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Histological Study for the Effect of Squalene Supplements on Muscles Rats Treated with Simvastatin

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Abstract

The present study aimed to reveal the biological vitality of squalene supplements and in combination with statins. Statins are a class of drugs that are regularly administered to decrease cholesterol. The main cause of statin discontinuation is the development of statin-associated muscle symptoms. Because of the statin-related myotoxicity phenotypes that are getting worse have been proposed, the study was conducted to examine the effects of squalene supplements in combination with statin. In the experiment, thirty male rats were divided into two group, (experimental one) five rats dosage with normal saline only and other rats administrated with cholesterol 1%/BW for 28 days. Then the second group was randomly divided into four groups (experimental two). The experiment lasted about during two months, the rats were administration as the following groups, first group: six rats used as a negative control group dosage with normal saline only; second group: six rats used as a positive control group dosage with normal cholesterol 1% only; third group: six rats administrated with cholesterol 1%/BW for 28 days then treated with simvastatin 40 mg/kg dosage orally every day for 4 weeks; forth group: six rats administrated with cholesterol 1%/BW for 28 days then treated with squalene 0.2 mg orally every day for 28 day, and fifth group: six rats will have administrated with cholesterol 1%/BW for 28 days then treated with simvastatin 40 mg/kg dosage orally with squalene every day for 28 day. In conclusion: we found from the study effect of squalene supplements on rats treated with statin to avoid side effect and protecting the muscles from damage caused by statin.

Keywords: Squalene, simvastatin, cholesterol, histopathological studies in muscles

Introduction

Squalene (SQ) is a natural substance belonging to the terpenoid family, widespread in nature, with various applications in many human life areas [1]. Squalene is the main triterpene hydrocarbon present in virgin olive oil (VOO). VOO is the highest source of SQ compared to other vegetable fats [2]. SQ is a metabolite involved in the biosynthesis of cholesterol and it is oxidized by monooxygenase in the early stages of metabolism. However, its monooxygenase activity is highly suppressed by the accumulation of cholesterol [3]. Therefore, when squalene is ingested by diet, squalene-derived sterols accumulate in the cells and β-hydroxy-βmethylglutaryl-CoA reductase is inhibited [3]. SQ has a lot of interesting activities, including antioxidant and antitumor effects [4], lowering the serum cholesterol level [5]. Statins inhibit HMG-CoA involved in cholesterol biosynthesis; precisely, they compete with HMG-CoA in the mevalonate pathway [6]. Statins are drugs frequently prescribed in patients suffering from dyslipidemia and, even, in patients with coronary artery disease, diabetes mellitus, stroke, blood hypertension, and chronic kidney disease with or without dyslipidemia. Advantages of using statins go beyond the simple reduction of cholesterol level, because of several additional positive effects, the so-called" pleiotropic effects", as anti-inflammatory, antioxidant, anti-proliferative, apoptotic, cell cycle regulatory, and immunomodulatory effects [7]. Statins are molecules of fungal origin that, by inhibiting the hydroxymethylglutaryl CoA (HMG-CoA) reductase enzyme, a key step in the sterol biosynthetic pathway, became powerful cholesterol-lowering medications [8].

Adverse effects on skeletal muscles occur in approximately 5–10% of patients taking statins [9]. Observational studies estimate that 10–15% of statin users develop statin-related muscular side effects, ranging from myalgia and fatigue to more severe muscle symptoms with significant creatine kinase (CK) elevations [10,11].

Materials and Methods

The study performed during the period from (October 2022 to December 2023). Mature rats hold in animal house of College of Veterinary Medicine, University of Kerbala. for adaptation in period ranging from two to three weeks then the study actual performance began starting. Thirty adult male rats aged (2-3 months) weighting 260-300 gm were obtained from the animal house of College of Pharmacy, University of Kerbala. They were set in the animal house with standard environment conditions temperature (25-28 C̊) relative humility 40٪ - 60٪ conditions room and the light system was 12 hrs. per day.[12]

Thirty rats were divided into two groups, (experimental one) five rats' were dosed with normal saline only and other rats administrated with cholesterol 1%/BW for 28 days. Then the second group randomly divided to four groups (experimental two) .

The experiment lasted about during two months; the rats were administration as the following groups :

1 -Fist group: Six rats were used as a negative control group dosage with normal saline only.
2 -Second group: Six rats were used as a positive control group dosage with normal cholesterol 1% only.
3 -Third group: Six rats were administrated with cholesterol 1%/BW for 28 days then treated with simvastatin 40 mg/kg dosage orally every day for 4 weeks.
4 -Forth group: Six rats were administrated with cholesterol 1%/BW for 28 days then treated with squalene 0.2 mg orally every day for 4 weeks.
5 -Fifth group: Six rats will have administrated with cholesterol 1%/BW for 28 days then treated with simvastatin 40 mg/kg dosage orally with squalene every day for 4 weeks.

Sample collection and tissue preparation

After the end of the experiment period muscles sample were taken after the animal sacrifice and they were immediately put into a 10% fixative formalin solution and left there for 48 hours before being processed. The samples were first dehydrated in graded alcohol at room temperature for 2 minutes each at 70%, 80%, 90%, and 100% concentrations before being submerged in xylene for 2 hours and then melted paraffin wax for 3 hours. The samples were then positioned and implanted in brand-new paraffin (paraffin blocks). To examine the sections under a microscope, the blocks were sectioned with a microtome at a thickness of 5 m. The sections were subjected to standard hematoxylin and eosin (H&E) staining procedures. Photomicrographs of each section were taken using a digital camera (canon, japan) using a light microscope to analyze the sections under examination [13].

Results and Discussion

The muscle of the control animals group showed normal histology (Figure 1) stained with (H&E), Photomicrograph of longitudinal section of skeletal muscle fibers of a control rat showing the normal morphology of parallel, cylindrical muscle fibers with visible transverse striations and multinucleated peripheral nuclei, and photomicrograph of cross section of skeletal muscle fibers of a control rat in (Figure 2) showing polygonal muscle fibers separated by endomysium. Bundles are separated by perimysium (collagen strands) .

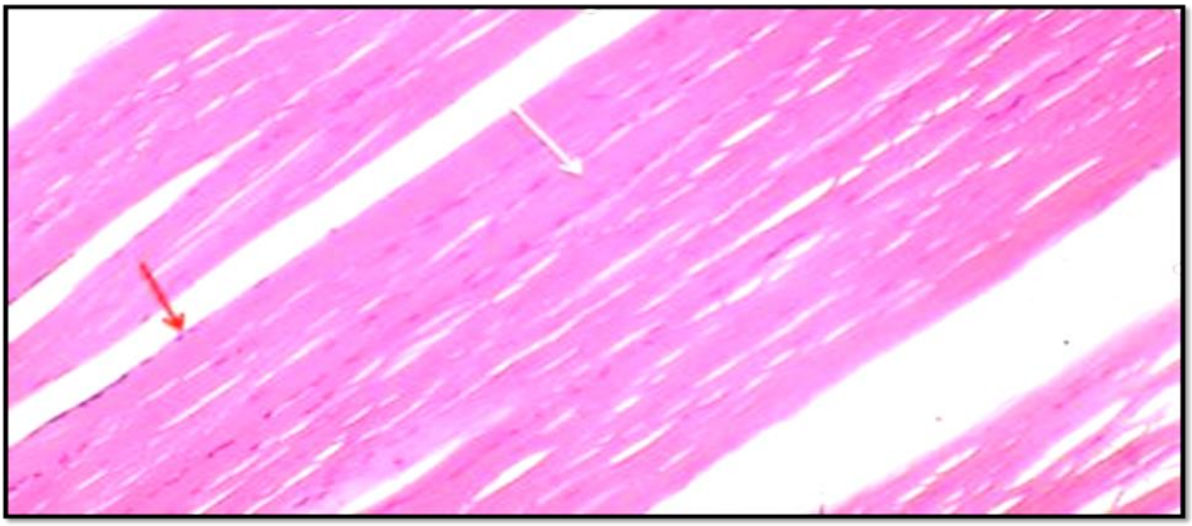

Figure (1): Photomicrograph of longitudinal section of skeletal muscle fibers of a control rat showing the normal morphology of parallel, cylindrical muscle fibers with visible transverse striations (white arrow) and multinucleated peripheral nuclei (red arrow) (H and E,10X).

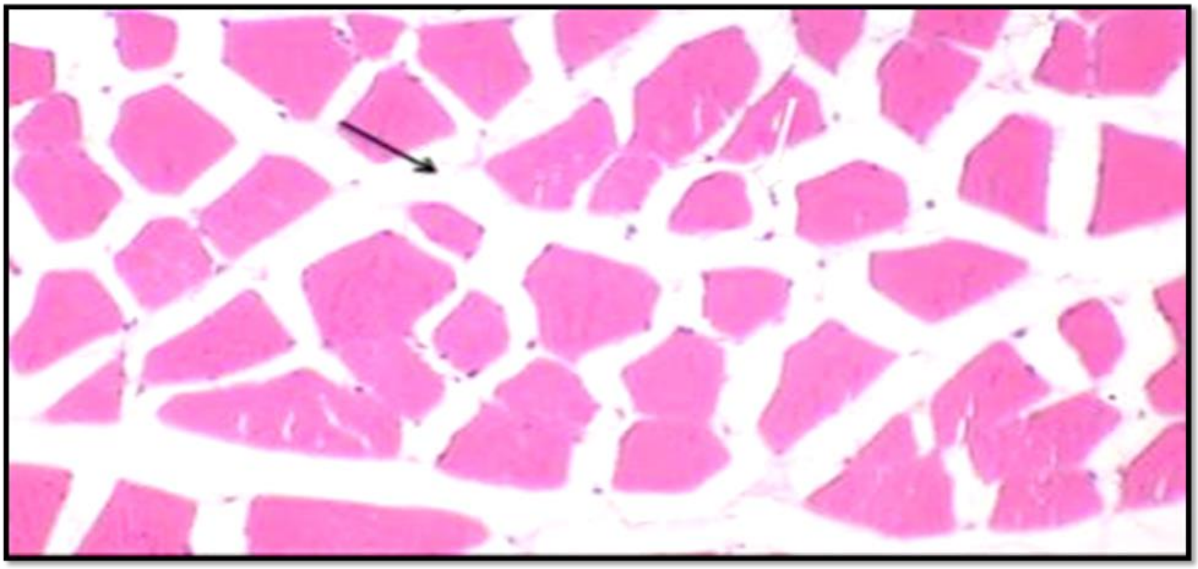

Figure (2): Photomicrograph of cross section of skeletal muscle fibers of a control rat showing polygonal muscle fibers separated by endomysium (white arrow). Bundles are separated by perimysium (collagen strands) (black arrow) (H and E,10X).

The histopathological examination from the second group of a longitudinal section of skeletal muscle fibers of cholesterol treated rat showed reverse parallel, branched, Considerably thin muscle fibers with distinct striations, increased perimysium between bundles, (H and E, 10X), (Figure 3). Photomicrograph for cross section of skeletal muscle fibers of cholesterol treated rat showing muscle fibers atrophy, with significant extended endomysium, (H and E,10X), (Figure 4).

Additionally, cholesterol plays a critical role in the development and maintenance of all cell membranes, including those in skeletal muscles. A decrease in the

amount of cholesterol in skeletal muscle cell membranes can cause them to become unstable and change the fluidity and excitability of ion channels. This can increase the sensitivity of skeletal muscle to HMGCoA reductase inhibition [21]. As a result, myocyte damage and myopathy may result from altered sodium, potassium, and chloride channel function [22]. Statins and Muscle Disease People with muscle disease often avoid statins because of known side effects. The cause of statin-induced myopathy is unclear, although several mechanisms have been proposed, including increased oxidative stress [23], activation of the atrogynous-1 muscle wasting pathway [24], and increased susceptibility to RyR1-induced Ca2+ leak in a malignant hyperthermia mouse model [25]. Although statins may be myotoxic, one study suggests that statins are highly beneficial in skeletal muscle affected by underlying conditions such as ischemia, oxidative stress, and inflammation. Statins reduce oxidative stress, inflammation and fibrosis, all processes associated with functional muscle decline in muscular dystrophy, especially Duchenne muscular dystrophy (DMD). Simvastatin has been shown to improve muscle strength and fatigue resistance in DMD mice, in DMD, the leading cause of death is heart failure[26] .

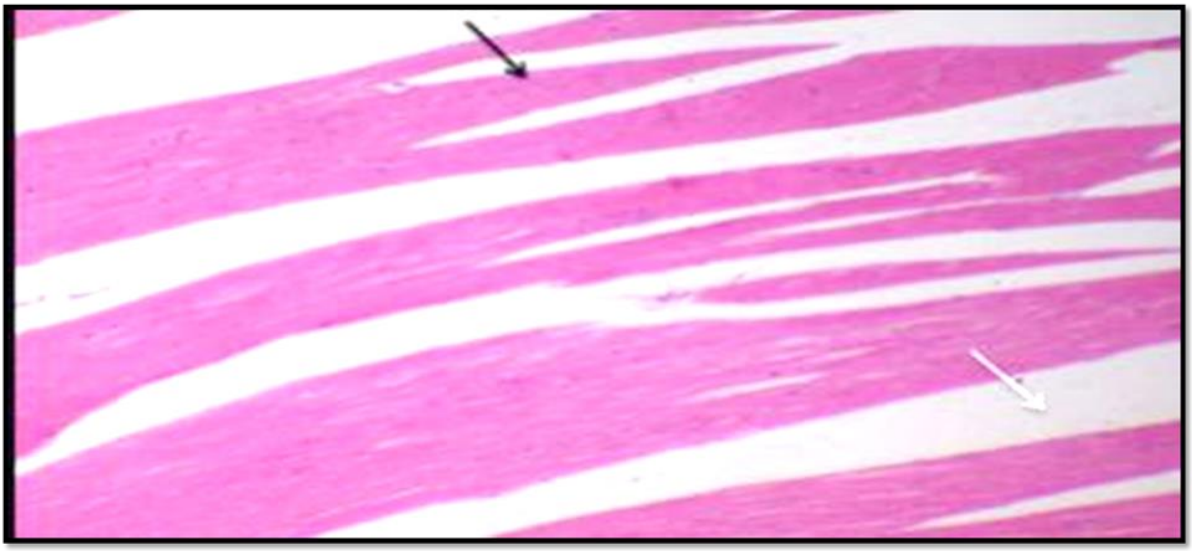

Figure (3): Photomicrograph for a longitudinal section of skeletal muscle fibers of cholesterol treated rat showing reverse parallel, branched, Considerably thin muscle fibers with distinct striations (black arrow), increased perimysium between bundles (white arrow) (H and E,10X) .

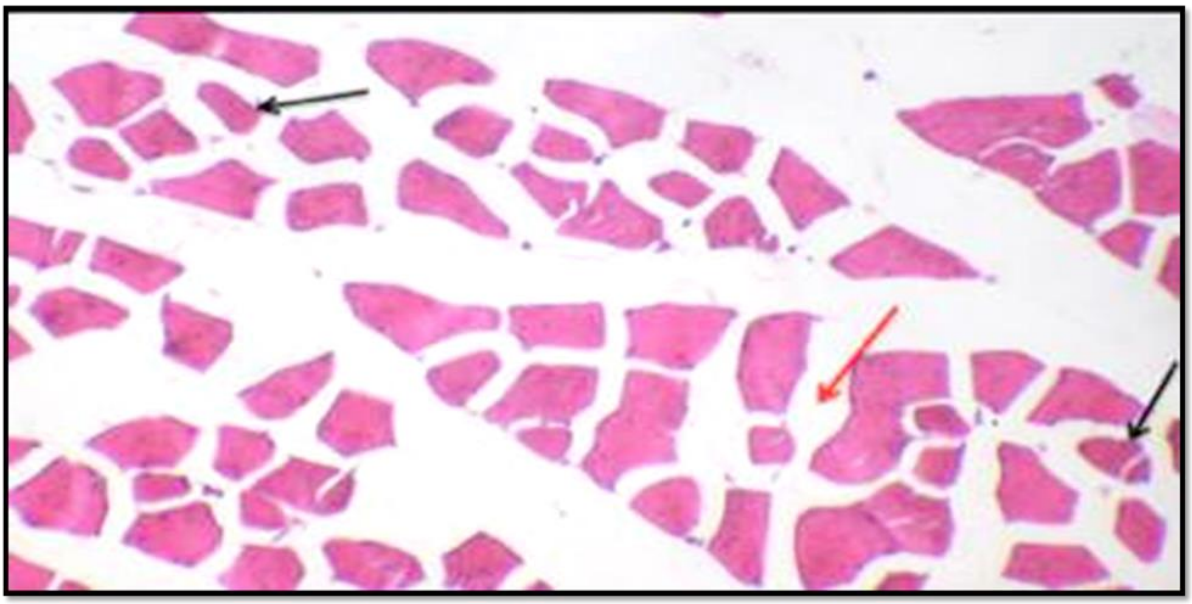

Figure (4): Photomicrograph for cross section of skeletal muscle fibers of cholesterol treated rat showing muscle fibers atrophy (black arrow), with significant extended endomysium (red arrow), (H and E,10X).

On the other hand, the statin treated group from the third group of longitudinal section of skeletal muscle fibers showed a significant pathological alterations represented by discontinuity and splitting in myofibrils, wavy appearance, marked atrophied myofibers with randomly arrangements of nuclei and mild infiltration of inflammatory cells, (H and E,10X), (Figure 5). Photomicrograph of transverse section of skeletal muscle fibers of statin treated rat showing significant pathological alterations represented by sever dilation in perimysium with increased collagen strands, markedly atrophied myofibers decrease intracellular spaces, some myofibers appeared with no nuclei and mild infiltration of inflammatory cells, (H and E, 10X). (Figure 6), as compared to the normal histological structure of the muscle (Figure1 and 2). Statins and Skeletal Muscle Adverse Effects. Statin intolerance is most frequently associated with a wide range of side effects in the skeletal muscle, the so-called "Statin-Associated Muscle Symptoms" (SAMS). SAMS are quite difficult to be diagnose and manage not only because there are no validated biomarkers or tests that can be used to confirm their presence, but also because muscle symptoms could originate from other comorbidities [14]. However, a study carried out by [15] showed that patients may have a statin-induced myotoxicity occurring as muscle necrosis due to statin exposure and manifesting with increased CK levels. The most important risk factors of SAMS are advanced age, female gender, Asian ethnicity, drugs altering statin plasma levels, excessive physical activity, muscle, liver or chronic kidney diseases, uncontrolled hypothyroidism, abdominal obesity and metabolic syndrome, and vitamin D deficiency. The risk of SAMS is higher with lipophilic statins such as simvastatin, atorvastatin, and lovastatin, because of their ability to not selectively diffuse into extrahepatic tissues as skeletal muscles. Typically, SAMS manifests with different muscle symptoms occurring after statin treatment (with or without eleva-

tions of serum creatine kinase) that might resolve after its interruption. SAMS has a highly variable clinical presentation ranging from a myopathic pattern, characterized by muscle tenderness, cramping and muscle aches, weakness and increased CK level (even 10 times higher the upper normal limit), to rhabdomyolysis [16]. Myopathy usually appears in patients who receive high doses of statins, especially when taking simvastatin 80 mg daily, which lead to higher plasma levels of active statins metabolites, especially in the first year of treatment or after having increased the dosage. Muscle disorders are often reversible after statin withdrawal [17]. Skeletal muscle is made up of fast and slow twitch muscle fibers, which have different compositions and react in various ways to outside substances like statins.

Statin treatment causes massive necrosis in fast-twitch, glycotic type IIB muscle in animals, while sparing the slower-twitch, oxidative type I fibers [18]. These changes were accompanied by ultrastructural changes to the muscle mitochondria, such as swollen mitochondria with disrupted cristae and increased vacuolation or degeneration, which led to vesicular bodies accumulating in the subsarcolemmal space [19]. T-tubular system vacuolization in statin-treated patients has also been seen in human studies.[20]

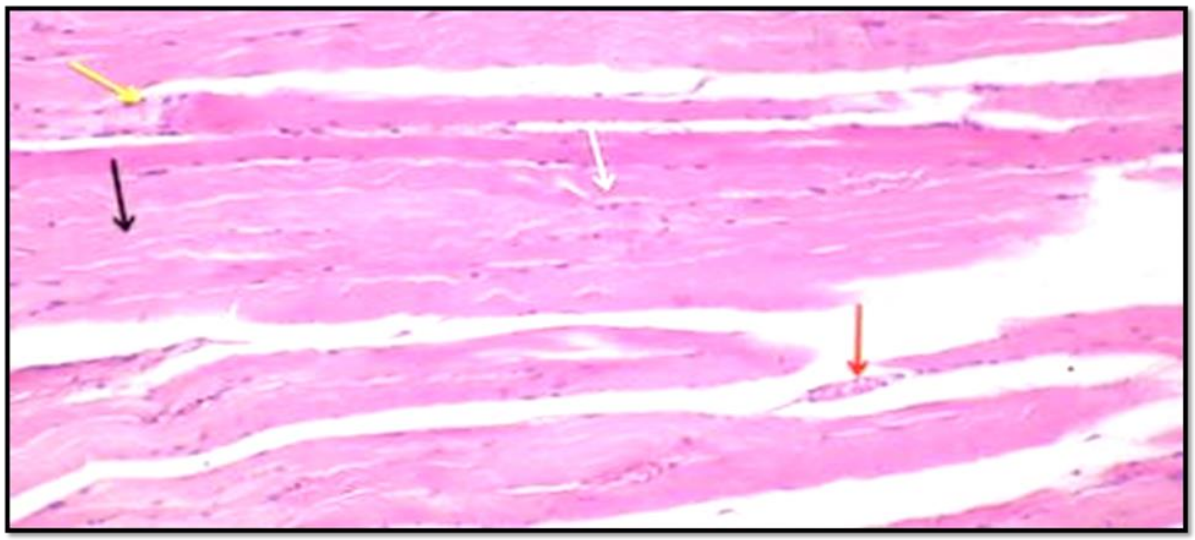

Figure (5): Photomicrograph of longitudinal section of skeletal muscle fibers of statin treated rat showing significant pathological alterations represented by discontinuity and splitting in myofibrils, wavy appearance (black arrow), marked atrophied myofibers (red arrow) with randomly arrangements of nuclei (white arrow) and mild infiltration of inflammatory cells (yellow arrow) (H and E,10X).

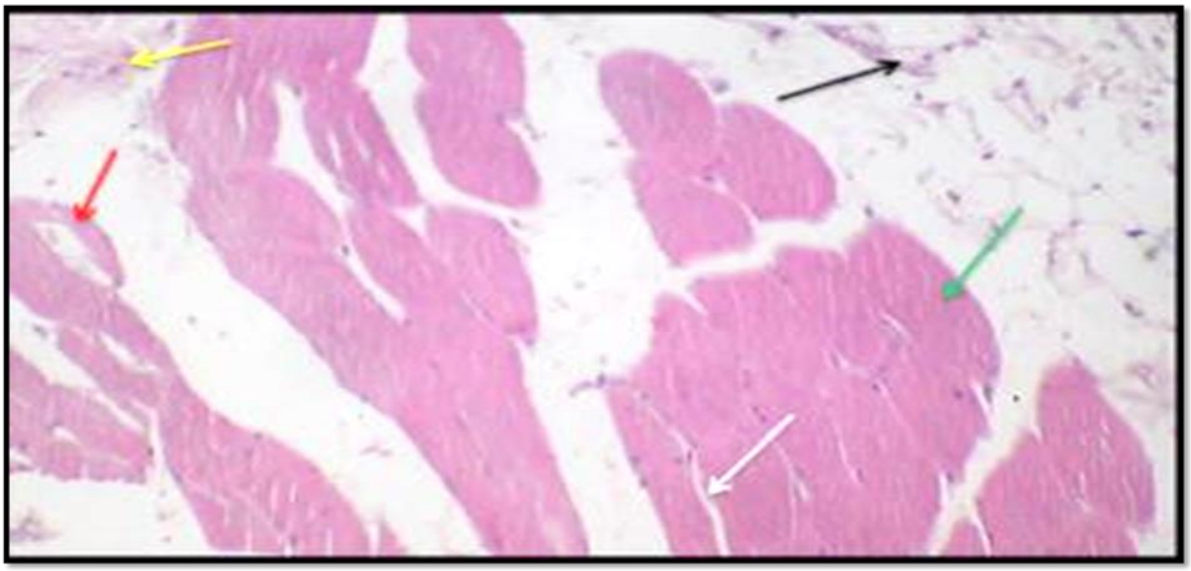

Figure (6): Photomicrograph of transverse section of skeletal muscle fibers of statin treated rat showing significant pathological alterations represented by sever dilation in perimysium with increased collagen strands (black arrow), marked atrophied myofibers (red arrow) decrease intracellular spaces (white arrow), some myofibers appeared with no nuclei (green arrow) and mild infiltration of inflammatory cells (yellow arrow) (H and E, 10X).

The photomicrograph for a longitudinal section of skeletal muscle fibers in figure (7) from a squalene treated rat which has parallel, cylindrical, considerably thin muscle fibers with significant striations, elongated and chain arranged nuclei, (H and E,10X). Photomicrograph for cross section of skeletal muscle fibers of squalene treated rat showing moderate increase in size, with significant extended nuclei and normal endomysium with collagen, (Figure 8) .

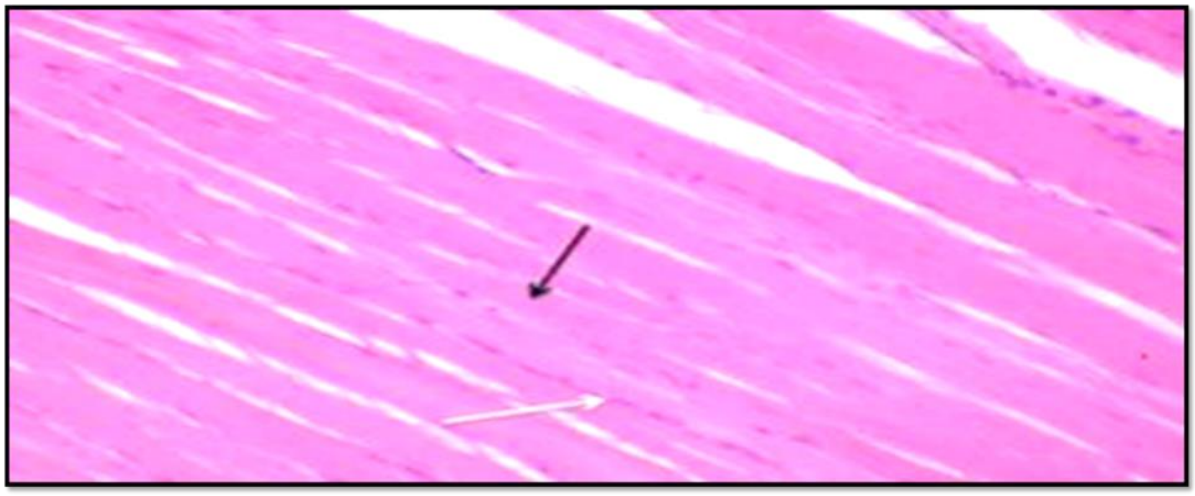

Figure (7): Photomicrograph for a longitudinal section of skeletal muscle fibers of squalene treated rat showing parallel, cylindrical, considerably thin muscle fibers with significant striations (black arrow), elongated and chain arranged nuclei (white arrow). (H and E,10X).

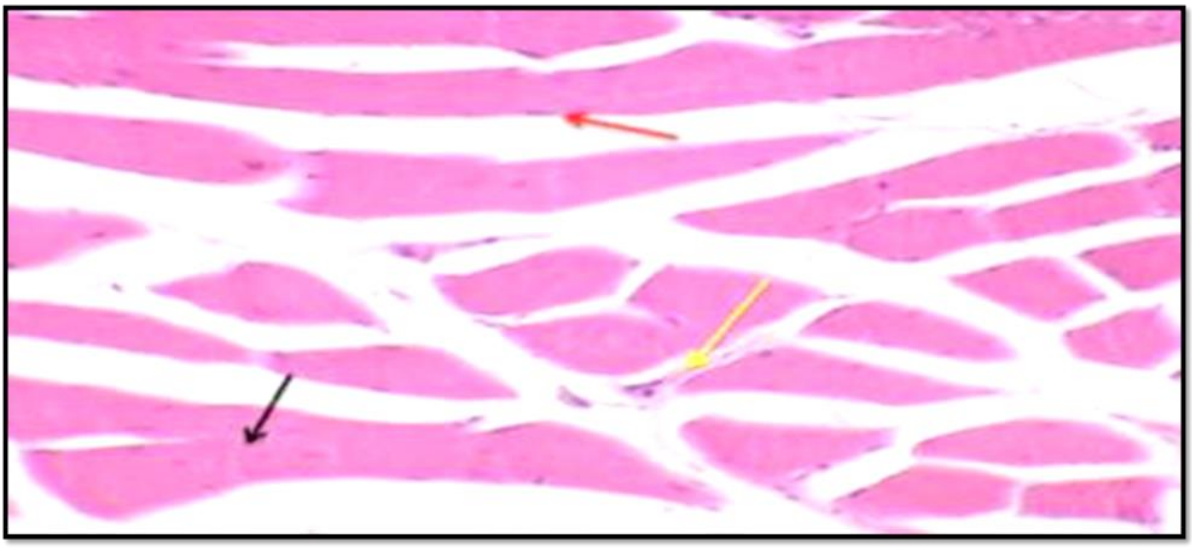

Figure (8): Photomicrograph for cross section of skeletal muscle fibers of squalene treated rat showing moderate increase in size (black arrow), with significant extended nuclei (red arrow) and normal endomysium with collagen (yellow arrow), (H and E,10X).

While photomicrograph for a longitudinal section of skeletal muscle fibers of statin and squalene treated rat from fifth group showing reverse histological picture resembling control, parallel muscle fibers with distinct striations and nuclei forming a nuclear chain, (H and E,10X), (Figure 9). The figure (10) group photomicrograph for a transverse section of skeletal muscle fibers of statin and squalene treated rat shows a reverse histological picture resembling control, muscle fibers with distinct boundaries and acidophilic sarcoplasm, nuclei is peripherally located and significant collagen fibers in endomysium, (H and E, 10X).

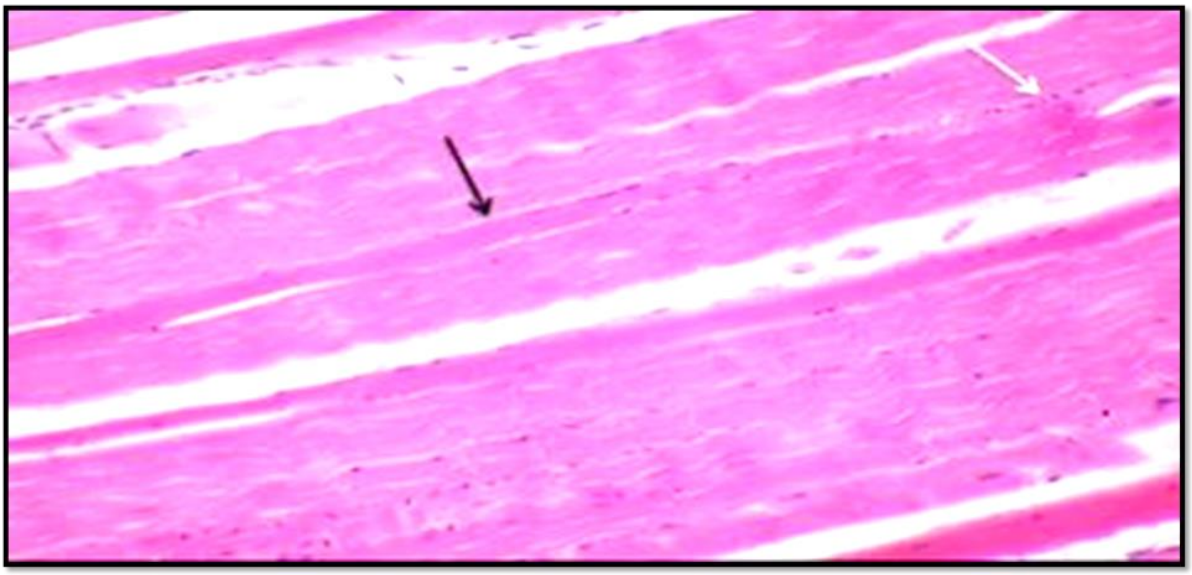

Figure (9): Photomicrograph for a longitudinal section of skeletal muscle fibers of statin and squalene treated rat showing reverse histological picture resem-

bling control, parallel muscle fibers with distinct striations (black arrow) and nuclei forming a nuclear chain (white arrow), (H and E,10X).

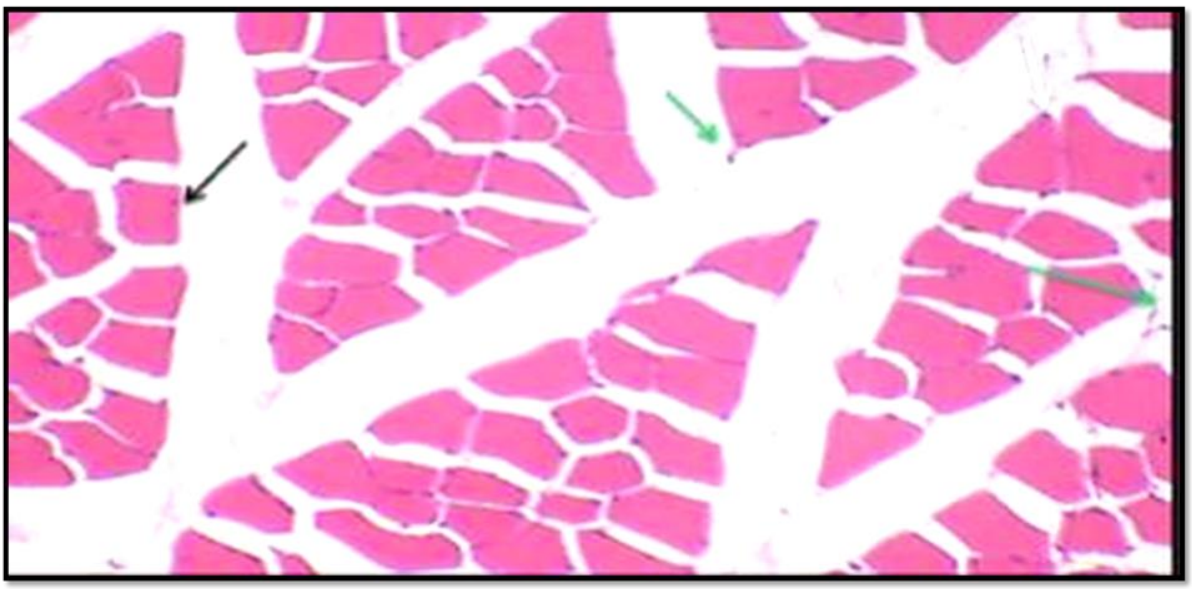

Figure (10): Photomicrograph for a transverse section of skeletal muscle fibers of statin and squalene treated rat showing reverse histological picture resembling control, muscle fibers with distinct boundaries and acidophilic sarcoplasm (black arrow), nuclei is peripherally located (white arrow) and significant collagen fibers in endomysium (green arrow), (H and E, 10X).

We conclude from the previous study the effect of squalene supplements on rats treated with statin to avoid side effect and protecting the muscles from damage caused by statin.

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