

## Histological Studies on Skeletal Muscles of Albino Rats under the Effect of Atorvastatin

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### ABSTRACT

**Introduction:**The purpose of this study was to evaluate the effect of atorvastatin on the structure of skeletal muscles in male albino rats and the possibility of recovery of any changes in the muscles.

**Drug used:** Atorvastatin (Lipitor 20mg) tablets.The daily single oral dose was 1.6 mg /kg body weight.

**Experimental animals:** Twenty five adult local strain male albino rats with average weight of 160 gm were chosen, divided into five equal groups and fed on ordinary rat diet: Group I (control group), Group II treated with atorvastatin (1.6 mg/kg/b.wt/day) for two weeks, Group III treated with atorvastatin for two weeks then stopped receiving treatment for two weeks (recovery group 1), Group IV treated with atorvastatin for four weeks and Group V treated with atorvastatin for four weeks then stopped receiving treatment for four weeks (recovery group II).

**Results:** Administration of atorvastatin for long duration resulted in some sort of myotoxic structural changes and apoptosis as evident by deformity in the mitochondria , lack of striation , degeneration of nuclei and splitting of muscle fibres in the adult male albino rat skeletal muscle.

**Conclusions:**Atorvastatin (statin) has many adverse effects on the skeletal muscle tissues with treatment for long duration (Group IV). So, patients should avoid the possible side effects.

**Keywords:** Albino Rats,Atorvastatin drug,skeletal muscles.

### INTRODUCTION

**Hyperlipidemia** is a metabolic disorder characterized by excess of lipid substances as cholesterol and triglycerides, in the blood. It is also called hyperlipoproteinemia because these lipid substances travel in the blood attached to proteins [1].Its complications such as atherosclerosis , myocardial infarction, stroke and peripheral vascular diseases remain important reasons of mortality and morbidity in industrialized and developing countries. Therefore, it is important to develop strategies for treating and preventing hyperlipidemia and its complications [2]. Drugs commonly used to treat high cholesterol level includes atorvastatin.

Atorvastatin is one of a group of drugs called 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, or "statins.". It reduces the levels of "bad" cholesterol (low-density lipoprotein, or LDL) and triglycerides in the blood, while increasing levels of "good" cholesterol (high-density lipoprotein, or HDL) [3]. These are usually the drugs of choice inspite of their side effects. They are easy to take and have few interactions with other drugs. Side effects can include myositis , joint pain, stomach upset, and liver damage. People who are pregnant or have liver disease should not take statins [4].

In addition, atorvastatin was reported to induce apoptosis in vascular smooth muscles

but the available literature demonstrated the apoptotic effect of atorvastatin on the skeletal muscle are scarce [2].

### MATERIAL AND METHODS

#### A-Materials

##### 1-Drug used:

Atorvastatin (Lipitor 20mg) tablets was purchased from Pfizer company for pharmaceutical & chemical industries .The drug was dissolved in distilled water and given orally by a gastric tube [4].The daily single oral dose was 1.6 mg /kg b. weight [5]

##### 2- Experimental animals:

The present study was carried out on twenty five adult local strain male albino rats with average weight of 160 gm were chosen to be the model of the present study. Rats were randomly divided into five equal groups and fed on ordinary rat diet.

##### Animal grouping :

- **Group I:** Rats received no treatment and served as control group.
- **Group II:** Rats were subjected to treatment with atorvastatin in a dose of 1.6 mg/kg/b.wt/day for two weeks (treated group 1).
- **Group III:** Rats were subjected to treatment with atorvastatin in the same dose

for two weeks, then stopped receiving treatment for two weeks (recovery group I).

- **Group IV:** were subjected to treatments with atorvastatin with the same dose for four weeks (treated group II).

- **Group V:** Rats were subjected to treatments with atorvastatin with the same dose for four weeks, then stopped receiving treatment for four weeks (recovery group II).

## B- Methods

### I- Histological study:

**1-Light microscopic examination:** Slides were stained with Hematoxylin&Eosin, Iron hematoxylin and P.A.S stains and examined using a light microscope.

**2- Electron microscopic examination :** The chosen regions were cut with glass knives for ultrathin sections (60-70 nm) which mounted on copper grids [6]. The ultra thin section poststained with uranyl acetate and lead citrate and examined in a Joel TEM CS 100.

### II- Laboratory study:

At the end of the experimental period, rats were anesthetized by ether and venous blood samples were withdrawn from the retro-orbital plexus by heparinized capillary tubes. Estimation of LDL (Low Density Lipoprotein) value was done. [7].

### III -Statistical analysis :

The Statistical data included were expressed as mean, standard error (SE $\pm$ ) standard deviation (SD  $\pm$ ) & the student T test was used to elucidate the differences between the treated groups and control group [8]. The obtained data were analysed by using SPSS software (V.15).

## RESULTS

### Group I (Control Group):

#### Light microscopic results

**1) Hematoxylin and Eosin:** Examination of H&E stained sections of the Lattismus dorsi muscle of the control group revealed the normal structure of skeletal muscle (Fig. 1).

**2) Iron hematoxylin stain:-**Examination of Iron Hx. of the control group revealed the normal structure of skeletal muscle (Fig.2).

**3) Periodic acid Schiff (PAS):** In PAS stained sections, two types of muscle fibers could be recognized. few fibers showed intense PAS+ve reaction while others had faint PAS+ve reaction, (Fig. 3).

**4) Semi thin sections (toludin blue ):** Showed normal striation in two types of fibres (dark and pale) with elongated peripheral nucleus (Fig. 4).

**Electron microscopic results:** The sarcoplasm noticed to be filled with myofibrils

which were arranged parallel to the longitudinal axis of the myofiber. The myofibrils showed regular arrangement of alternating light (I) and dark (A) bands. A pale narrow region, the H band, could be seen transecting the A band with a dark M line within it. Z line was seen bisecting the light band. Oval nuclei were seen under the sarcolemma, with their heterochromatin distributed along the inner surface of the nuclear envelope and one or two nucleoli could be noticed (Fig. 5 &6).

- **Laboratory assessment :** The main value of LDL in normal group was 45 ug/dl.

**GroupII** subjected to treatments with atorvastatin in a dose of 1.6 mg/kg/body.wt/day for two weeks.

### Light microscopic results

**1)Hematoxylin and Eosin :** Muscle showed mild focal histological changes. Some fibers showed variations in size and shape. Some nuclei were elongated in shape and internal in position. Splitting of some fibers were also observed (Fig. 7).

**2)Iron haematoxlin stain:** longitudinal sections in group II rat skeletal muscle showed splitting of the myofibers, Mitochondria and nuclei were blue to black, and some of the peripheral elongated nuclei disappeared and striation was lost in some muscle fibers (Fig. 8).

**3)Periodic acid Schiff (PAS):**Rat skeletal myofibres shows moderate decrease in PAS+ content (Fig. 9).

**4)Semi thin sections (toludin blue):**(Fig.10) Skeletal myofibril shows peripheral elongated nuclei with minimal loss of striation in the dark fibres and complete loss of striations in the pale type

### Electron microscopic results:

**Electron microscopic** examination of rat skeletal myofibril showed few areas of myofibrillar loss and few mitochondria vacuolation, intact outer mitochondrial membrane and minimal destruction of its cristae. (Fig. 11).

### Statistical results :

a) Rats receiving atorvastatin for two weeks in group II revealed a significant decrease ( $P < 0.05$ ) in diameter of skeletal muscle compared to those group I control rats (Table 1& Fig. 24).  
b) Rats receiving atorvastatin for two weeks in group II revealed a significant decrease ( $P < 0.05$ ) in P.A.S positive reaction compared to those of group I control rats (Table 2& Fig. 25).

c) Rats receiving atorvastatin for two weeks in group II revealed a non significant change in serum L.D.L level (Table 3& Fig. 26) compared to those of group I control rats .

**Group III** :Subjected to treatment with atorvastatin in a dose of 1.6 mg/kg/b.wt/day for two weeks then stopped receiving treatment for two weeks.

#### Light microscopic results

**1) Hematoxylin and Eosin** : Longitudinal sections showed intact muscle fibers. occasional areas of cellular infiltration were noticed (**Fig. 12**).

**2)Iron hematoxylin stain:-**Longitudinal sections in skeletal muscle Group III showed cellular infiltration and splitting of myofibers. fragmentation of the sarcoplasm, the dense central nuclei striation is lost in some muscle fibers(**Fig. 13**)

**3)Periodic acid Schiff (PAS)** :Rat skeletal muscle showed more decrease in PAS+ve content (**Fig. 14**).

#### Statistical results

a) Significant decrease ( $P < 0.05$ ) in diameter of skeletal muscle in comparison with rats in group I control (Table 1& Fig. 24).

b) Significant decrease ( $P < 0.05$ ) in P.A.S positive reaction in comparison with those in group I control rats (Table 2& Fig.25).

c) Non significant change in serum L.D.L level compared to those of group I control rats (Table 3& Fig. 26).

**Group IV** :Subjected to treatments with atorvastatin in adose of 1.6 mg/kg/b.wt/day for four weeks.:

#### Light microscopic results

##### 1)Hematoxylin and Eosin :

There was mild focal changes observed in sections. Muscle fibers showed irregular variation in size with rounding of some of them. Nuclei were internal in position rather than peripheral and some appeared rounded in shape rather than oval Splitting of some fibers was also observed which appeared as a transverse invagination or complete separation. In addition, few muscle fibers appeared atrophied (**Fig. 15**).

**2) Iron hematoxylin stain:-**Longitudinal sections in skeletal muscle showed lost striations in some muscle fibers , fragmentation of the sarcoplasm , and the dense central nuclei (**Fig. 16**).

**3)Periodic acid Schiff (PAS)**: Skeletal showed marked decrease in PAS+ve contents (**Fig. 17**).

**4) Semi thin sections (Toludin blue )**: Skeletal myofibril showed loss of striation of fibers and vacuolation of muscle fibers of both types (**Fig. 18**).

#### Electron microscopic results :

Skeletal myofibril showed areas of yofibrillar loss with hypercontactedmyofibers and mitochondria showing disfigured vacuolated appearance with beaded outer membrane and destruction of most of cristae (**Fig.19 and 20**).

#### Statistical results

a)There was significant decreas ( $P < 0.05$ ) in diameter of skeletal muscle in comparison with group I control rats (Table 1& Fig. 24).

b) There was significant decrease ( $P < 0.05$ ) in P.A.S reaction in comparison with group I control rats (Table 2& Fig. 25).

c)There was a non significant change in serum L.D.L level compared to those of group I control rats (Table 3& Fig. 26).

**Group V** :Subjected to treatments with atorvastatin in adose of 1.6 mg/kg/b.wt/day for four weeks then stopped receiving treatment for four weeks.

#### Light microscopic results

**1)Hematoxylin and Eosin** : The muscle fibers were almost similar to the control group but showed regenerating areas with peripherally located nuclei and limited splitting of myofibers .Striated nature of muscle fibers was resumed again (**Fig. 21**)

**2)Iron hematoxylin stain:-** Skeletal muscle showing muscle fibers with peripherallylocated nuclei Mitochondria and nuclei blue to black, limited splitting of myofibers& striated nature of muscle fibers is resumed again .(**Fig. 22**)

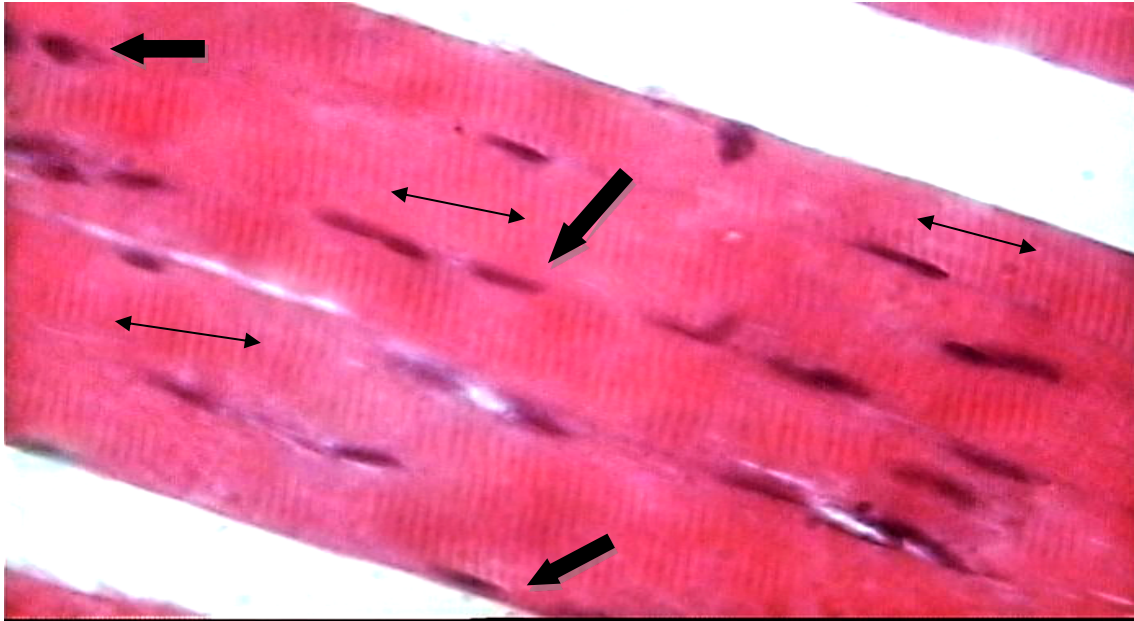
**3) Periodic acid Schiff (PAS)**: Skeletal muscle showed marked restoration of PAS+ve content and reappearance of striation in a wide range (**Fig. 23**).

#### Statistical results

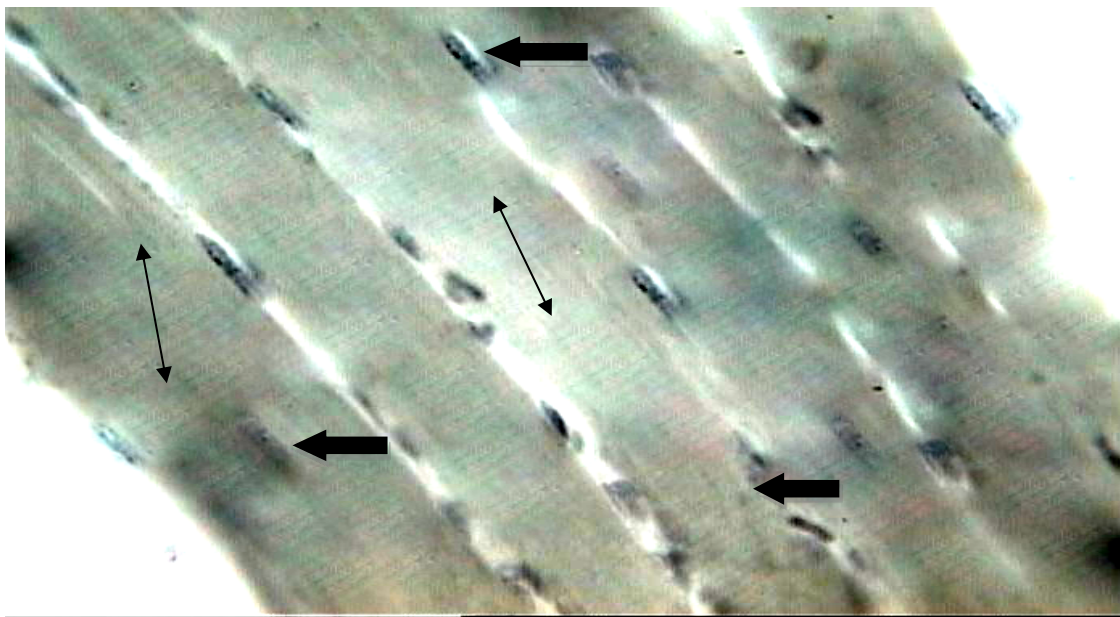
a) There was significant decrease (  $P < 0.05$ ) in diameter of skeletal muscle in comparison with group I control rats (Table 1& Fig. 24).

b) There was significant decrease (  $P < 0.05$ ) in P.A.S reaction in comparison with group I control rats (Table 2 & Fig. 25).

c) There was a non significantchange in serum L.D.L level compared to those of group I control rats (Table 3& Fig.26).

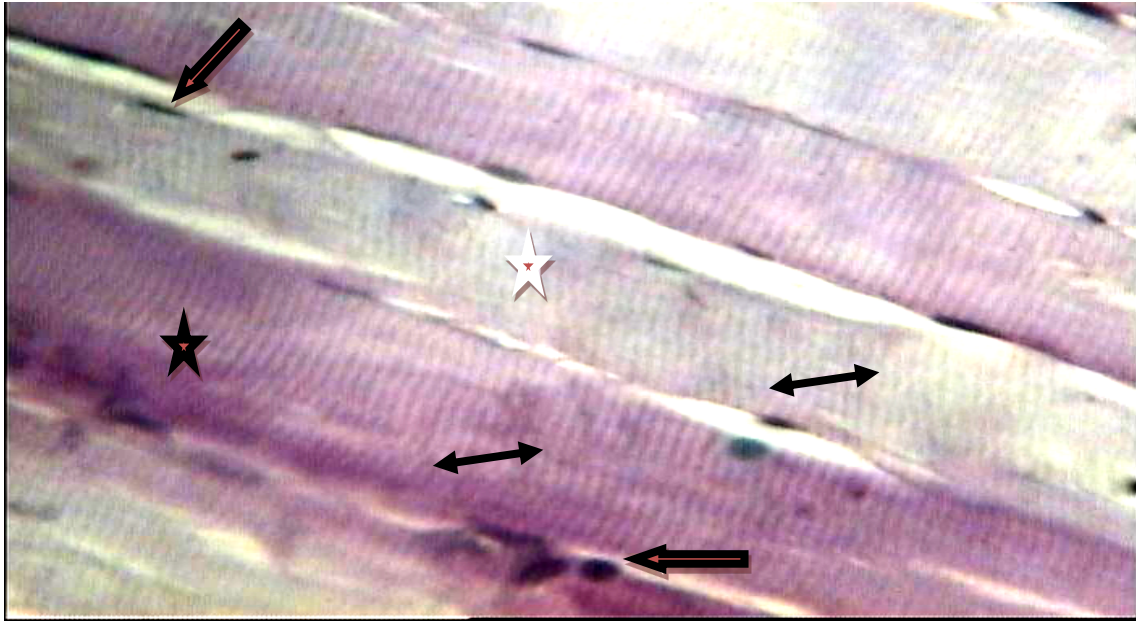


(Fig. 1): A photomicrograph of longitudinal section of skeletal muscle in control group showing multiple peripheral elongated nuclei (**thick arrow**) and striated character of normal muscular structure (**crossed arrow**) (Hx& E X 400).

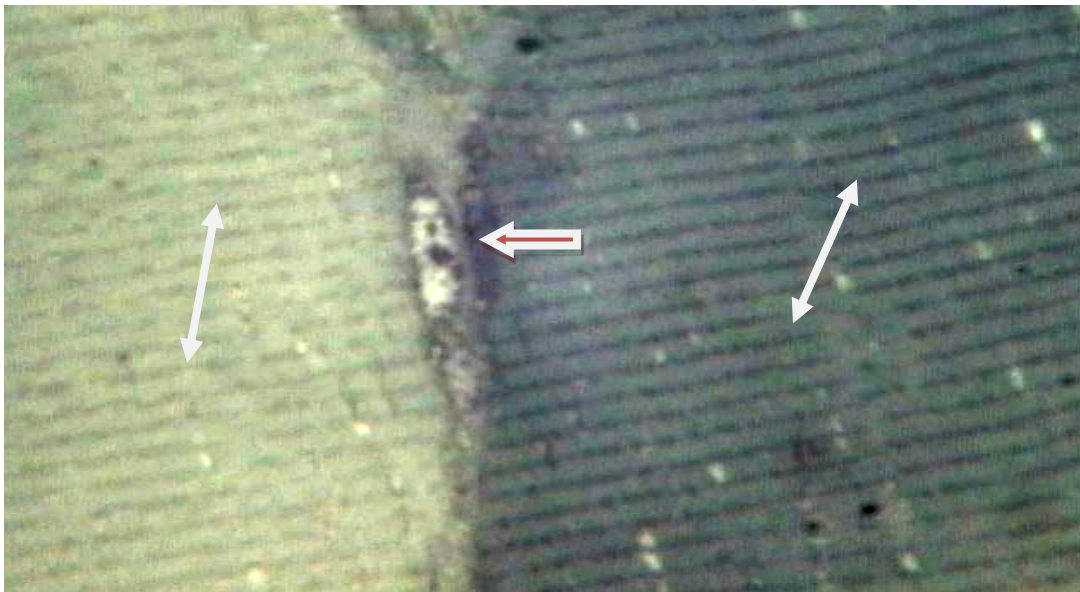


(Fig.2): A photomicrograph of LS in stained section of the Lattismusdorsi muscle of the control group revealed the normal structure of skeletal muscle showing striated character of normal muscular structure (**crossed arrow**) Mitochondria and Nuclei , blue to black (**thick arrow**) (iron HxX 400).



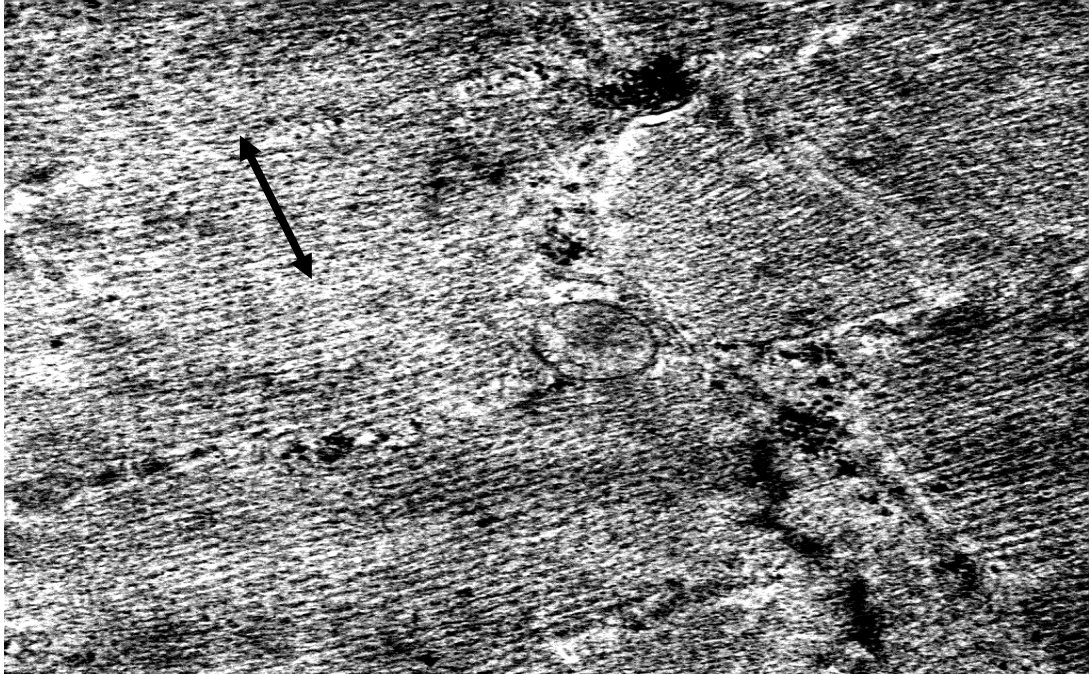


(**Fig. 3**): A photomicrograph of longitudinal section of skeletal muscle in control group showing parallel muscle fibers with multiple peripheral elongated nuclei (**thick arrow**) with normal distributions of PAS stain in 2 types of fibres (type IIB) pas +ve (**black star**) and (type I) pas – vefibres (**white star**)& appearance of normal transverse striations(**crossed arrow**). (**PAS X 400**) .

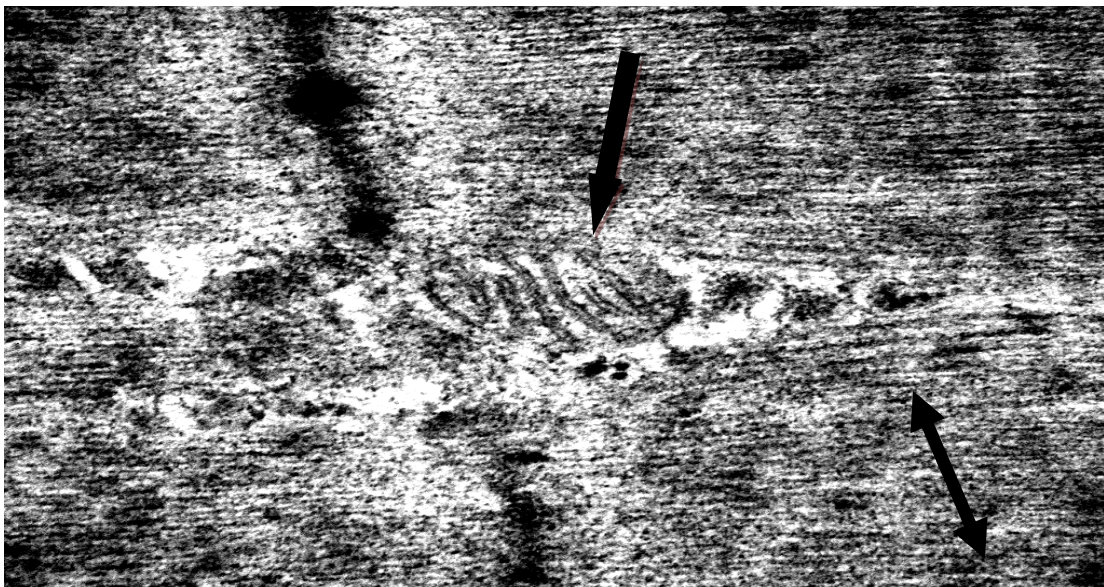


(**Fig. 4**): A photomicrograph of semithin section of skeletal muscle in control group showing myofibril with normal striation in two types of fibres (**crossed arrow**) and elongated peripheral nucleus (**thick arrow**) (**Toluidin blue X 1000**).



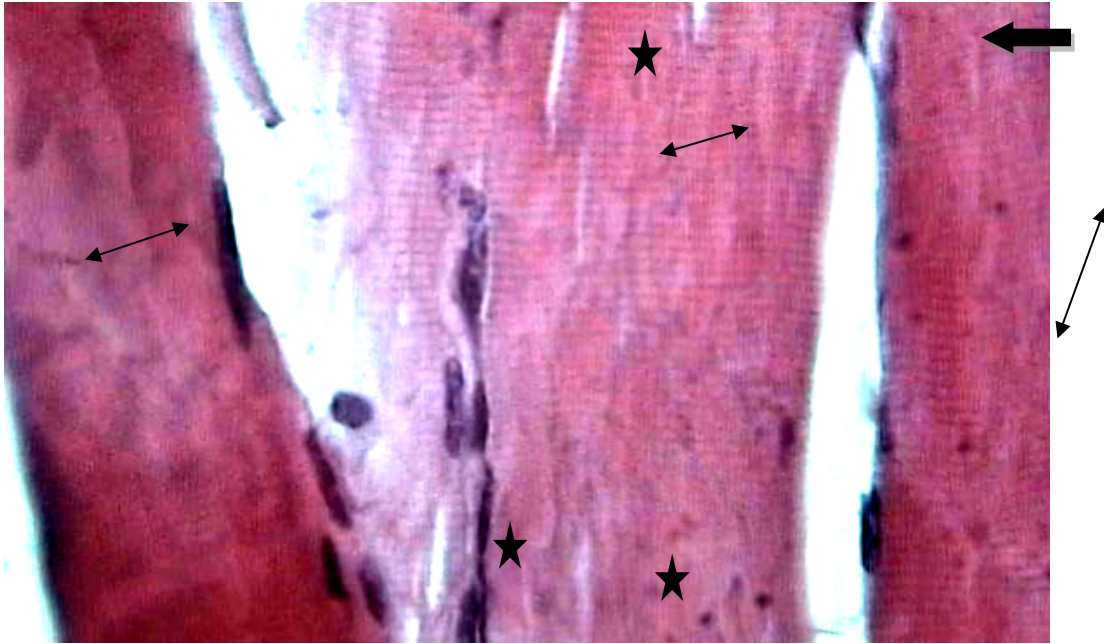


(Fig. 5): An electron photomicrograph of control rat skeletal myofibril shows normal striation (crossed arrow)(X50000).

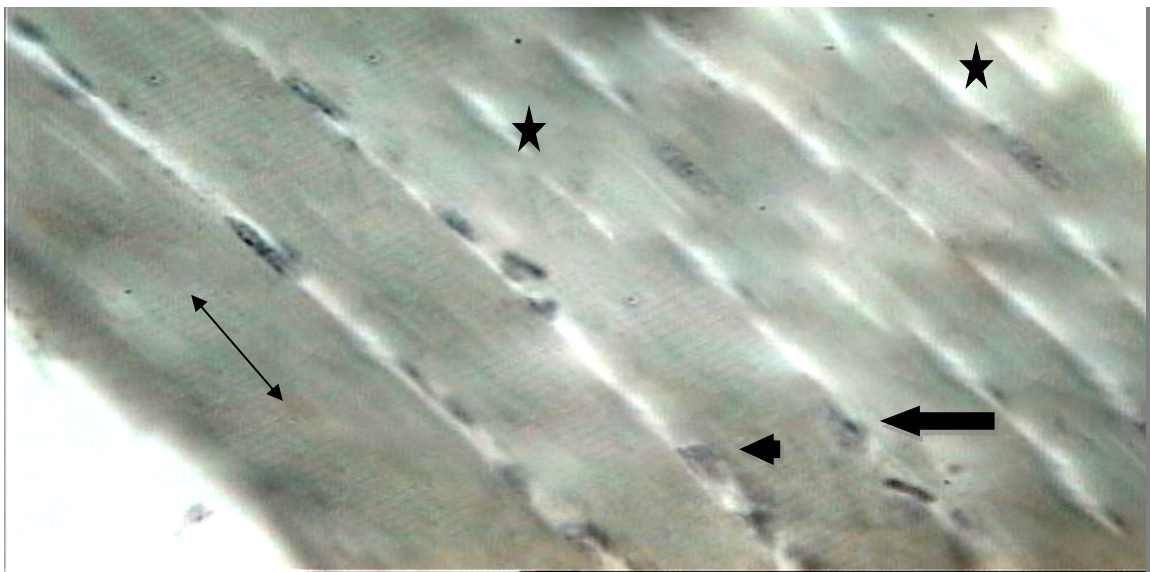


(Fig. 6): An electron micrograph of a transverse section of latissimus dorsi muscle of a control rat of group1 showing subsarcolemmal normal mitochondria (thick arrow), and normal striations of myofibres(crossed arrow)(X 100000).

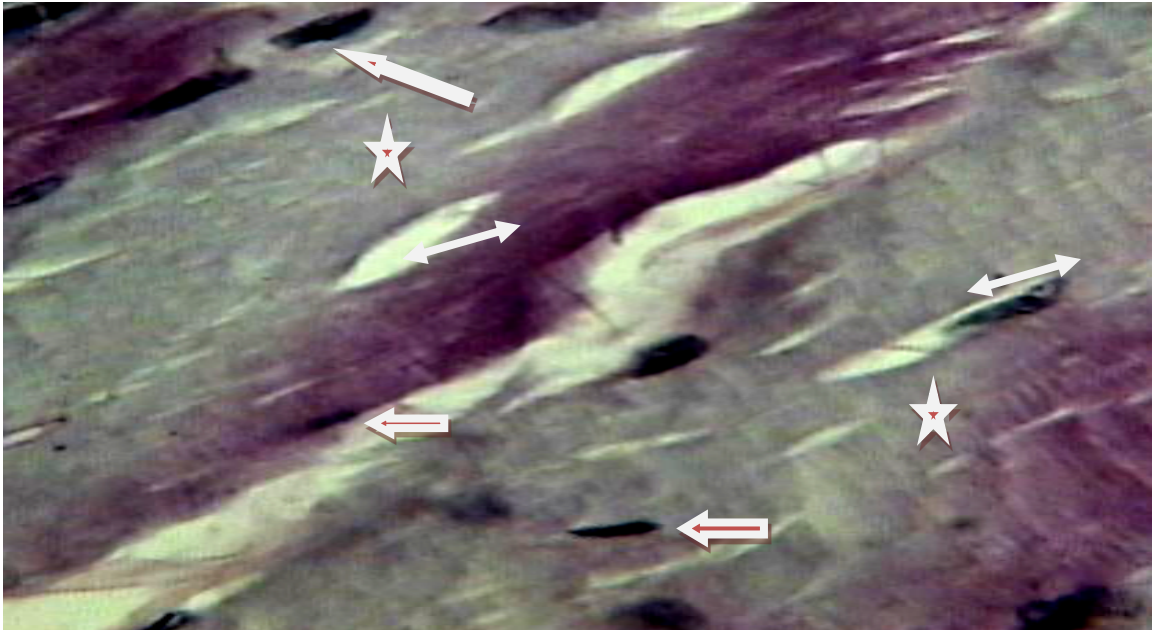




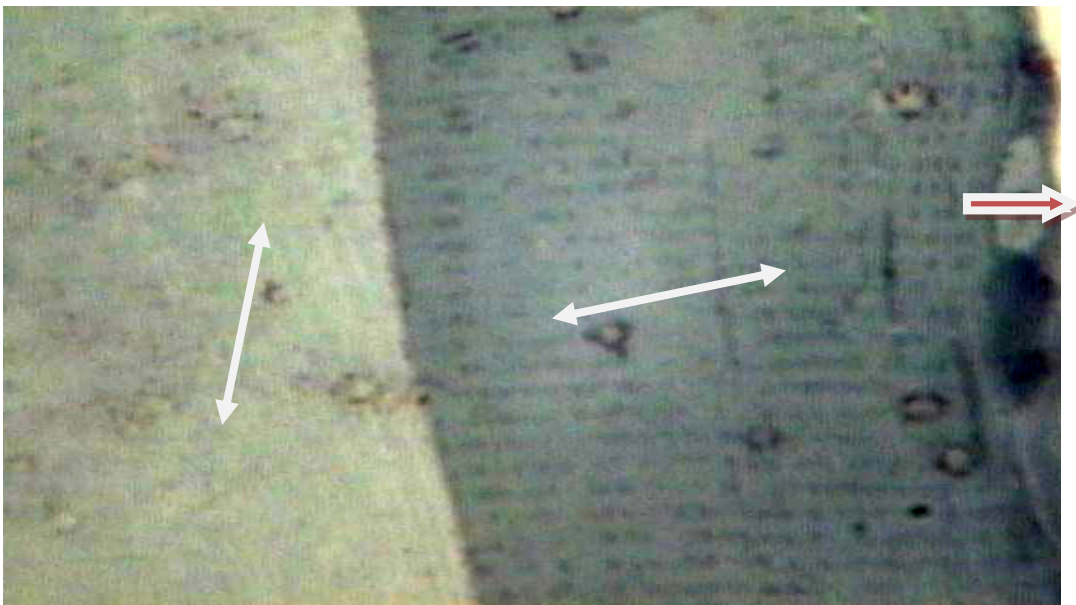
(Fig.7):A photomicrograph of longitudinal section of skeletal muscle in groupII showing splitting of the myofibers(**star**), some of peripheral elongated nuclei disappeared (**thick arrow**) and striation is lost in some muscle fibers (**crossed arrow**) (Hx&EX 400).



(Fig.8):A photomicrograph of longitudinal section of skeletal muscle in group II showing splitting of the myofibers(**star**), Mitochondria and Nuclei , blue or black (**arrow head**) some of peripheral elongated nuclei disappeared (**thick arrow**) and striation is lost in some muscle fibers (**crossed arrow**) (Iron HxX 400).

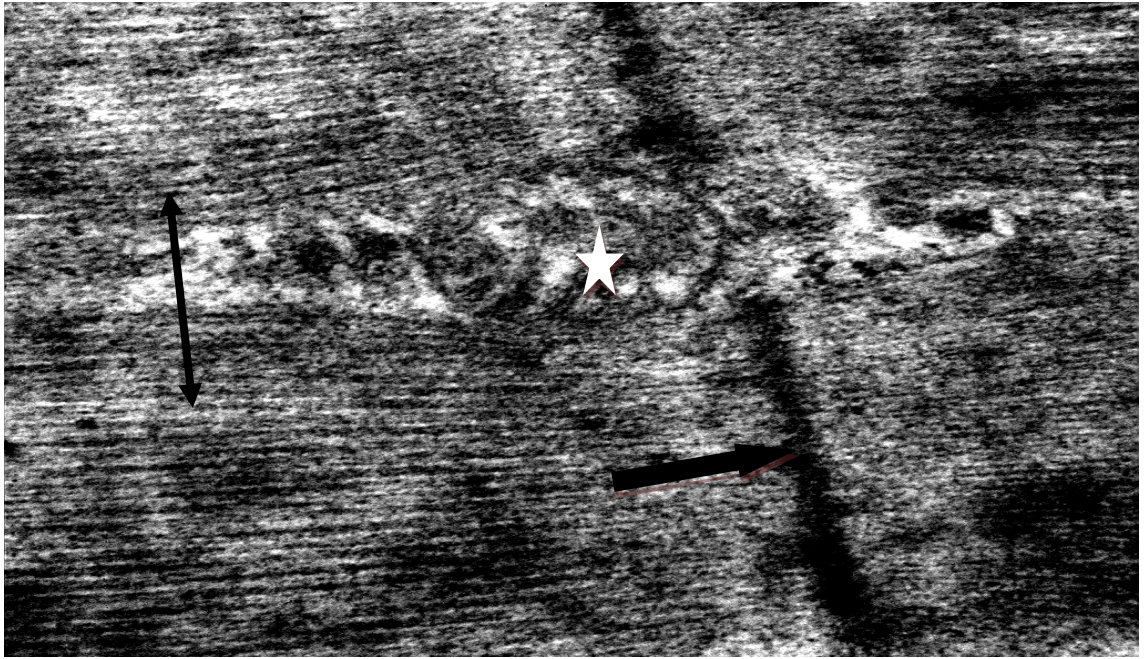


(**Fig. 9**):A photomicrograph of longitudinal section of skeletal muscle fibers in group II showing multiple peripheral elongated nuclei dense central nuclei , disappearance of some peripheral nuclei (**thick arrow** ) moderate decrease in PAS+ content (**star**) more splitting of (type I) myofibres (**crossed arrow**) (PAS X 400).

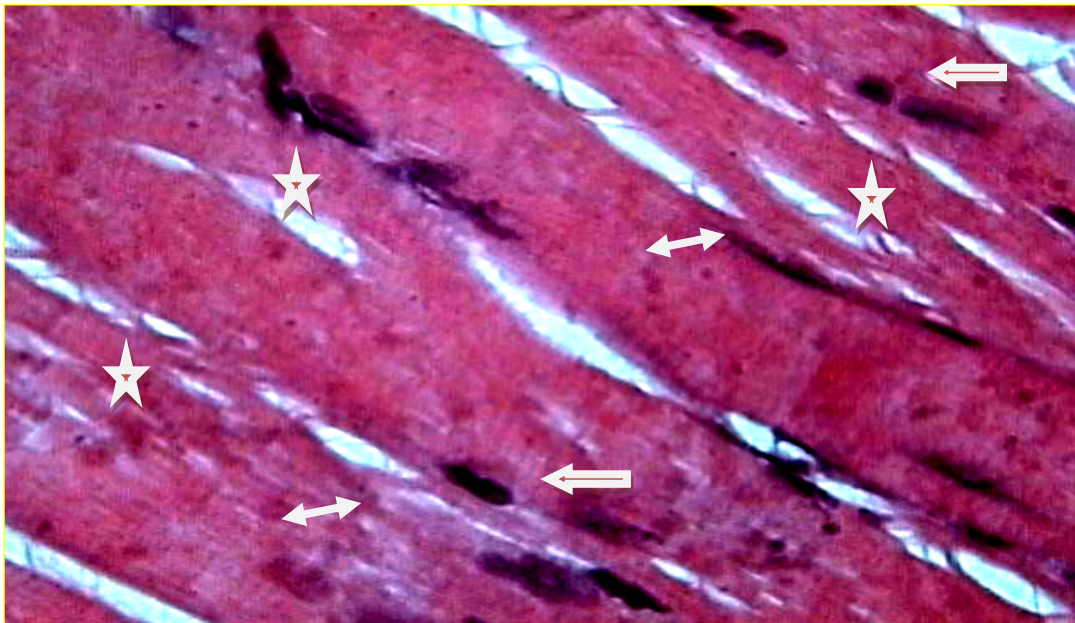


(**Fig. 10**):A photomicrograph of semithin section of skeletal muscle in group II showing peripheral elongated nucleus(**thick arrow** ) with minimal striation loss in the dark fibres and complete loss of striations in the pale type (**crossed arrow**)(Toluidin blue X 1000).



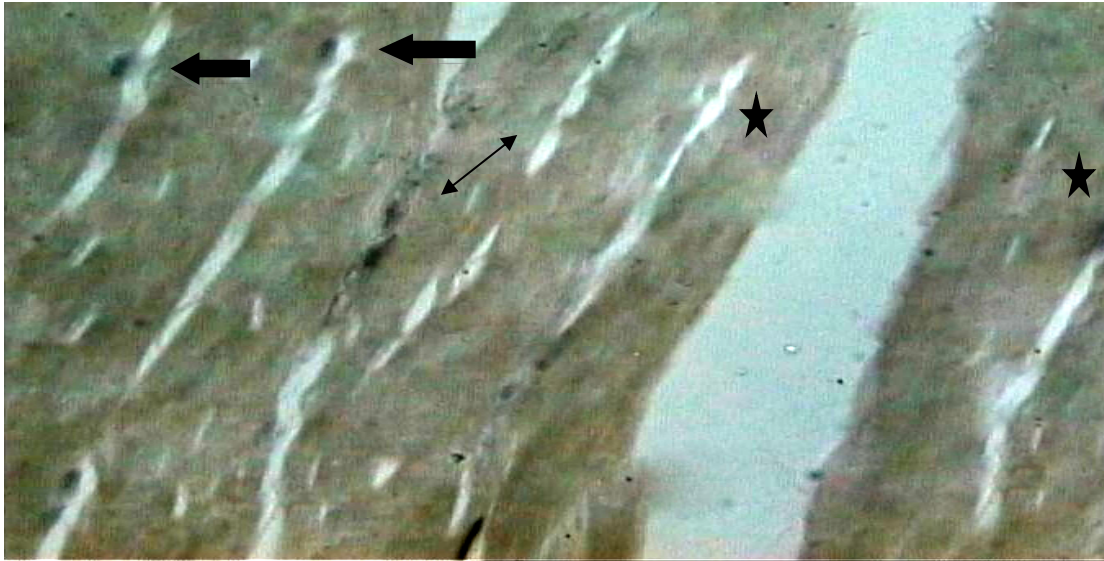


(**Fig.11**): An electron photomicrograph of skeletal muscle myofibril of group II showing few areas of myofibrillar loss (**crossed arrow**) and minimal mitochondrial vacuolation(**star**) with intact outer mitochondrial membrane & minimal destruction of its cristae and normal sarcomere (**thick arrow**). (X 70000).

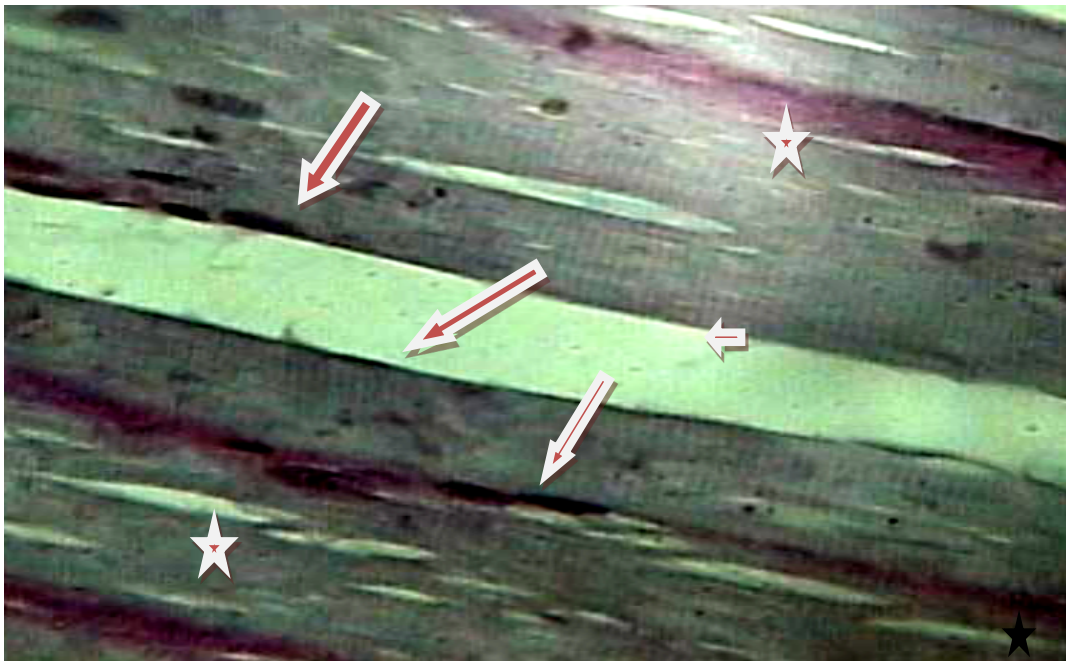


(**Fig.12**): A photomicrograph of longitudinal section of muscle fibers in group III showing splitting of the myofibers (**star**) in addition to focal areas also striation stile lost in some muscle fibers(**crossed arrow**) with sarcomere fragmentation (Hx&E X 400).

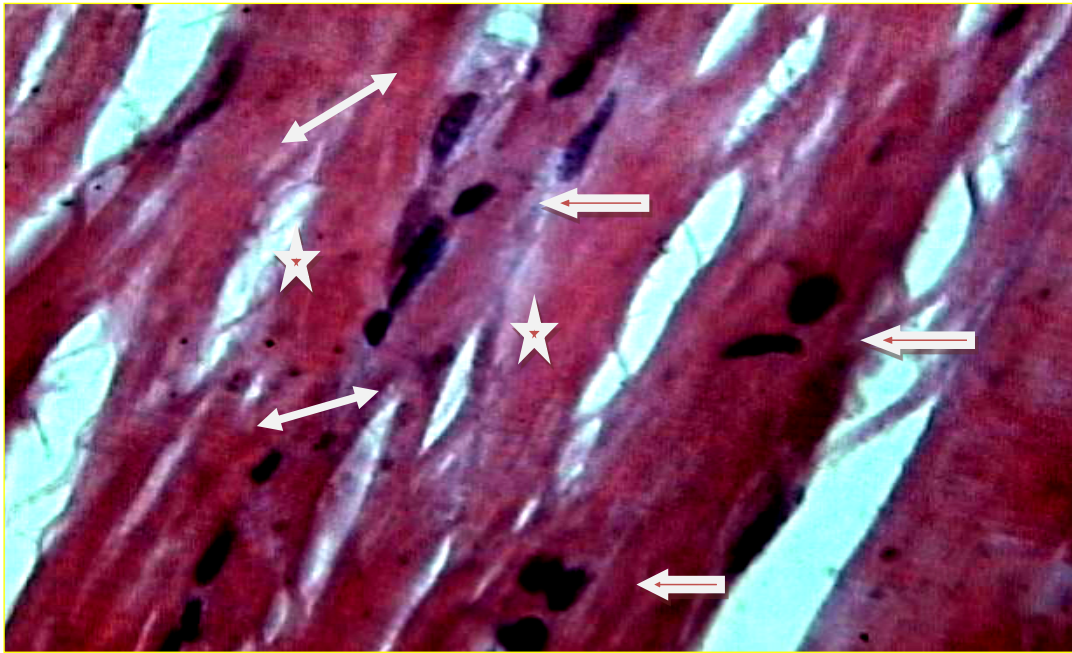




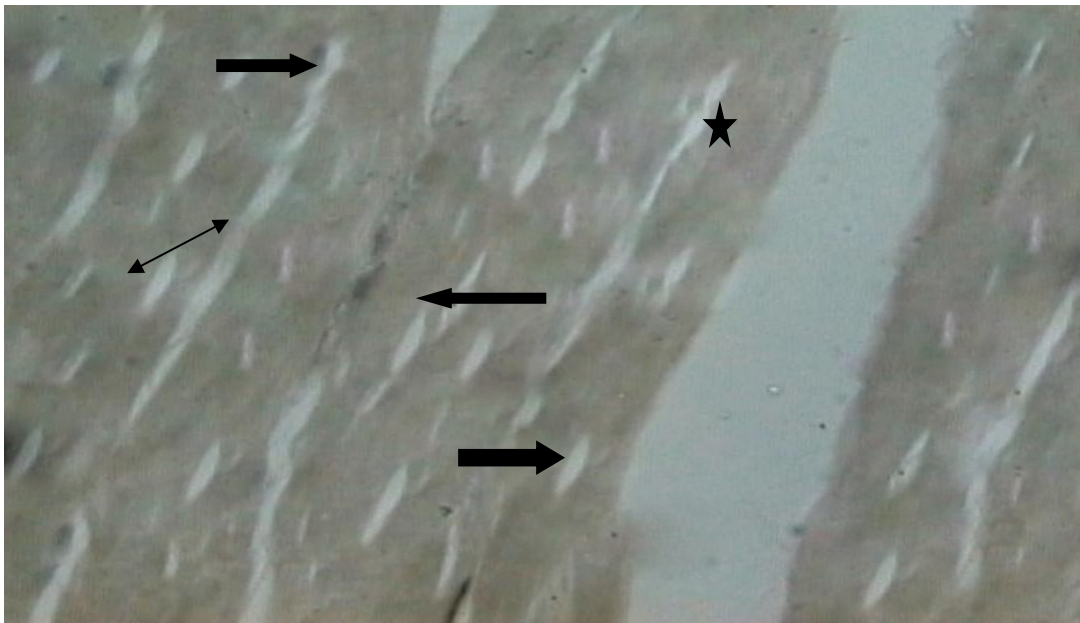
(Fig. 13): A photomicrograph of longitudinal section of muscle fibers of **group III** showing splitting of myofibers. (stars), dense central nuclei (**thick arrow**) striation is lost in more muscle fibers (**crossed arrow**) (Iron Hx X 400.).



(Fig.14): A photomicrograph of longitudinal section of muscle fibers in group III showing very few peripheral elongated nuclei, disappearance of many peripheral nuclei (**thick arrow**) decrease in PAS+ content (**star**) limited splitting of myofibers (PAS X 400).

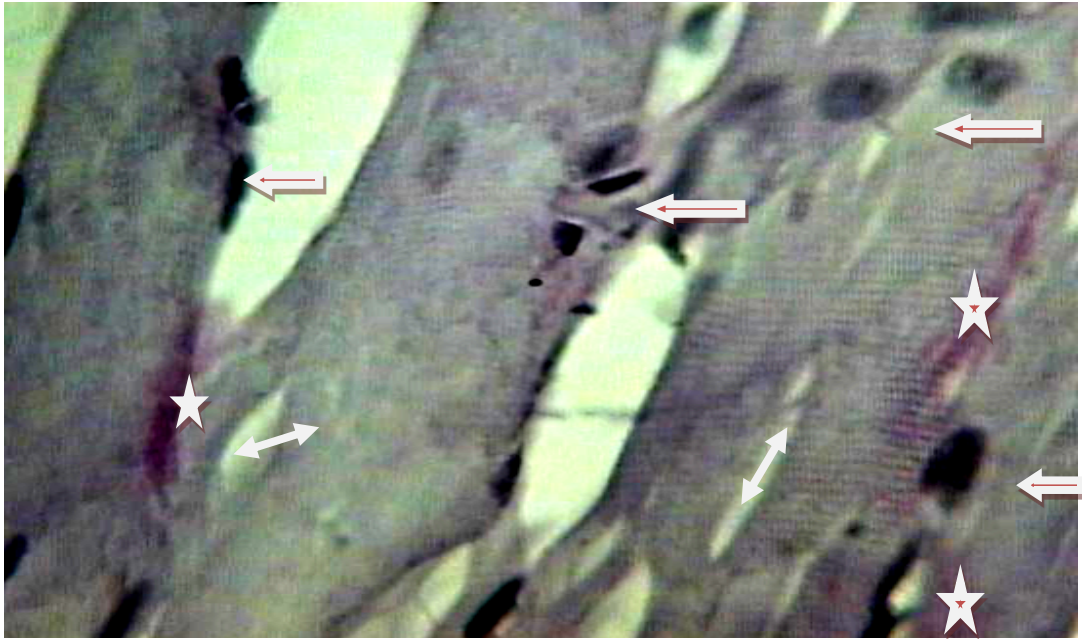


**(Fig.15):** A photomicrograph of longitudinal section of muscle fibers in group IV showing marked splitting of the myofibers (**star**) and fragmentation of the sarcoplasm. Loss of striation (**crossed arrow**) and nuclear degeneration with dense centrally located nuclei (**thick arrow**) (Hx&EbX 400).

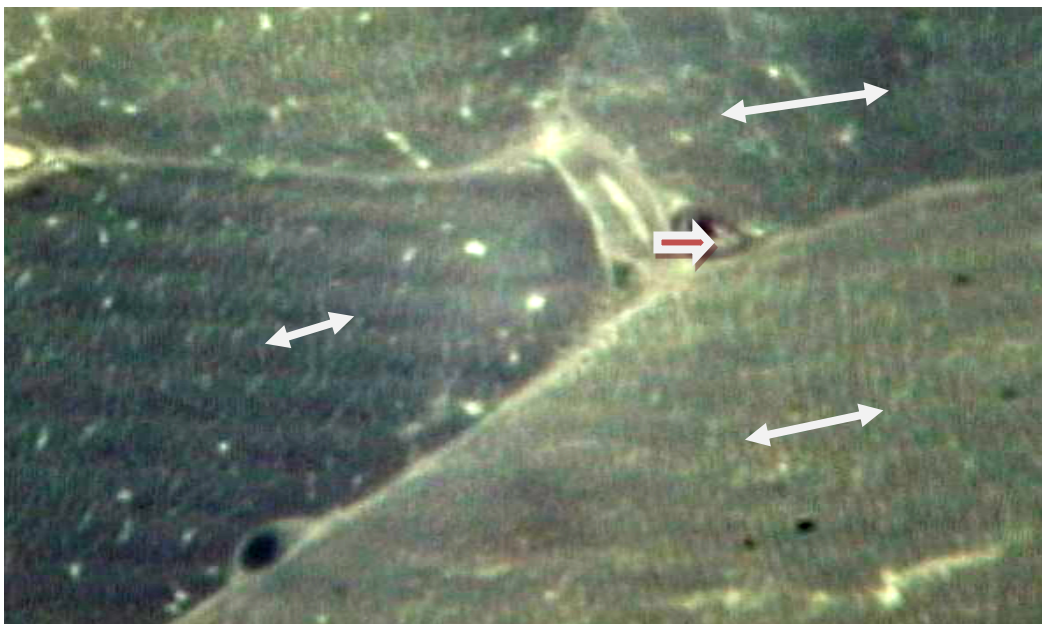


**(Fig.16):** A photomicrograph of longitudinal section of muscle fibers in group IV showing striation is lost in some muscle fibers (**crossed arrow**). Note the fragmentation (**star**) of the sarcoplasm, the dense central nuclei and the remnants of nucleivacuolation of mitochondria(**thick arrow**)(Iron HxX 400).

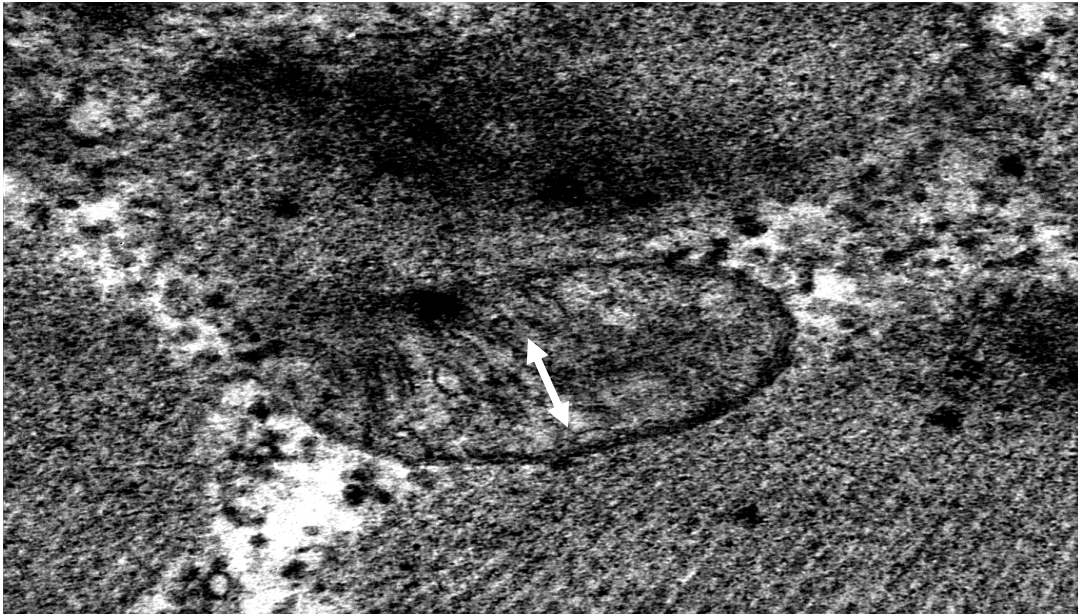




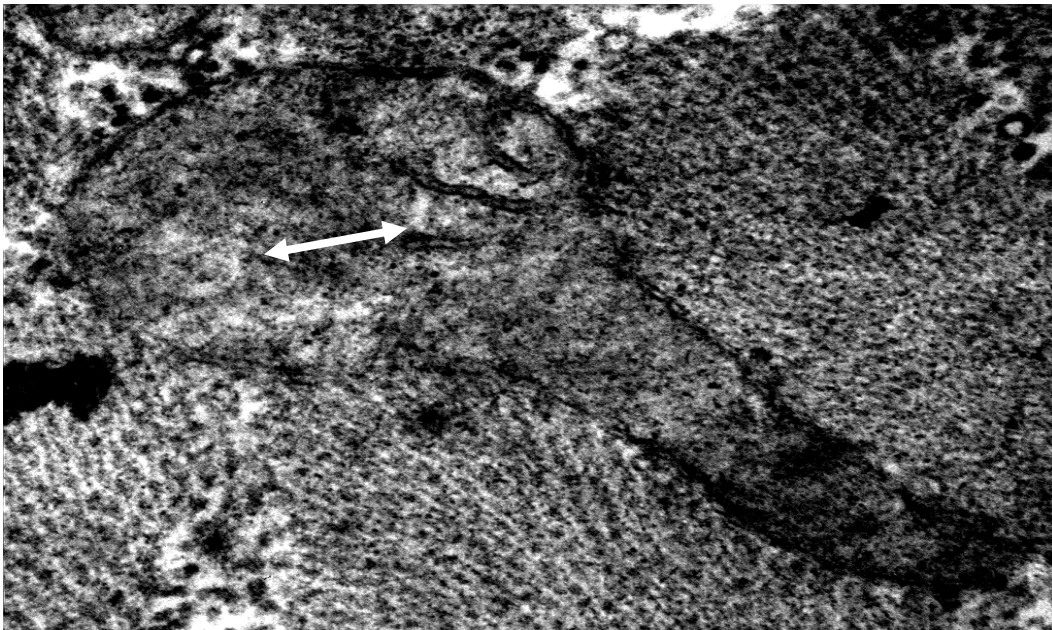
(Fig.17):A photomicrograph of longitudinal section of muscle fibers in group IV showing few peripheral elongated nuclei, dense central nuclei , disappearance of some peripheral nuclei (**thick arrow** ) and marked decrease in PAS+ content (**star**) (PASX 400).



(Fig. 18):A photomicrograph of semithin section of muscle fibers in group IV showing peripheral elongated nucleus(**thick arrow** ) , loss of striation and vacuolation of muscle fibers (**crossed arrow**) (Toluidin blue X 1000).

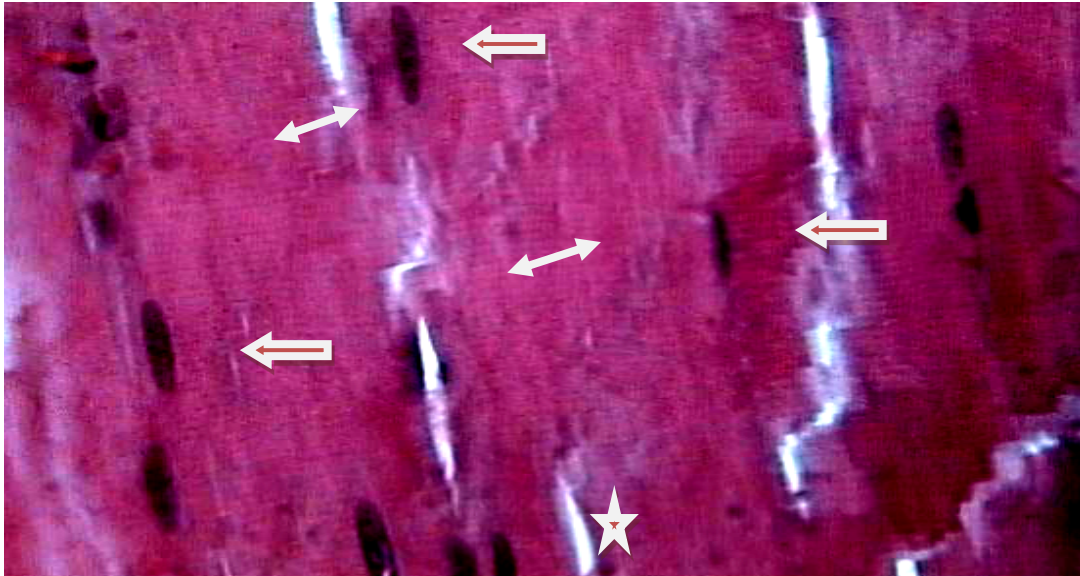


**(Fig.19):** An electron photomicrograph of muscle fibers of group IV showing areas of myofibrillar loss with hyper contacted myofibers and mitochondria vacuolation with destruction of most of cristae (crossed arrow). (X70000).

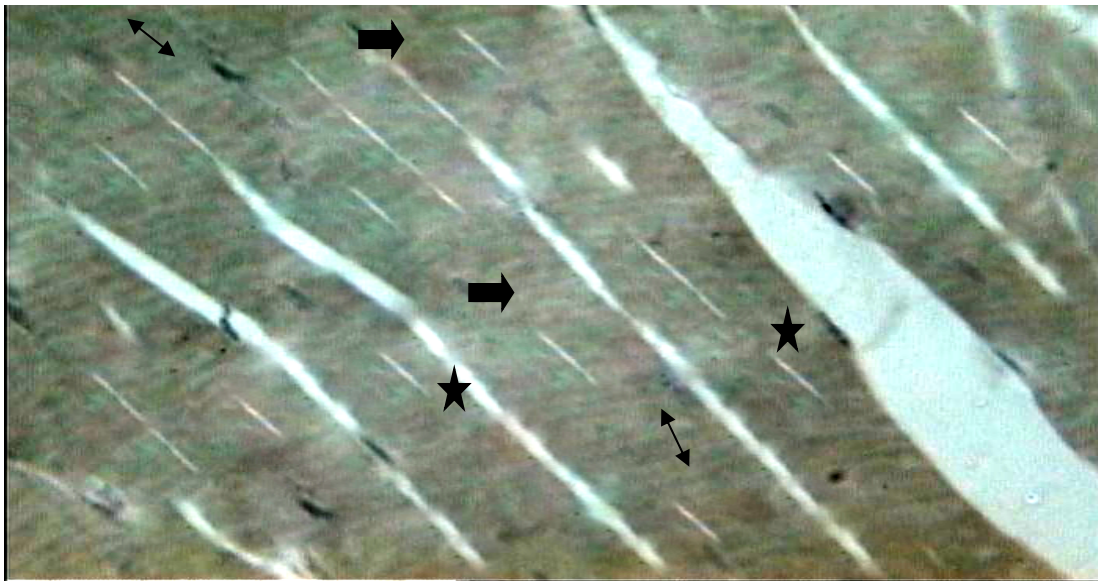


**(Fig. 20):** An electron micrograph of a transverse section of latisimusdorsi muscle of atreated rat of group IV showing disfigured vacuolated mitochondria (crossed arrow) with beaded outer membrane and destruction of most of cristae, (X 100000).



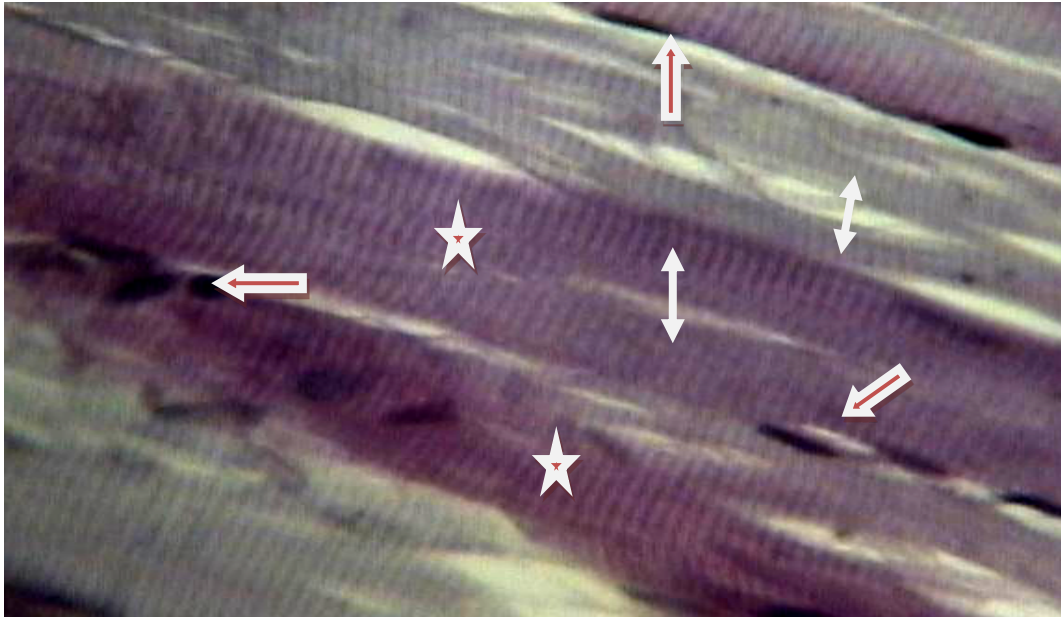


(Fig. 21): A photomicrograph of longitudinal section of myofibers in group V showing muscle fibers with peripherally located nuclei(**thick arrow**), limited splitting of myofibers (**star**) & striated nature of muscle fibers was resumed again (**crossed arrow**) (Hx&E X 400).



(Fig. 22): A photomicrograph of longitudinal section of myofibers in group V showing muscle fibers with centrally and peripherally located nuclei and Mitochondria, blue to black (**thick arrow**), limited splitting of myofibers (**star**) & striated nature of muscle fibers was resumed again (**crossed arrow**)(Iron Hx.X 400).

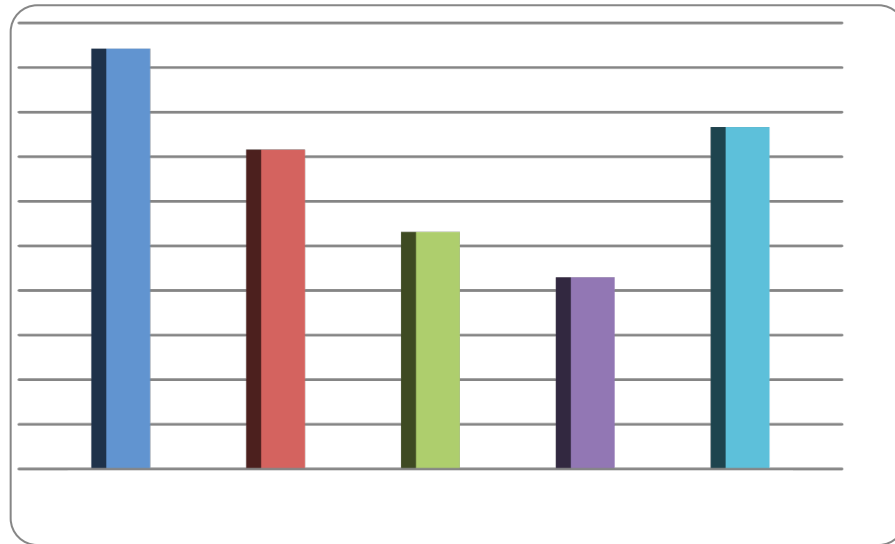




**(Fig. 23):** A photomicrograph of longitudinal section of myofibers in group V showing multiple peripheral elongated nuclei (**thick arrow**) restoration of PAS+ content (**star**) mild splitting of myofibers(**crossed arrow**)(PAS X 400).

	Group I	Group II	Group III	Group IV	Group V
	Control	Treated 2 weeks	Recovery 2 weeks	Treated I month	Recovery I month
Mean	0.34	0.3	0.27	0.25	0.31
N	10	10	10	10	10
SD±	0.04	0.03	0.07	0.05	0.04
P Value		0.05	0.05	0.05	0.05
Sgnificance		p<0.05 Sig.	p<0.05 Sig.	p<0.05 Sig.	p<0.05 Sig.

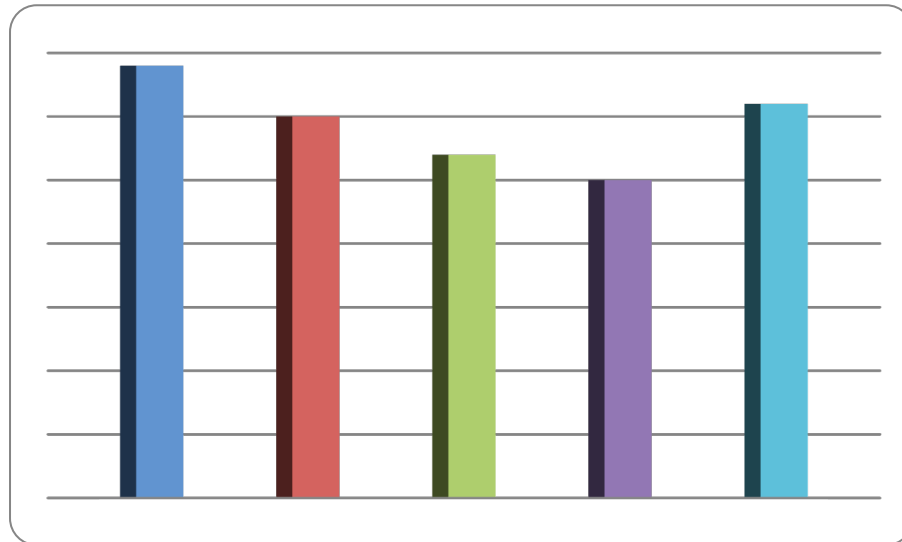
**(Table 1)** Changes in th diameter of skeletal muscles under the effect Atorvastatin



(Fig. 24) Histogram showing the changes in the diameter of skeletal muscles under the effect Atorvastatin in the different groups of the study

	Group I	Group II	Group III	Group IV	Group V
	Control	Treated 2 weeks	Recovery 2 weeks	Treated 1 month	Recovery 1 month
Mean	47.12	35.81	26.58	21.49	38.33
N	10	10	10	10	10
SD±	8.62	2.78	4.45	5.22	12.23
P Value		0.05	0.05	0.05	0.05
Significance		p<0.05 Sig.	p<0.05 Sig.	p<0.05 Sig.	p<0.05 Sig.

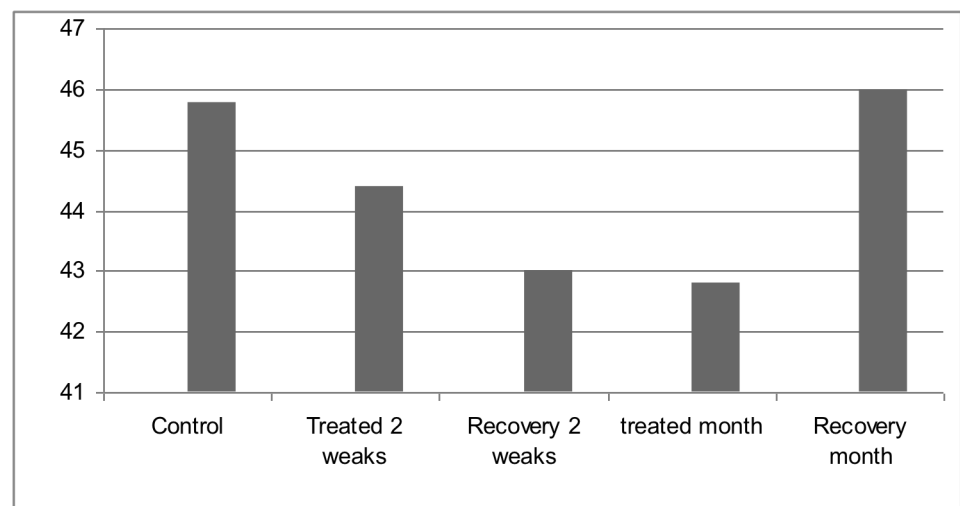
(Table 2) Changes in the optical density of skeletal muscles under the effect Atorvastatin



(Fig. 25) Histogram showing the changes in the optical density of skeletal muscles fibers under the effect Atorvastatin in the different groups of the study

Mean	Group I	Group II	Group III	Group IV	Group V
	Control	Treated 2 weeks	Recovery 2 weeks	treated month	Recovery month
Mean	45.80	40.20	43.80	43.20	46.00
SD±	11.08	11.34	9.23	9.42	9.19
SE±	3.50	3.59	2.92	2.98	2.91

(Table 3): Changes in the L.D.L value of skeletal muscles under the effect Atorvastatin in different groups.



(Fig. 26) Histogram showing the changes of the L.D.L in different groups under the effect Atorvastatin.

## DISCUSSION

Hyperlipidemia is a common metabolic disorder results from genetic predisposition interacting with an individual's diet. The best-known lipoproteins are LDL (low density lipoprotein) and HDL (high density lipoprotein). Excess LDL cholesterol contributes to the blockage of arteries, which eventually leads to heart attack [9].

Statins are the most widely used lipid-lowering agents and best tolerated drugs for treating Hyperlipidemia. By inhibiting the key enzyme in cholesterol biosynthesis (HMG-CoA) reductase in the liver This induces an increased hepatic uptake of plasma LDL with a reduction in circulating levels [10].

Hepatic, renal and muscular systems were reported to be affected during statins therapy. Generalized muscular aches are the commonest adverse effect in humans associated with statin therapy occasionally leading to a frank myotoxicity, which ranges from mild myopathy to frank rhabdomyolysis[11]. It was stated that statin-associated myotoxicity is complex, involving effects on cell membrane structure and function, mitochondrial dysfunction and impaired myocyte regeneration [12]. The present study aimed to know the effect of atorvastatin because of its worldwide.

The mitochondria as a main source of cellular energy are considered a regulatory center of apoptosis. It can release cytochrome C into the cytosol, which then forms an "apoptosome" with Apaf-1, ATP, and procaspase-9. Once the apoptosome is formed, procaspase-9 can cleave and activate itself into caspase-9. Caspase-9 can then cleave and activate procaspase-3, leading to apoptosis. This process is highly regulated. The Bcl-2 family of proteins was the first described to affect the release of cytochrome c. This family consists of several proteins, which are antiapoptotic or proapoptotic. For example, Bcl-2 and Bcl-XL protect against cytochrome c release and are therefore anti-apoptotic while Bax, Bak, Bad, and Bid favor cytochrome c release and are therefore proapoptotic[13].

Another regulatory protein that can regulate the release of cytochrome c release from the mitochondria is apoptosis repressor

with card (ARC) [14]. Upon stimulation, ARC can translocate from the cytosol to the mitochondrial membrane to inhibit the release of cytochrome c. Recent data show that ARC may prevent apoptosis by binding to Bax and interfering with its activation, which would ultimately protect against cytochrome c release [15].

Preliminary evidence suggests that statin treatment in humans results in decreased skeletal muscle mitochondrial enzyme activity [16].

Examination of the skeletal muscles of male albino rats especially low back muscles as many patients complain of low back pain after long duration use of statins. Lattismus dorsi was chosen as an example of the back extensor skeletal muscles as it is a slow twitch muscle & contains two types of fibres (oxidative / glycolytic) metabolic nature help it in contraction in usual daily habits and sustained contraction all of the day[17].

In the current study, different structural changes were detected in the skeletal muscle by light microscope in group II rats receiving atorvastatin. Focal areas with cellular infiltration and containing myofibers characterized by splitting of the myofibers fragmentation of the sarcoplasm and dense centrally located nuclei or even remnants of nuclei were detected. The centrally located myonuclei have been used as a marker for fiber damage, this findings were in agreement with **Rosenblatt & Woods [18], Sugarman et al., [19] and Rodine [20]**.

They demonstrated central nuclei in skeletal muscle in degenerative myopathies. In addition central nuclei were also reported in cases of muscle fiber regeneration,

In this study, measuring the transverse diameter of the muscle fibers reported a decreased in the diameter of the most treated and recovery groups which approve the myopathic changes in the muscle. In toluidin blue stain used in the current study showed loss of cross striation in the affected fibers. Loss of cross striations has been considered as an important indicator reflecting myofilament degeneration [21]. P.A.S stain used in this study showed that the lattismus dorsi muscle has two types of

fibres(I and II B) and indicated a differential fibre sensitivity to statin-induced muscle degeneration.

The fibres showed a degenerative response to statin administration that matched their oxidative / glycolytic metabolic nature and that was approved by the optical density of its glycogen contents :

Least sensitive  $\rightarrow$  I  $\leftrightarrow$  IIB  $\leftarrow$  most sensitive. Type I and IIB fibres represent metabolic extremes of a continuum of metabolic properties through the fibre types with type I fibres most oxidative in metabolism and type IIB fibres most glycolytic.

There was a continuum of muscle fibre types in adult mammalian skeletal muscles from the slow twitch type I to the fastest twitch type IIB:

I  $\leftrightarrow$  IC  $\leftrightarrow$  IIC  $\leftrightarrow$  IIA  $\leftrightarrow$  IIAD  $\leftrightarrow$  IID  $\leftrightarrow$  IIDB  $\leftrightarrow$  IIB (pure fibres shown in bold[22].

The type I and II B fibres represent the 2 metabolic extremes with a continuum of metabolic properties through these fibre types [23]. Type I fibres have slow contraction times, are oxidative in metabolism, have a high content of mitochondria and myoglobin, and have low quantities of glycogen and myosin AT-Pase.

In contrast, type II B fibres have fast contraction times, are glycolytic in metabolism, have a low content of mitochondria and myoglobin, and have high quantities of glycogen and myosin ATPase. Type I fibres are considered slow oxidative fibres, type IIB fast glycolytic fibres, and types IIA and IID fast oxidative/glycolytic fibres. In addition, by electron microscopical examination in some (non necrotic) glycolytic fibres from muscles showed early multifocal single fiber degeneration as well as changes were noticed in mitochondrial[24].

These changes were characterized by an increased incidence of vacuolation accumulated in the subsarcolemmal areas , These findings suggest the important of early involvement of mitochondria in selective glycolytic muscle fibre necrosis following inhibition of the enzyme HMG-CoA reductase. These histological features are entirely consistent with findings of other investigators using a variety of statins including lovastatin, pravastatin, simvastatin and cerivastatin[25],[26].

Throughout this study ,we noticed that changes in the lattismusdorsi muscle in the

form of degeneration and ultrastructural changes. The lattismus dorsi was a slow twitched muscle containing predominantly slow oxidative type I fibres and the more oxidative of the fast fibres IIB. We can also report that in muscles with a high content of fast glycolytic fibres there can be quite severe necrosis of the type II fibres with essentially total sparing of the type I fibres, which also show no ultrastructural changes, supporting reports from other investigators [27].

In addition, our results illustrated that when muscles showing acute multifocal single fibre degeneration are assessed by P.A.S & Toluidin blue staining there is a differential sensitivity to statin-induced necrosis between the different type of fibres. So, our studies have shown that muscles showing the most severe degeneration following administration of atorvastatin contained a substantial proportion of type I fibres (rich in mitochondria), and in these muscles it is the type I fibres that become degenerative first because of early destruction of mitochondria .

In other type of fibre, type II B, and these muscles were relatively spared of degeneration because its high glycogen contents. Taken together, these observations illustrate a continuum of fibre sensitivity to statins that mirrors their oxidative/glycolytic metabolic properties: Least sensitive fibres type IIB while type I fibres are Most sensitive. Therefore, a clear relationship between the metabolic nature of individual muscle fibres and their sensitivity to statin-induced necrosis. As mitochondria play a central role in metabolism they may be important to this process. Ragged red fibres have been reported in humans [28]. following administration of statins. This change indicates the presence of subsarcolemmal accumulations of abnormal mitochondria.

Further studies in rats have shown a close relationship between muscle degeneration and ultrastructural abnormalities in mitochondria although there is some discrepancy concerning the time of earliest mitochondrial change[14].

Schaefer et al[26] stated that mitochondrial injury does not appear to be the primary cause of skeletal muscle necrosis induced by atorvastatin but are likely secondary to necrosis or degeneration.



Also, in some (nonnecrotic) glycolytic fibres from muscles showing very early multifocal single fibre necrosis, the only subcellular alterations present in isolation of any other changes were in the mitochondria. They were characterized by an increased incidence of mitochondrial vacuolation and further mitochondrial degeneration resulting in myelinoid and vesicular bodies, which tended to accumulate in the subsarcolemmal areas. So the low back muscles (oxidative fibres) are more affected with atorvastatin treatment because of their high mitochondrial contents to give them the energy to contract for long duration as those muscles showing the most severe necrosis following administration of atorvastatin contained a substantial proportion of type I fibres (rich in mitochondria), and in these muscles it is the type I fibres that become necrotic first because of early destruction of mitochondria[29].

#### CONCLUSION:

Administration of atorvastatin for long duration resulted in some sort of myotoxic structural changes and apoptosis as evident by deformity in the mitochondria, lack of striation, degeneration of nuclei and splitting of muscle fibres in the adult male albino rat skeletal muscle.

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