin+omega-3 fatty acids treated group) showed high substantial (P<0.001) increase as compared to group II (simvastatin treated group) in the mean value of the percentage area of the positive myogenin immune-stained area for satellite stem cells. The mean value of the percentage area of the positive myogenin immune-stained area for satellite stem cells in group IV (simvastatin+omega-3 fatty acids treated group) showed substantial (P=0.022) increase as compared to group III (simvastatin+L-carnitine treated group) **(histogram 2).**

DISCUSSION

Simvastatin is a well-known antihyperlipidemic drug. However, myopathy is a common side effect of simvastatin in the form of myopathy, myalgia, rhabdomyolysis, mild creatine kinase elevation, and necrotizing myopathy mediated by the immune system [4]. In the current investigation, rats of group II received simvastatin for 3 weeks to investigate its effect on their skeletal muscle. Histological examination by H&E of longitudinal sections of group II treated with showed muscle fiber disorganization in the form of absence of the regular cross striations, different muscle fiber size and presence of clear vacuolations. Some muscle fibers showed apoptotic features as fragmentation into apoptotic blebs containing part of the sarcoplasm and nuclei. The transverse sections of the muscle fibers showed sarcoplasmic fragmentation with some rounded shrunken muscle fibers. These results mirrored those of those describes by **Ruscica et al** [28]. Simvastatin mechanism of action depends on inhibition of HMG-CoA reductase. This in turn will decrease mevalonate production which is responsible for cholesterol synthesis [29]. Therefore, the cell membrane structure and function of the extrahepatic tissues as the skeletal muscle will be affected by the reduction in cholesterol synthesis. Because the end products of the mevalonate pathway contribute to the maintenance of cell proliferation and the prevention of apoptosis, statins will exacerbate these degenerative characteristics of the muscles [30]. Simvastatin is also accountable for myopathic alterations and excessive calcium ion Ca2+ leakage from the sarcoplasmic reticulum, which disrupts the calcium signaling system [31]. Simvastatin was also proven to have increase the oxidative stress on the tissues by increasing reactive oxygen species formation at the expense of endogenous antioxidants, which led to the described microscopic findings [32].

In the current investigation H&E microscopic examination of group II (simvastatin treated group) showed presence of some nuclei in the center of the muscle fiber instead of the normal peripheral site. Insufficient metabolite exchange and oxygen transport to muscle fibers caused by simvastatin toxicity is responsible for this nuclear migration. A critical component of the pathophysiology of the subsequent muscle fiber splitting is the nuclear migration. An adaptive response occurs when a muscle fiber splits after experiencing too much strain because Once it attains a certain size, the efficiency of oxygen delivery and metabolite exchange begins to decline [33]. Moreover, there was mononuclear cells in between the muscle fibers because of the release of certain inflammatory mediators after muscle fibers damage [34].

To distinguish between the three varieties of skeletal muscle fibers, histochemical analysis of succinic dehydrogenase enzyme activity was performed. The skeletal muscle tissue is mainly composed of type I (oxidative) muscle fibers that are small in diameter and deeply blue stained with strong SDH activity. Type IIa (intermediate) muscle fibers has intermediate diameter and moderate SDH activity. Type IIb (glycolytic) muscle fibers has large diameter and weak SDH activity [35].

In the present work, group II showed weak succinic dehydrogenase activity in the majority of muscle fibers and only few type I (oxidative) muscle fibers with strong SDH activity. Similar findings were mentioned by **Borges et al** [36].Our findings were confirmed by the statistical analysis as the percentage area of succinic dehydrogenase enzyme activity in group II (simvastatin treated group) showed high substantial (P<0.001) decrease in contrast to that of subgroup Ia (negative control subgroup).

The decrease in SDH activity is explained by mitochondrial dysfunction due to the high oxidative stress, coenzyme Q10 depletion and calcium ion (Ca^{+2}) leakage in skeletal muscles exposed to simvastatin. This is responsible for energy depletion and altering muscle protein [37]. Because it inhibits complex I of the respiratory chain, simvastatin causes mitochondrial dysfunction. This is because a monocarboxylate transporter is responsible for statin absorption in skeletal muscles. An increase in cytoplasmic Ca+2 caused myotoxicity and reactive oxygen production in mitochondria by simvastatin. So that, simvastatin is responsible for inhibition of respiration and β-oxidation **[38].**

Myogenin immunohistochemical stained sections of group II (simvastatin treated group) displayed an

immunological response in the cytoplasm when few satellites stem cells. Compared to subgroup Ia (the negative control group), group II (the group treated with simvastatin) exhibited a substantial (P=0.024) reduction in the mean value of the percentage area of the positive myogenin immune-stained area for satellite stem cells. This indicates suppression of satellite stem cell regenerative capacity along with the muscle fibers degeneration with simvastatin administration [39].

Electron microscopic examination of group II (simvastatin treated group) showed marked disruption of the sarcomeres and loss of the regular alternation of the light and dark bands. The cell membrane showed discontinuity and Z lines were irregular. Similar ultrastructure findings were described by **Chen et al** [40]. Simvastatin is associated with oxidative stress that is responsible for protein and nucleic acid damage. Moreover, inhibition of HMG-CoA reductase results in coenzyme Q10 depletion which is considered as a fundamental electron carrier and antioxidant. This in turn plays a role in more muscle fiber pathogenesis [37]. As a result of cholesterol depletion in the sarcolemma and the nuclear membrane, the electron transport system is impaired in membrane ion channels [29]. The sarcolemma affection also leads to marked dilatation of the transverse tubules. This can result in skeletal muscle's calcium signaling and excitation-contraction coupling being disrupted, as well as the damaged muscle fiber's contraction strength being reduced [41].

In the current study, multiple mitochondria were observed with variable size and shape by electron microscopic examination of group II (simvastatin treated group). Simvastatin decreases complex I activity, which affects the mitochondrial electron transport chain and ATP synthesis. Additionally, uncoupled oxidative phosphorylation is reduced, which impairs the potential of the mitochondrial membrane [38]. Simvastatin also disrupts calcium homeostasis. Excess intracellular calcium impairs oxidative phosphorylation, inhibits mitochondrial respiration, and results in mitochondrial malfunction. Ion transport mechanisms malfunction and ATP is depleted as a result [42]. In this investigation, the electron microscopy analysis of group II (simvastatin treated group) revealed accumulation of excess electron dense glycogen granules. Glucose transporter 1, the insulin receptor β subunit, glucose transporter 4, pyruvate dehydrogenase, glycogen synthase, and glycogen synthase kinase 3β might all be adversely affected by simvastatin. These elements have a role in insulin signaling pathways and glucose metabolism; if they decline, cells are less able to absorb glucose, which increases the amount of glycogen in the liver and muscles[40].

In the current study, L-carnitine was suggested as a protective drug against the pathological effect of simvastatin on the skeletal muscle. Therefore, rats of group III received L-carnitine with simvastatin for three weeks. Histological examination of longitudinal sections (LS) of group III (simvastatin+L-carnitine treated group) skeletal muscles stained by H&E showed less pathological changes that observed in group II (simvastatin treated group). Many of the muscle fibers revealed features that were similar to those observed in group I (control group). They were cylindrical, parallel, cross striated with deep acidophilic sarcoplasm. Their nuclei were multiple, elongated, vesicular and peripherally located just under the sarcolemma. However, many degenerated muscle fibers were still noticed. In some fibers there were nuclei forming a nuclear chain in the center of the muscle fibers. In between the muscle fibers there were myotubes of fused myoblasts that appeared as long, cylindrical multinucleated cells with centrally located elongated nuclei. Transverse sections (TS) revealed that most skeletal muscle fibers were polygonal, with deeply acidophilic sarcoplasm and nuclei situated peripherally; nevertheless, group II (the group treated with simvastatin) showed significant cytoplasmic fragmentation. The loose connective tissue endomysium was noted between the fibers of the muscle. The dense connective tissue perimysium was surrounding the muscle bundles and appeared thinner than group II (simvastatin treated group) with some congested blood capillaries. Satellite stem cells were seen across the muscle fibers These findings were described also by **Lee et al** [43].

L-carnitine supplementation decreases muscle damage and apoptosis by suppression of atrogin-1 mRNA expression and downregulation of the ubiquitin-proteasome system (UPS) genes that responsible for skeletal muscle protein breakdown [44]. On the other side, L-carnitine increases the concentration of insulin like growth factor-1 (IGF-1), which is an important modulator of UPS activity and inhibits the breakdown of the contractile proteins [45]. As one of the proven etiologies of statin induced myopathy is the oxidative damage of the mitochondrial respiratory chain, it has been demonstrated that taking simvastatin and L-carnitine at the same time acting as a radical scavenger through dramatically lowering the levels of the oxidative stress markers and raising levels of the antioxidant reduced glutathione [46]. Additionally, by making it easier for long-chain fatty acids to be transported into mitochondria, L-carnitine enhances mitochondrial β-oxidation and protects against oxidative stress caused by free fatty acids. s [47] .

Group III (simvastatin+L-carnitine treated group) showed increase in SDH activity and blue staining intensity of the muscle fibers as compared to group II

(simvastatin treated group). The type I oxidative fibers were more than type IIa and type IIb fibers. By statistical analysis, Group III (simvastatin+L-carnitine treated group) showed substantial (P=0.002) decline in the average value of the succinic dehydrogenase enzyme's activity % area as compared to group I (control group). However, Group III (simvastatin+L-carnitine treated group) showed high substantial (P<0.001) increase in the mean value of the percentage area of succinic dehydrogenase enzyme activity as compared to group II (simvastatin treated group). This could be explained by the ability of L-carnitine to suppress apoptosis by elevating ATP as a result of facilitation of β-oxidation of fatty acids and therefore, inhibition of free fatty acid toxicity to the mitochondria [48]. L-carnitine can also stimulate mitochondrial biogenic gene expression [49].

Myogenin immunohistochemical stained sections of Group III (simvastatin+L-carnitine treated group) showed positive cytoplasmic immune reaction of many stem cells situated across the basal lamina and the sarcolemma. By statistical analysis, the percentage area of the positive myogenin immune-stained area for satellite stem cells in Group III (simvastatin+L-carnitine treated group) showed high substantial (P<0.001) increase in contrast to group I (control group). Also, Group III (simvastatin+L-carnitine treated group) showed high substantial (P<0.001) increase as compared to group II (simvastatin treated group) in the mean value of the percentage area of the positive myogenin immune-stained area for satellite stem cells. These findings can to explain itself because L-carnitine has the capacity to elevate the oxygen consumption and provide energy to the bone marrow derived stem cells and therefore their activation and differentiation into myoblasts and myotubes. L-carnitine promotes the expression of myogenic regulatory factors myogenin and skeletal muscle protein myosin heavy chain. So that, it enhances satellite cells mitotic activity and myotubes differentiation [50]. **Lee et al** [43] showed the role of L-carnitine in increasing myogenic differentiation, fusion of myocytes and myotube growth. Additionally, L-carnitine stimulates mesenchymal stem cells (MSCs) to differentiate into fat cells, myocytes, chondrocytes and other types of mesenchymal cells [51]. Additionally, it suppresses degradation encoded proteins that are part of the UBS, which promotes the differentiation of satellite cells [52].

Using an electron microscope to analyze the skeletal muscle of group III (simvastatin+L-carnitine treated group) showed less pathological changes than those observed at group III (simvastatin treated group). regular peripheral euchromatic nuclei with prominent nucleolus just under the sarcolemma. Some sarcomeres were regular with some disrupted areas. Some mitochondria were regular in shape and size, while others were not. Many Z lines were intact and triad tubules were not widely dilated. Additionally, these results were detailed by **Moustafa et al** [5]. The regression of electron microscopic pathological features in group III (simvastatin+L-carnitine treated group) as compare to group II (simvastatin treated group) is because L-carnitine has antioxidant and anti-inflammatory effects. This can be explained by its role of eliminating the byproducts of fatty acid metabolism via transporting the long-chain fatty acid through the inner mitochondrial membrane for β-oxidation in the matrix in order to produce ATP by oxidative phosphorylation [8]. Moreover, L-carnitine increases protein biosynthesis and downregulates both protein degradation genes in skeletal muscle phosphodiesterase type I and musculoskeletal components, RING-finger protein-1 (MuRF1) responsible for protein catabolism [50].

In the current study, omega-3 fatty acids were suggested to have a role in attenuation of simvastatin induced myopathy so that, rats of group IV received omega-3 fatty acids with simvastatin for three weeks. Histological examination of longitudinal sections (LS) of group IV showed the majority of the muscle fibers group I to a greater or lesser extent as they long, parallel with regular transverse striations. The nuclei were elongated, vesicular and peripherally located. By the transverse sections (TS) the majority of the muscle fibers were well organized, polyhedral with acidophilic sarcoplasm and peripheral nuclei. In both sections, Multiple satellite stem cells were present across the sarcolemma and the basal lamina. Similar findings were also described by **Wu et al** [53].

Omega-3 fatty acids and their potential protective function can be explained by their anti-inflammatory mechanisms. First, omega-3 fatty acids as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) decrease the pro inflammatory omega-6 derivatives as arachidonic acid [54]. Second, EPA & DHA are precursors to specialized resolving mediators (SPM) like resolvins, protectins and maresins that suppress the inflammatory response inhibit immune cell activation. Also, EPA & DHA inhibit TNF-α, IL-1β, and IL-6 which are inflammatory cytokines [55]. Omega-3 fatty acids have a potent antioxidant effect. They neutralize damaging free radicals and reduce oxidative stress to the cells. EPA & DHA activate Nuclear factor erythroid 2-related factor 2 (Nrf2) pathway that controls3 multiple genes responsible for detoxification and antioxidant activity [56].

In this investigation, group IV showed increase in SDH activity in comparison to both group II

(simvastatin treated group) and group III (simvastatin+L-carnitine treated group). The type I oxidative fibers were more than type IIa and type IIb fibers. Group IV (simvastatin+omega-3 fatty acids treated group) showed insubstantial (P=0.824) difference in the mean value of the percentage area of succinic dehydrogenase enzyme activity as compared to subgroup Ia (negative control subgroup). However, group IV (simvastatin+omega-3 fatty acids treated group) showed high substantial (P<0.001) increase in the mean value of the percentage area of succinic dehydrogenase enzyme activity as compared to group II (simvastatin treated group). The mean value of the percentage area of succinic dehydrogenase enzyme activity in group IV (simvastatin+omega-3 fatty acids treated group) showed substantial increase (P=0.039) as compared to group III (simvastatin+L-carnitine treated group). These histochemical findings can be explained by the emerging role of omega-3 fatty acid in improving the neuromuscular function by enhancing muscle size and strength, preventing muscle mass loss and improving mitochondrial respiration. Incorporation of omega-3 fatty acids into sarcolemma and mitochondrial membrane phospholipids increases muscle protein production and prevents its breakdown [57]. It is well known that omega-3 fatty acids can affect mitochondrial membranes in addition to the sarcolemma by increasing mitochondrial EPA and DHA content and improving ADP sensitivity. In turn, reactive oxygen species (ROS) are released less when ADP-stimulated oxidative phosphorylation occurs [58].

Myogenin immunohistochemical stained sections of group IV (simvastatin+omega-3 fatty acids treated group) showed positive cytoplasmic immune reaction of excess number of the satellite stem cells across the sarcolemma and basal lamina. This was confirmed by the statistical analysis as the mean value of the percentage area of the positive myogenin immune-stained area for satellite stem cells in group IV (simvastatin+omega-3 fatty acids treated group) showed high substantial (P<0.001) increase as compared to subgroup Ia (negative control subgroup). Also, group IV (simvastatin+omega-3 fatty acids treated group) showed high substantial (P<0.001) increase as compared to group II (simvastatin treated group) in the mean value of the percentage area of the positive myogenin immune-stained area for satellite stem cells. The mean value of the percentage area of the positive myogenin immune-stained area for satellite stem cells in group IV (simvastatin+omega-3 fatty acids treated group) showed substantial (P=0.022) increase as compared to group III (simvastatin+L-carnitine treated group). Omega -3 fatty acids may stimulate differentiation of satellite cells by increasing expression of myogenic markers as IGF-II, IGFBP-5, MyoD for differentiation of satellite stem cells into myoblasts and myotubes that are responsible for muscle repair and regeneration [53]. At the same time, omega-3 fatty acid can inhibit mitogen-activated protein kinase (MAPK) which is responsible protein breakdown and satellite stem cell apoptosis leading finally to more satellite stem cells support and proliferation [59].

Electron microscopic examination of the skeletal muscle of group IV (Simvastatin+Omega-3 fatty acids treated group) showed regular peripheral euchromatic nuclei with prominent nucleolus just under the sarcolemma. The majority of the sarcomeres were regular with few localized areas of disruption. Most of the mitochondria, Z lines and triad tubules were regular in size and shape. This can be attributed to the previously discussed antioxidant [56] and anti-inflammatory [54] properties of omega-3 fatty acids along with their role in maintaining the integrity of cellular membranes [55].

CONCLUSION

This study showed that, simvastatin administration resulted in histopathological and morphometric negative consequences on the musculoskeletal system. Both L-carnitine and omega-3 fatty acids showed less pathological changes in the simvastatin induced myopathy by microscopic examination. However, omega-3 fatty acids had better effect than L-carnitine in attenuation of simvastatin induced myopathy and more activation of endogenous muscle satellite stem cells.

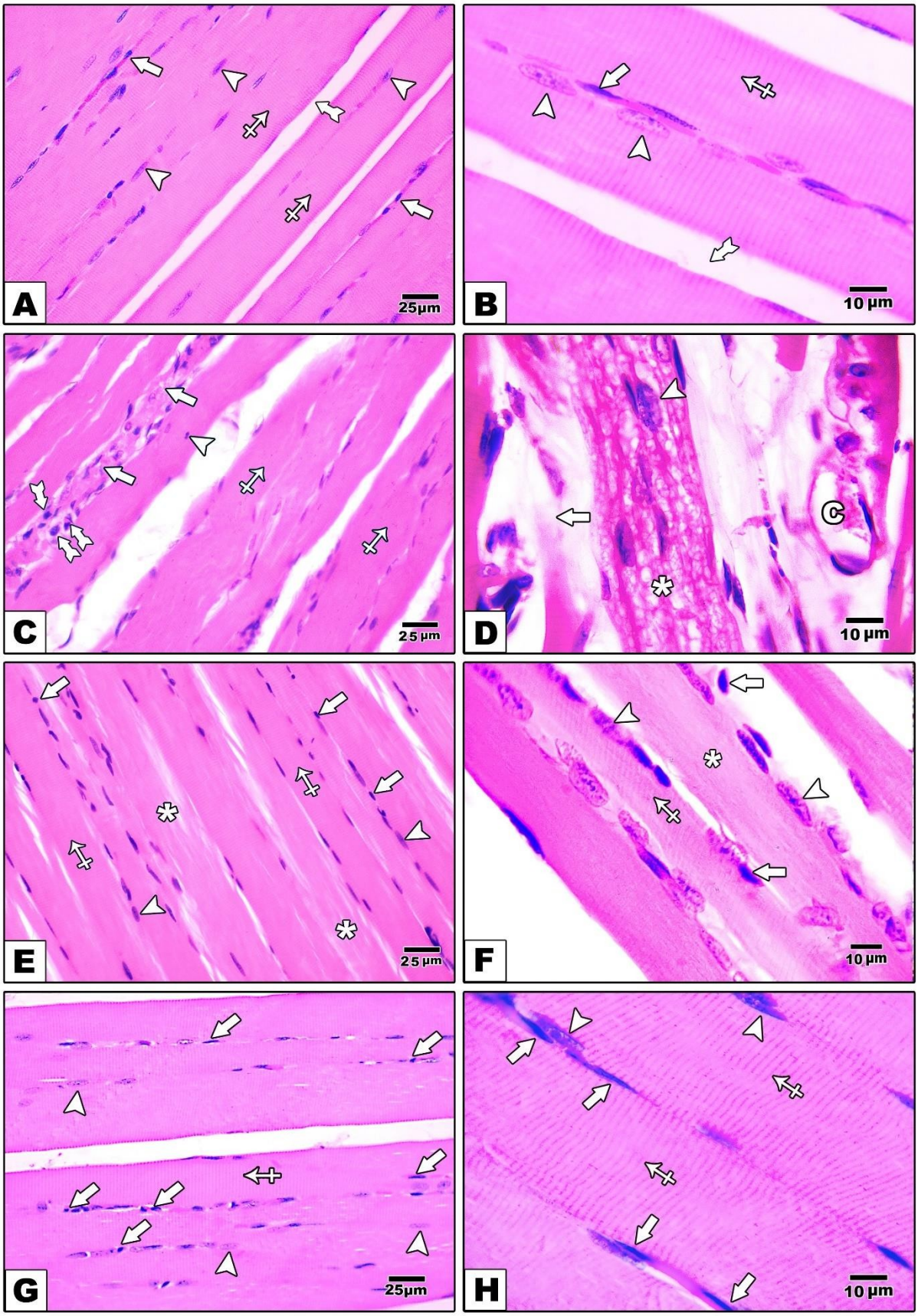


Figure 1: Photomicrographs of H&E stained longitudinal sections in the skeletal muscle of all groups. **A and B:** group I (control group) show long parallel striated muscle fibers with acidophilic sarcoplasm (crossed arrows).

The nuclei of the muscle fiber are multiple, elongated and vesicular just under the sarcolemma (arrow heads). The satellite stem cells (arrows) are small cells with dark nuclei outside the sarcolemma and under basal lamina. Note the loose connective tissue of the endomysium (bifid arrows) between the muscle fibers. **C:** group II (simvastatin treated group) shows disorganization of the muscle fibers and loss of the sarcoplasmic transverse striations (crossed arrows) with splitting and vacuolation (arrows). There is fragmentation of the muscle fibers into blebs that have small amount of the sarcoplasm and nuclei (bifid arrows). Some nuclei are displaced (arrow head). **D:** group II (simvastatin treated group) shows splitting (arrow) and vacuolations (stars) of the muscle fibers with displaced nuclei (arrow head). Note the congested blood capillary (C) in the endomysium. **E and F:** Group III (simvastatin+L-carnitine treated group) show that many muscle fibers are cylindrical, parallel with regular transverse striations (crossed arrows) while some show splitting (stars). Most of nuclei are elongated, vesicular and peripherally located (arrowheads). Many satellite stem cells (arrows) are present between the sarcolemma and the basal lamina. **G and H:** group IV (Simvastatin+Omega-3 fatty acids treated group) show that the majority of the muscle fibers are well organized with regular transverse striations (crossed arrows). The nuclei are elongated, vesicular and peripherally located (arrowheads). Multiple satellite stem cells (arrows) are present between the sarcolemma and the basal lamina. **(H&E stain A,C,E,G X400, B,D,F,H X1000).**

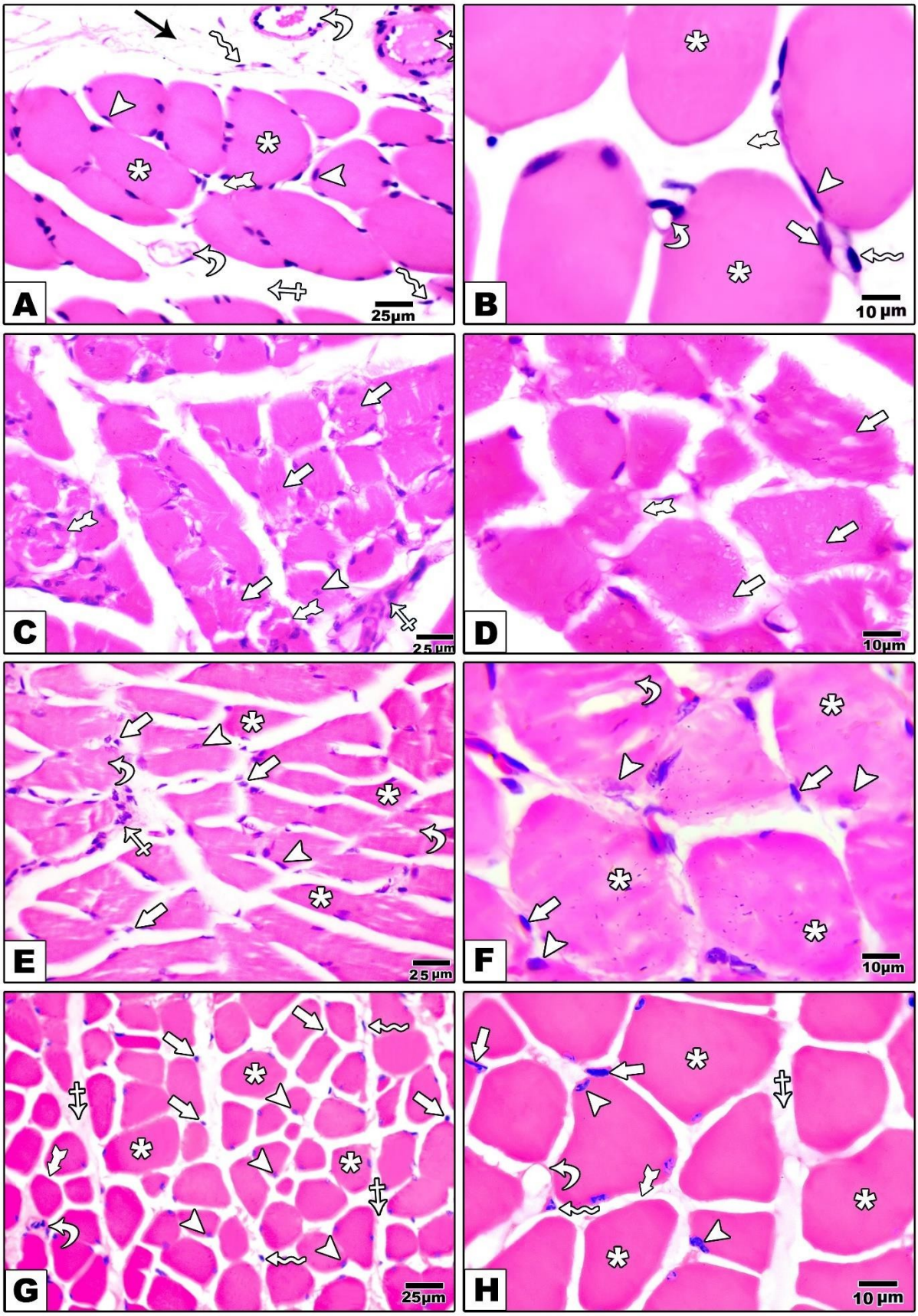


Figure 2: Photomicrographs of H&E stained transverse sections in the skeletal muscle of all groups. **A and B:** group I (control group) show polyhedral acidophilic skeletal muscle fibers (stars) with peripheral basophilic nuclei

(arrowheads).

The whole muscle is surrounded by dense connective tissue epimysium (black arrow) and each muscle bundle is surrounded by a dense connective tissue perimysium (crossed arrow) and the muscle fibers are separated by loose connective tissue endomysium (bifid arrows). The fibroblasts (zigzag arrows) and the blood vessels (curved arrows) are present in the connective tissue. Note the satellite stem cells with dark nuclei in between the basal lamina and the sarcolemma (arrow) in **B. C and D:** group II (simvastatin treated group) show disorganization of the muscle fibers with vacuolation and fragmentation of the sarcoplasm (arrows). Some rounded shrunken fibers are present (bifid arrows). Note the centrally displaced nuclei (arrow head) and markedly thick perimysium (crossed arrow) in **C. E and F:** Group III (simvastatin+L-carnitine treated group) show some well-organized polyhedral acidophilic muscle fibers (stars) and some disorganized fragmented muscle fibers (curved arrows). The muscle fibers nuclei are mostly peripheral and vesicular (arrow heads) and many satellite stem cells (arrows) are present between the sarcolemma and the basal lamina. Note the thick perimysium (crossed arrow) in **E. G and H:** group IV (Simvastatin+Omega-3 fatty acids treated group) show that the majority of the muscle fibers are well organized, polyhedral with acidophilic sarcoplasm (stars). The muscle fibers nuclei are peripheral and vesicular (arrow heads) and multiple satellite stem cells (arrows) are present between the sarcolemma and the basal lamina. Note the fibroblasts (zigzag arrows) and the blood vessels (curved arrows) in the connective tissue of the perimysium (crossed arrows) and endomysium (bifid arrows). **(H&E stain A,C,E,G X400, B,D,F,H X1000).**

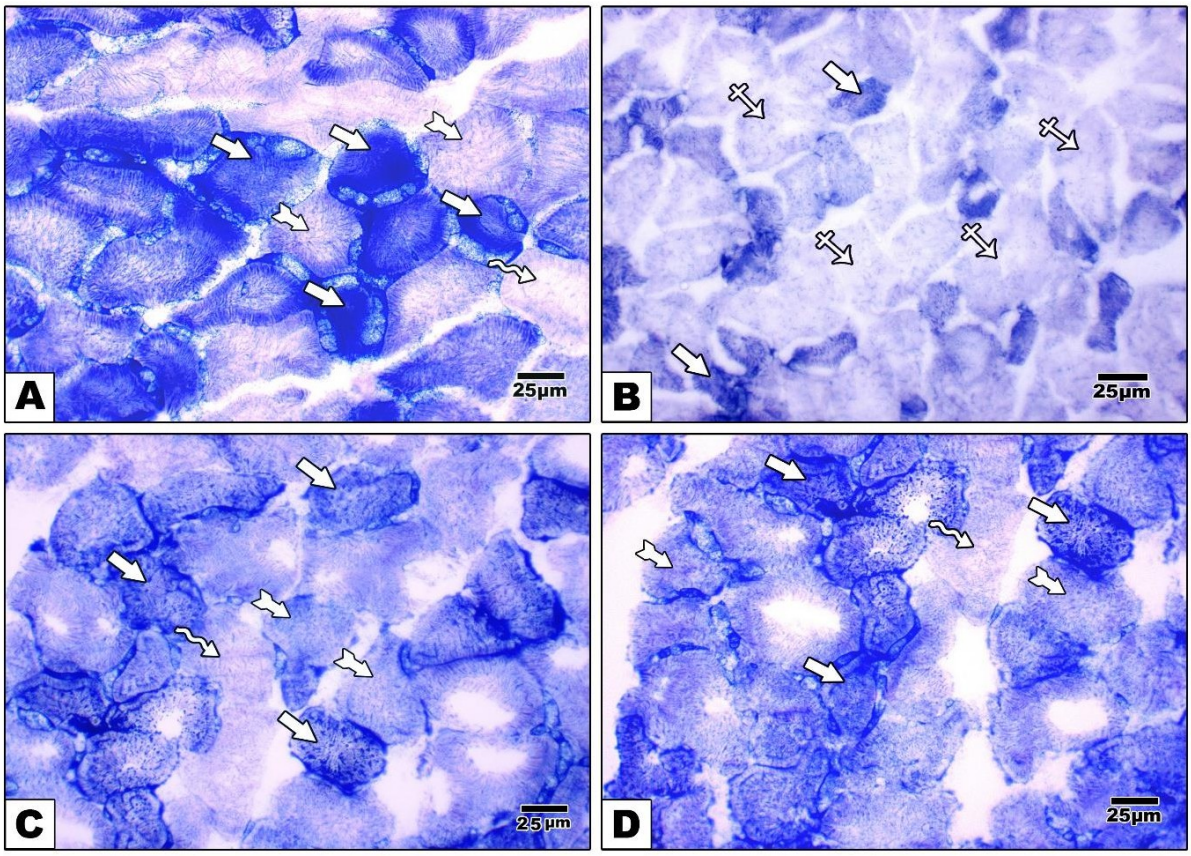


Figure 3: Photomicrographs of frozen transverse sections in the skeletal muscle of all groups. **A:** group I (control group) shows that the majority of the muscle fibers are type I (oxidative) muscle fibers that are small in diameter and deeply blue stained with strong SDH activity (arrows). Some type IIa (intermediate) muscle fibers with intermediate diameter and moderate SDH activity are found (bifid arrows).

Type IIb (glycolytic) muscle fibers with large diameter and weak SDH activity are also seen (zigzag arrows). **B:** group II (simvastatin treated group) shows weak succinic dehydrogenase activity in the majority of muscle fibers (crossed arrows). Few type I (oxidative) muscle fibers show strong SDH activity (arrows). **C:** Group III (simvastatin+L-carnitine treated group) shows increase in SDH activity of the muscle fibers in comparison to group II. **D:** group IV (Simvastatin+Omega-3 fatty acids treated group) shows increase in SDH activity in comparison to both group II and

group III. In **C and D:** Type I has strong SDH activity (arrows), type IIa moderate SDH activity (bifid arrows) and Type IIb has weak SDH activity (zigzag arrows). **(SDH X400).**

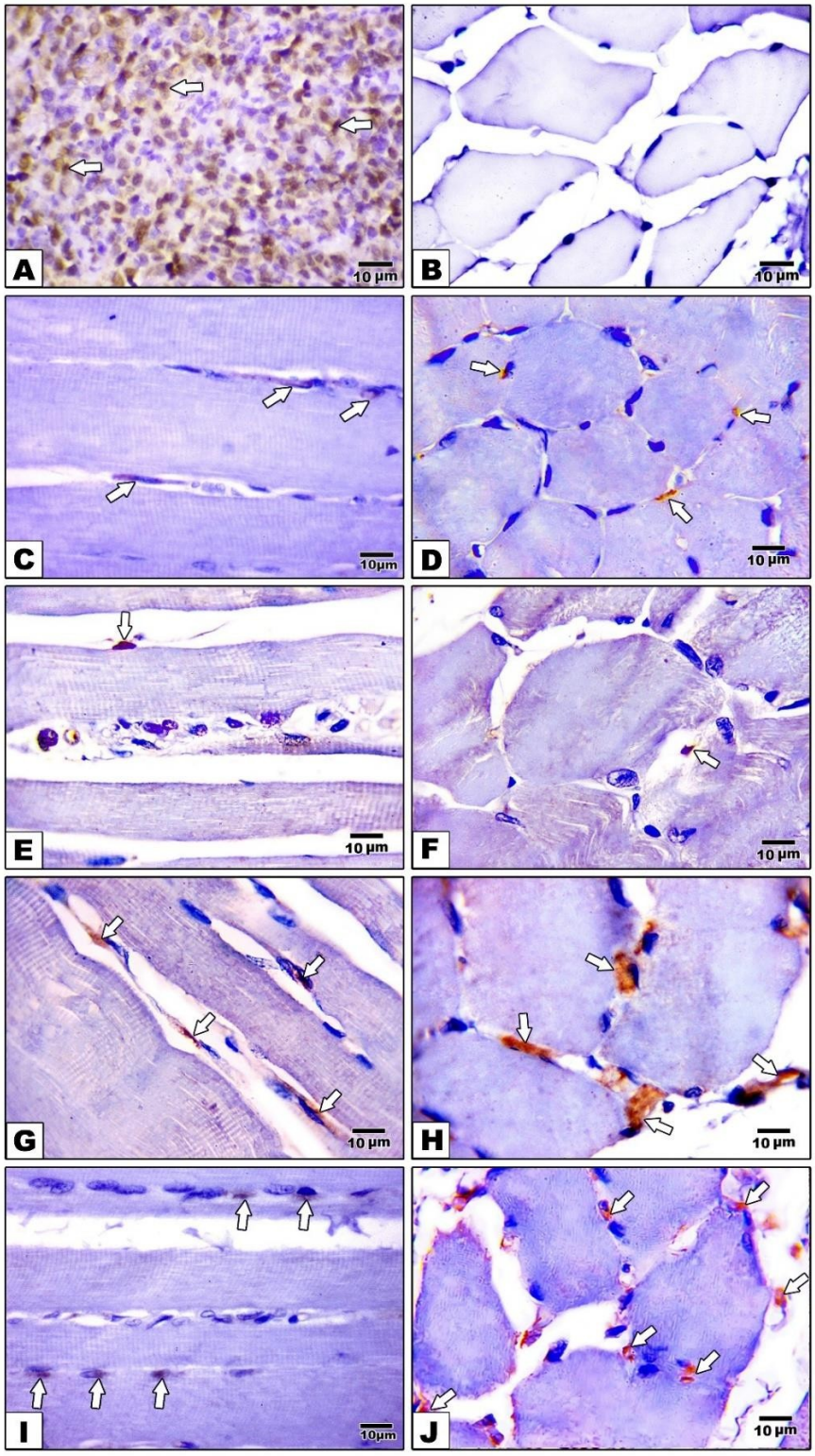


Figure 4: Photomicrographs of myogenin immunohistochemical stained sections. **A:** rhabdomyosarcoma positive control shows brown positively stained cytoplasmic and/or nuclear myogenin immune reaction (arrows). **B, D, F, H and J** are transverse sections, while **C, E, G and I** are longitudinal sections in the skeletal muscle of all groups. **B:** skeletal muscle negative control after omitting the 1ry Ab shows no immune reaction.

C, D: group I (control group) show positive brown myogenin immune reaction in the cytoplasm of satellite stem cells in between the sarcolemma and the basal lamina (arrows). **E, F:** group II (simvastatin treated group) show positive immune reaction the cytoplasm of few satellite stem cells. (arrows). **G and H:** group III (simvastatin+L-carnitine treated group) show positive immune reaction in the cytoplasm of many satellite stem cells. **I and J:** group IV (Simvastatin+Omega-3 fatty acids treated group) show positive immune reaction in the cytoplasm of multiple satellite stem cells. **(Myogenin immunohistochemical staining X1000).**

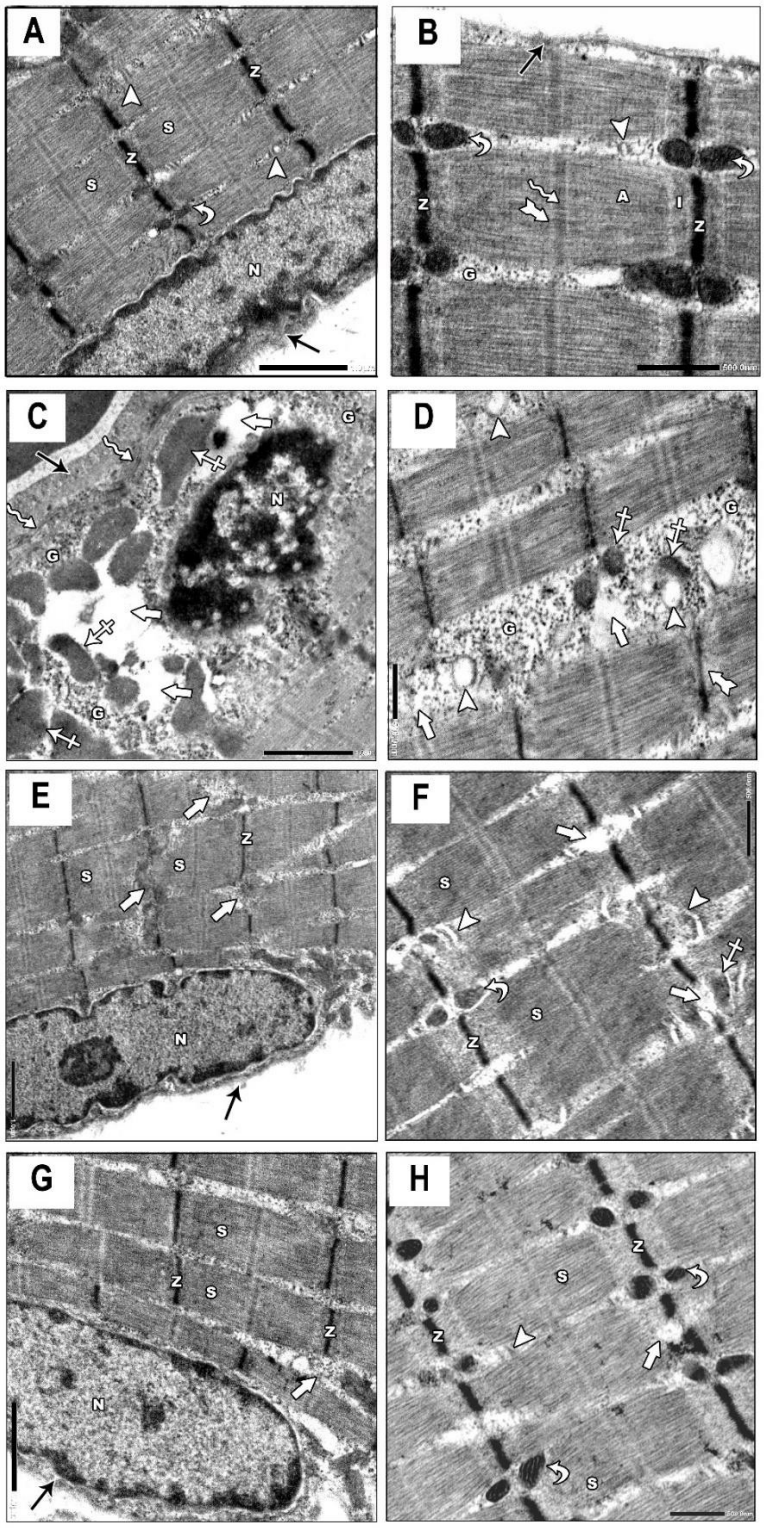
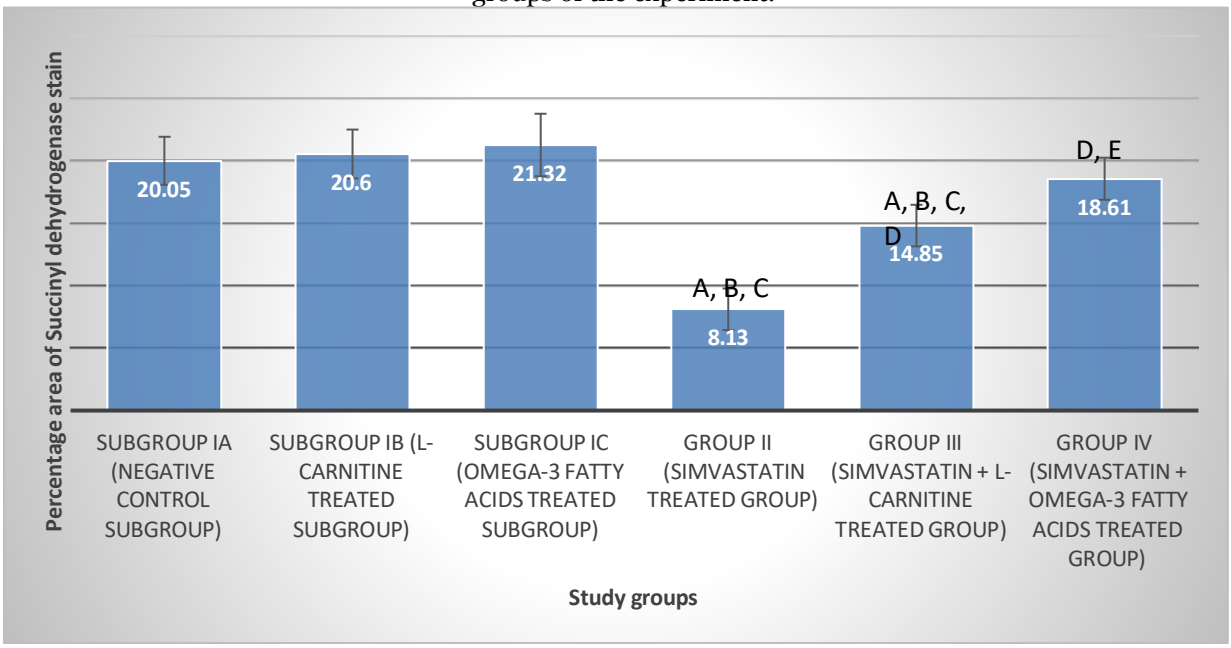


Figure 5: Electron photomicrographs of ultrathin longitudinal sections in the skeletal muscle of all groups.

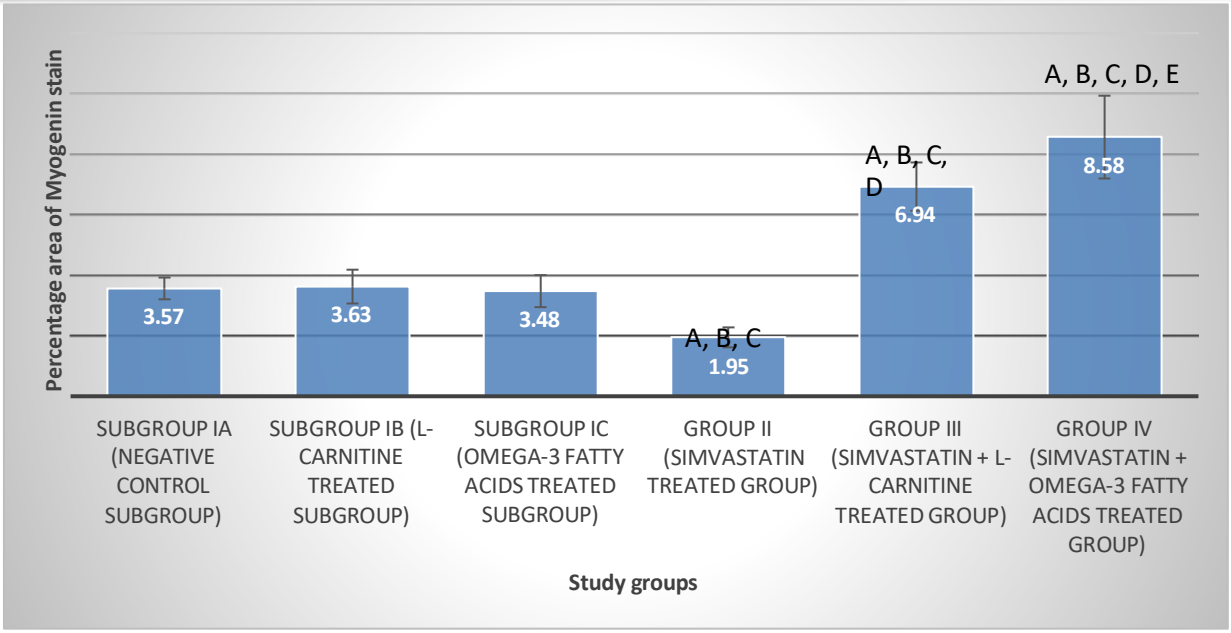
A and B: Group 1 (control group), **C and D:** group II (simvastatin treated group), **E and F:** Group III (simvastatin+L-carnitine treated group), **G and H:** group IV (Simvastatin+Omega-3 fatty acids treated group). **A:** shows regular peripheral euchromatic nucleus (N). **A and B:** show regular sarcolemma (black arrows) and intact sarcomeres (S) with regular light bands (I), dark bands (A), H zone (bifid arrow), M line (zigzag arrow), Z lines (Z) and mitochondria (curved arrows), triad tubules (arrow heads) and few glycogen granules (G). **C:** shows irregular heterochromatic nucleus (N) with disrupted nuclear envelope & displaced away from the sarcolemma (black arrow) with collagen fibers deposition (zigzag arrows). **C and D: s**how disrupted areas of the muscle fibers (arrows) with irregular shaped mitochondria (crossed arrows) and large amount of glycogen granules (G). **D:** shows wide triad tubules (arrow heads) and disorganized Z lines (bifid arrow). **E:** shows regular peripheral euchromatic nucleus (N) with prominent nucleolus just under the sarcolemma (black arrow). **E and F:** show some regular sarcomeres (S) with some disrupted areas (arrows). Some mitochondria are regular in shape and size (curved arrow), while others are not (crossed arrow). Many Z lines are intact (Z) and triad tubules (arrow heads) are not widely dilated. **G:** shows regular peripheral euchromatic nucleus (N) with prominent nucleolus just under the sarcolemma (black arrow). **G and H:** show that the majority of the sarcomeres (S) are regular with few localized areas of disruption (arrows). Most of the mitochondria (curved arrows), Z lines (Z) and triad tubules (arrowhead) are regular in size and shape. **(TEM A,C,E,G X8000, B,D,F,H X10000).**

Histogram (1): Percentage area of succinic dehydrogenase enzyme activity (mean ± SD) within the different groups of the experiment:



SD: standard deviation, P: probability
* statistically substantial if P ≤0.05
** highly statistically substantial result if P ≤0.001
A: comparison in relation to subgroup Ia (negative control subgroup).
B: comparison in relation to subgroup Ib (L-carnitine treated subgroup).
C: comparison in relation to subgroup Ic (omega-3 fatty acids treated subgroup).
D: comparison in relation to group II (simvastatin treated group).
E: comparison in relation to group III (simvastatin+L-carnitine treated group).

Histogram (2): Percentage area of the positive myogenin immune-stained area for satellite stem cells (mean ± SD) within the different groups of the experiment:



SD: standard deviation, P: probability
* statistically substantial if P ≤0.05
** highly statistically substantial result if P ≤0.001
A: comparison in relation to subgroup Ia (negative control subgroup).
B: comparison in relation to subgroup Ib (L-carnitine treated subgroup).
C: comparison in relation to subgroup Ic (omega-3 fatty acids treated subgroup).
D: comparison in relation to group II (simvastatin treated group).
E: comparison in relation to group III (simvastatin+L-carnitine treated group).

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