

Effect of Atorvastatin (ATOR) on the Cardiac Muscle Fibres in Hyperlipidemic Adult Male Albino Rats (Structural and Biochemical Study)

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ASBTRACT

Background: Atorvastatin (ATOR) used as a cholesterol-lowering drug. Ator is one of statins widely used, commercially available and increasingly used day by day. Few studies and limited data were existed on atorvastatin induced histological and ultrastructural changes in the cardiac muscle fibres. The present study was conducted to evaluate the potential toxicity of the human equivalent therapeutic doses of Atorvastatin on cardiac muscle fibres in induced hyperlipidemic adult male albino rats. **Material and Methods:** thirty adult male albino rats were used in this study, the rats used weighing (150±10) gm. Rats were divided into groups, Group one (5 rats), served as the control group (C). Other twenty five rats were subjected to high fat diet (25% fat and 2% cholesterol) for 3 weeks and then they subdivided into three groups. Group two (five rats) was considered as a hyperlipidemic group (H). Group three, treated group one (T1) (ten rats) received atorvastatin (Ator 20mg) (1.5 mg / day / rat) for three weeks, by the end the third week five rats were sacrificed and other five rats were considered as the recovery group one (R1), they received normal diet only for another three weeks. Group four, treated group two (T2) (ten rats) received Atorvastatin (Ator 20mg) (1.5 mg / day / rat) for other six weeks, by the end of the 6th week five rats were sacrificed and other five rats were considered as the recovery group two (R2), they received only normal diet for another six weeks. At the end of each step of the experiment, rats were sacrificed and specimens of cardiac muscle fibres prepared by different methods for light and electron microscopic studies. Also blood sampling was obtained for the biochemical study. **Results:** the histological data obtained from the different groups showed many changes, in hyperlipidemic group (H) indistinct and distorted striation and separation of cardiac muscle fibres, vesicular nuclei, presence of myofibroblasts with congested and dilated blood vessels, loss of some nuclei and condensation of nuclear chromatin with normal mitochondria were detected. Treated group one (T1) showed that cardiac muscle fibres restored their striation with separated and splitted myofibres and pyknotic nuclei, increased myofibroblasts, lymphocytic infiltration, congested blood vessels and mitochondrial vacuolation with disorganization of their cristae. In treated group two (T2) patchy loss of striation was observed with splitted cardiac muscle fibres, pyknotic nuclei of cardiomyocytes, margination of nuclear chromatin, congested blood vessels, lymphocytic infiltration, swelling of mitochondria with disorganization of their cristae. In recovery group one (R1) cardiac muscle fibres restored their striation. In recovery group two (R2) cardiac muscle fibres restored their normal architecture. **The statistically evaluated data** showed a significant difference in parentage of collagen and optical density of PAS + ve materials in the different groups when compared to the control group specially in T2. **The laboratory evaluated data** showed significant increase in levels of cholesterol (C), triglycerides (TG), low density lipoprotein- cholesterol (LDL-C), Very low density lipoprotein (vLDL), Atherogenic index (AI) and decrease in high density lipoprotein- cholesterol (HDL-C) between the control and the hyperlipidemic group. Administration of ATOR to the treated groups induce significant decrease in levels of C, TG, LDL-C, (vLDL), (AI) and increase of HDL-C when compared with the hyperlipidemic group. Also there were significant decrease in levels of C, TG, LDL-C and increase of HDL-C in recovery groups when compared with the hyperlipidemic group. **Conclusion:** administration of ATOR for long time led to adverse degenerative effects on structure of the cardiac muscle fibres, although it has a potent lowering effect on lipid profile in hyperlipidemia. **Key words:** Atorvastatin (ATOR), Cardiac muscle fibers, Induced hyperlipidemia, Adult male albino rats.

INTRODUCTION

Different studies on the experimental animals had clearly demonstrated the common risk factor for cardiovascular diseases in case of hyperlipidemia.^[1]

High blood cholesterol and hyperlipidemia are major risk factors. Epidemiological studies have shown that elevation of serum total cholesterol, LDL-cholesterol, triglycerides, fibrinogen and platelets count are major risk factors for cardiovascular disease (CVD) which lead to morbidity and mortality in adult human being in different sites of the world even in the developing countries.^[2]

Treatment of hypercholesterolemia may reduce the risk or development of cardiovascular disease.^[3]

Various treatments for high blood cholesterol are available. Medication, patient education, dietary changes and exercise are all recommended treatments for hyperlipidemia.^[4]

Statins (3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors) are the primary class of medication used to lower serum cholesterol concentration. The most commonly prescribed, atorvastatin, commonly known as Lipitor^[5] From 1996 to 2012, atorvastatin became the world's best-selling drug.⁴ Atorvastatin is made in tablet form by generic drug makers under various brand names.^[6] In Egypt the drug has a different marketing name, the most common known as Ator.

Atorvastatin (member of lipophilic statins) is classified as a drug that has concentration-dependent adverse effect on muscle cell viability and promote cell disruption via proteolysis and apoptosis.^[7] Hypolipidemic drugs cause potentially serious side effects and include digestive disturbances, nausea and vomiting.^[8]

Using of atorvastatin safely requires regular monitoring for side effects. Atorvastatin has the potential to cause adverse events which can result in muscular inflammation (myositis).^[9] In the last few years there has been an increased interest regarding the side effects of statins specially atorvastatin.^[10]

Skeletal muscle side effects that are associated with atorvastatin-use involve muscle cramping, soreness, fatigue, weakness and, in rare cases, rapid muscle

breakdown that can lead to death (Rhabdomyolysis).^[11]

Atorvastatin (ATOR) is known to cause skeletal muscle side effects, but it is currently unclear if it also affects the structure of cardiac muscle fibres or not.

MATERIALS AND METHODS

Thirty adult male albino rats were used in this study; rats used weighing (150±10) gm. They were left for two weeks in the laboratory room before any experimental interference for acclimatization. All rats were kept under normal conditions of light, temperature and relative humidity, and provided with normal diet pellets and drinking tap water during the whole experimental period.

Following that, rats were divided into the experimental groups. Group one (five rats) was considered as a control group (C).

Other twenty five rats were subjected to high fat diet (HFD) (25% fat and 2% cholesterol) for 3 weeks.^[12] After induction of hyperlipidemia the rats weighted about (200 ± 10) gm. Then they were subdivided into three groups. Group two (five rats) was considered as a **hyperlipidemic group (H)**.

Before start the treatment with atorvastatin (Ator 20mg / tablet) (Product of EPICO company), it was dissolved in distilled water and given orally by a gastric tube.^[13] Atorvastatin human dosage about 80mg, which equal about 7.2mg/kg in rats.^[14] and^[15] So the daily single oral dose in this study is about 1.5 mg / day / rat.

Group three, **treated group one (T1)** (ten rats) were similar to the second group, but received atorvastatin (Ator 20mg) (1.5 mg / day / rat) for three weeks orally. At the end the third week five rats were sacrificed and other five rats were left without any other treatment and considered as the **recovery group (R1)** and received normal diet only for another three weeks.

Animals of group four (**Treated group two, T2**) (ten rats) were similar to the second group, but they received atorvastatin (Ator 20mg) (1.5 mg / day / rat) for six weeks orally. At the end of the sixth week five rats were sacrificed and other five rats were left without any other treatment and considered as the **recovery group two (R2)** and received normal diet only for another six weeks.

At the end of each step of the experiment, all animals were anaesthetized by ether inhalation, sacrificed and small pieces of hearts were extracted and fixed in 10% neutral buffered formalin for 48 hours and prepared for paraffin sections. Another piece of specimens were fixed in glutaraldehyde for E/M technique.

Parameters of the study

1- Histological study:

Paraffin sections (5µm thickness) were prepared and stained with haematoxylin and eosin (H&E), Masson's trichrome stain and periodic acid – Schiff technique.^{[16]and[17]}

2- Electron microscopic study:

Semithin sections were cut and stained with toluidine blue for studying the structure of the cardiac muscle fibres. The chosen regions were cut with glass knives for ultrathin sections (60-70 nm) which mounted on copper grids.^[18] The ultra-thin sections were post stained with 2% uranyl acetate and lead citrate and examined with Joel TEM CS 100 electron microscope.

3- laboratory tests:

Venous blood samples were withdrawn from the retro-orbital plexus in heparinized capillary tubes, by pressing the thumb behind the angle of the jaw resulting in the engorgement of the retro-orbital plexus.^[19]

After separation of plasma from blood, the various biochemical parameters were estimated as the following.

- a- Total serum cholesterol (TC).^[20]
- b- Triglycerides (TG).^[21]
- c- Low density lipoprotein cholesterol, (LDL)(as LDL-C = Total cholesterol (TC) – (HDL-C + VLDL-C).^[22]
- d- High density lipoprotein cholesterol, (HDL).^[23]
- e- Very low density lipoprotein (vLDL)(as VLDL-C = Triglycerides / 5).^[22]
- f- Atherogenic index (AI) (as Atherogenic Index (AI) = TG/HDL-C).^[22]

4- statistically evaluation:

The statistical data included were expressed as mean, standard deviation (SD ±) and the student T test was used to elucidate the differences between different groups and the control group.^[24] The obtained data were analyzed by using SPSS (Statistical package for social studies) software (V.16), the level of significance was adopted at $P \leq 0.05$.

RESULTS

I- The histological results:-

1- Control group (C) (Figs. 1-8).

- **Light microscope study (LM):** branched striated cardiac muscle fibers were detected in the control group with single centrally located oval nuclei and intercalated discs.
- Normal fine collagen fibres were observed between the cardiac muscle fibres with normal distribution of PAS + ve materials (magenta red) in sarcolemma of the cardiac muscle fibres.
- Semithin sections revealed visible cross striations and central vesicular nuclei surrounded by a clear perinuclear zone.
- **Electron Microscope study:** (EM) revealed that cardiomyocytes contain strands of myofibrils formed of light bands, Z lines, dark bands, H zone and M line bisecting H zone and mitochondria with normal organization of cristae.

2- Hyperlipidemic group (H) (Figs. 9-17).

- LM revealed indistinct and distorted striation of fibres, separation of muscle fibres with vesicular nuclei and presence of myofibroblast.
- Mild to moderate increase in collagen fibres with decreased PAS+ ve materials in the cardiac muscle were observed regarded to statistically significance data in comparison with the control group as $P < 0.05$.
- Semithin sections revealed loss of striation of muscle fibres, congested and dilated blood vessels, loss of some nuclei and presence of myofibroblasts.
- EM revealed an area of loss of striation, appearance of nucleus of myofibroblast, splitting of muscle fibres with condensation of nuclear chromatin, normal intercalated discs and Z line with normal mitochondria.

3- Treated group one (T1) (Figs. 18-24).

- LM revealed that cardiac muscle fibers restored their striation with pyknotic nuclei, increased myofibroblasts and appearance of lymphocytic infiltration.
- Mild decrease in collagen fibres with mild increase in PAS + ve materials in cardiac muscle were observed regarded to statistically significant data in comparison with hyperlipidemic group.
- Semithin sections revealed myofibroblast nucleus and lymphocytic infiltration.
- EM revealed nucleus of myofibroblast, congested blood vessels with areas of loss of striation, nuclear damage with separated and

splitted myofibres, mitochondrial vacuolation and disorganization of their cristae.

4- Treated group two (T2) (Figs. 25-34).

- LM revealed patchy loss of striation with splitted muscle fibres, some nuclei become pyknotic with margination of nuclear chromatin (Retipersion), congested blood vessels with presence of lymphocytic infiltration.
- Increased collagen fibres was noticed & focal areas of excessive collagen fibres deposition were also seen with decreased PAS + ve materials in the cardiac muscle were observed regarded to statistically significance data in comparison with hyperlipidemic and treated 1 groups.
- Semithin sections revealed loss of striation , dilated and congested blood vessels with vacuolation and nuclear damage.
- EM revealed indentured degenerative pyknotic nuclei of cardiomyocytes with patchy loss of striation, nucleus of myofibroblast ,swollen mitochondrial with expansion of the inter-membranous spaces and disorganization of their cristae.

5- Recovery group one (R1) (Figs. 35-37).

- LM revealed that cardiac muscle fibres restored their normal striation and myofibroblast.
- Decreased collagen fibres with increased PAS + ve materials in the cardiac muscle were observed regarded to statistically significant data in comparison with hyperlipidemic , treated 1&2.

6- Recovery group two (R2)(Figs. 38-40).

- LM revealed that cardiac muscle fibres restored their normal striation with decreased collagen fibres with increased PAS + ve materials in the cardiac muscle were observed regarded to statistically significant data in comparison with hyperlipidemic , treated 1&2 and recovery 1 groups.

II-Statistically and laboratory evaluated data:-

- 1- Table. 1 :** showing a significant difference in percentage of collagen fibres between the cardiac muscle fibers in the different groups when compared to the control group specially in T2 group as $p < 0.05$.
- 2- Table. 2:** showing a significant difference in optical density of PAS + ve materials in the cardiac muscle fibres in the different groups when compared to the control group especially in T2 group as $p < 0.05$.

- 3- Tables. 3 & 4 & 5 & 6&7 and 8:** showing significant increase in levels of cholesterol , Triglycerides, Low Density Lipoprotein Cholesterol (LDL-C) very low density lipoprotein (vLDL) and atherogenic index(AI) and significant decrease of High Density Lipoprotein Cholesterol (HDL-C) between the control and hyperlipidemic groups as $p < 0.05$.

Administration of ATOR to treated groups induced significant decreases in the serum levels of cholesterol , Triglycerides, Low Density Lipoprotein Cholesterol (LDL-C) very low density lipoprotein (vLDL) and atherogenic index(AI) and significant increase of High Density Lipoprotein Cholesterol (HDL-C) when compared with hyperlipidemic group as $p < 0.05$.

Also there were significant decreases in the serum levels of cholesterol , Triglycerides, Low Density Lipoprotein Cholesterol (LDL-C) very low density lipoprotein (vLDL) and atherogenic index(AI) and significant increase of High Density Lipoprotein Cholesterol (HDL-C) in recovery groups when compared with hyperlipidemic and treated groups ($p < 0.05$).

DISCUSSION

Hyperlipidemia is a heterogeneous disorder commonly characterized by an increased flux of free fatty acids (FFAs), raised cholesterol ,triglycerides (TGs), low-density lipoprotein-cholesterol and apolipoprotein B (Apo-B) levels, as well as by a reduced plasma high-density lipoprotein -cholesterol concentration because of metabolic effects or dietary and lifestyle habits .^[25]

High-fat diet as well as hyperlipidemia might induce oxidative stress and lipid oxidation that contributes to the high risk of cardiovascular diseases (CVD) .^[26]

Atorvastatin (Ator) (HMG-Co-A reductase inhibitor) was shown to be a highly effective member of statins which are used to reduce cardiovascular-related morbidity and mortality in patients with or without coronary artery disease .^[27]

Indications for statins therapy as recommended by The National Institute for Health and Clinical Excellence make statins one of the world's most prescribed drugs. One of these indication was hypercholesterolemia .^[28]

Reducing the level of cholesterol in blood plasma is an effective method to treat hyperlipidemia-related diseases. The currently used medicines, such as fibrates and statins, are characterized by their high lipid-lowering speed and efficacy.^[29]

Myotoxic effects of atorvastatin are known to be dose and time dependent. As pointed out, impaired metabolism of statins, pharmacokinetic interactions and/or genetic effects are all probable causes of the myotoxicity.^[30]

Little studies are available about Ator effect on structure of the cardiac muscle fibres. The present study tried to evaluate the adverse effects of Ator on cardiac muscle fibres in rats, at the first induction of hyperlipidemia by high fat diet for 3 weeks, following by use of Ator in dose equivalent to human dose for 3 and 6 weeks, then the treatment was stopped for 3 and 6 weeks with using normal diet, to explain if these adverse effects are reversible or not, the main parameters of this study depend on light and electron microscopy examination of the cardiac muscle fibres.

The obtained results in the present study revealed that the main histological changes in hyperlipidemic group (**H**) at level of LM, showed indistinct and distorted striation in the cardiac muscle fibres with separation of muscle fibres, vesicular nuclei, loss of some nuclei, congested and dilated blood vessels and presence of myofibroblast. But at level of EM there were condensation of nuclear chromatin with normal mitochondria.

These results are in agreements with **Haddad *et al.***^[31] who reported that the heart showed some sorts of haemorrhages and wavy appearance of myofibres, as well as the sarcoplasm of some fibres was characterized by granular appearance, large areas of degenerated muscle fibers with evident loss of transverse striations and wide interfascicular spaces in hyperlipidemic group compared to the control group.

Ekhteraei *et al.*^[32] reported that there were a close relationship between muscle degeneration and ultrastructural abnormalities in mitochondria although there was a discrepancy concerning the time of earliest mitochondrial change.

Evans and Rees^[33], reported that statin-associated myotoxicity is complex, involving effects on cell membrane structure and

function, mitochondrial dysfunction and impaired myocyte regeneration.

In this study the main histological changes in Ator treated group one (**T1**) at level of LM involving restoration of the cardiac muscles striation and pyknotic nuclei, increased myofibroblasts and lymphocytic infiltration. But at level of EM there were congested blood vessels with areas of loss of striation and nuclear damage with separated and splitted myofibres, mitochondrial vacuolation and disorganization of their cristae.

In this study the main histological changes in Ator treated group two (**T2**) at level of LM, involving patchy loss of striation with splitted muscle fibres, some nuclei became pyknotic with margination of nuclear chromatin (Retipersion), congested blood vessels and presence of lymphocytic infiltration. But at level of EM there were many degenerated and pyknotic nuclei of cardiomyocytes, nucleus of myofibroblast and mitochondrial destructive swelling disorganization of cristae.

The obtained results in this study are in arrangement with the work of **Joseph *et al.***^[34], who observed ultrastructural changes in the cardiac muscle fibres after long-term Ator treatment. They reported that treated hearts presented scattered, misorganized and swollen mitochondria, protein aggregates as well as inclusion bodies and enlarged, mislocalized T-tubular structures.

In correlation with the present work **Noory *et al.***^[35], reported in his work that atorvastatin administration in rats led to ultrastructural changes in the cardiac muscle fibres with clear rupture of some muscle fibres with separation of them from each other together with mitochondria swelling and cristae destruction. Discontinuous and elongation of the cardiac muscle fibres were observed which might be due to occurrence of partial decomposition in these muscles.

In this study the main histological changes after stopping treatment with Ator in the treated groups with normal diet, in recovery group one (**R1**) showed restored striation and myofibroblasts. Recovery group two (**R2**) showed nearly normal structure of the cardiac muscle fibres.

The above obtained results are in arrangement with those of **Catapano**^[10], who reported that after stopping of atorvastatin

side effects disappeared and this usually leading to a full recovery.

In this study appearance of myofibroblast in different groups may be support results in increase of collagen fibres in different percentage, also tougher with lymphocytic infiltration may be indicated heart injury in response either to hyperlipidemia and Ator treatment.

The above obtained results are in arrangement with those of **Jennifer and Heather** ^[36], who reported that in the case of ischemia or heart failure, the myofibroblasts can be found in large areas of the heart as myofibroblasts are not part of normal cardiac tissue and appear only following cardiac injury. Myofibroblasts produce and secrete a number of cytokines themselves which help to maintain the inflammatory response to injury.

The statically evaluated data in this study declared a significant difference between all groups in collagen fibres percentage especially in treated group two as it shown a marked increase of collagen fibres. Also the optical density of PAS + material showed a significant difference between all groups especially in treated group two as it shown a marked decrease in PAS + materials.

In this study successfully induced hyperlipidemia in rats modulating the biochemical parameters of lipid profile, enhancement levels of cholesterol, triglycerides, low density lipoprotein cholesterol (LDL-C) very low density lipoprotein (vLDL) and atherogenic index(AI) were elevated and high density lipoprotein cholesterol (HDL-C) was decreased. These results consistent with **Farhana et al.** ^[37], who reported that induction of hyperlipidemia resulted in several alterations in serum cholesterol, triglycerides, LDL-c, VLDL, atherogenic index and HDL-c levels.

The hypercholesterolemic effect induced by HFD may be due to the activity of the rate-determining enzyme in cholesterol biosynthesis, HMG-CoA reductase, stimulating the cholesterologenesis rate ^[38]. **Liu et al.** ^[39], has suggested that increased lipogenesis in cholesterol-fed rats due to decreased oxidation of fatty acids and decreased secretion of VLDL as causes for the accumulation TG and decrease lipolysis of fat cells. **Yokogoshi**

et al. ^[40], thought that increased cholesterol synthesis in the high-cholesterol-fed rats. **Xuet al.** ^[41], has also reported that the impaired hepatic lipid homeostasis because of lipid accumulation attributed to the increasing activity of the enzymes involved in fatty acid biosynthesis in the rats by the dietary cholesterol.

In the present study administration of Ator to the treated groups induced significant decreases in the levels of cholesterol, triglycerides, low density lipoprotein cholesterol (LDL-C) very low density lipoprotein (vLDL) and atherogenic index(AI) with a significant increase of high density lipoprotein cholesterol (HDL-C), these result are in accordance with those of **Bethet al.** ^[42], who reported that administration of Ator to rats fed HFD caused a reduction in serum TC and LDL-c levels, VLDL level with a consequence reduction in atherogenic index, while serum HDL-c levels were elevated.

These findings could be interpreted by the inhibitory effect of Ator on HMG-CoA reductase enzyme which catalyzes the conversion of HMG-CoA to mevalonate, a rate-limiting step in the formation of endogenous cholesterol leading to the decrease in the intracellular stores of cholesterol. This in turn results in the up-regulation of LDL receptors on the cell membrane, thus increasing the clearance of LDL-c from plasma. ^[43] Another possible explanation of our results is that Ator may lower LDL-c level by inhibiting hepatic cholesterol synthesis in very low density lipoprotein (VLDL) which is the source of LDL-c. Thus may impair VLDL particle assembly and secretion from liver, decrease the VLDL levels in plasma, and further decrease the LDL level in plasma. ^[44]

Other proposed mechanism for Ator in lowering serum cholesterol level is by inhibiting the absorption of dietary cholesterol. Statins may reduce the cholesterol content in the intestinal mucosal cells, with a subsequent reduction in acyl-CoA cholesterylacyltransferase (ACAT) activity, which catalyzes the intracellular esterification of cholesterol and formation of cholesteryl esters, and thus decrease the absorption of cholesterol. ^[45] The reduction in HDL cholesterol level in animals fed high fat diet may be due to the

decrease in lecithin-cholesterol acyltransferase (LCAT) activity, the enzyme involved in the transesterification of cholesterol, the maturation of HDL and the flux of cholesterol from cell membranes into HDL. [46] Atorvastatin (Ator) treatment could restore HDL-c levels may be attributed to the reduction of cholesteryl ester transfer protein (CETP) which plays an important role in HDL metabolism. [47] Atorvastatin (Ator) appeared to reduce triglyceride levels by increasing LDL clearance and by inhibiting triglyceride synthesis. It also increases HDL cholesterol levels. [48]

Conclusion: this study shed light on Ator side effects on cardiac muscle fibres, so it should not stop on its role in modulating lipid profile. So patients on Ator treatment need to adjust time and dose or try to use another method to modulate lipid profile as change diet habits or use another safe medication as fibrates and herbal medicinal plants. Also advice for another researches on Ator adverse effect and comparison between the different kinds of statins to select the most safest one on the cardiac muscle fibres.

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The Histological Results:

1-Control group (C)

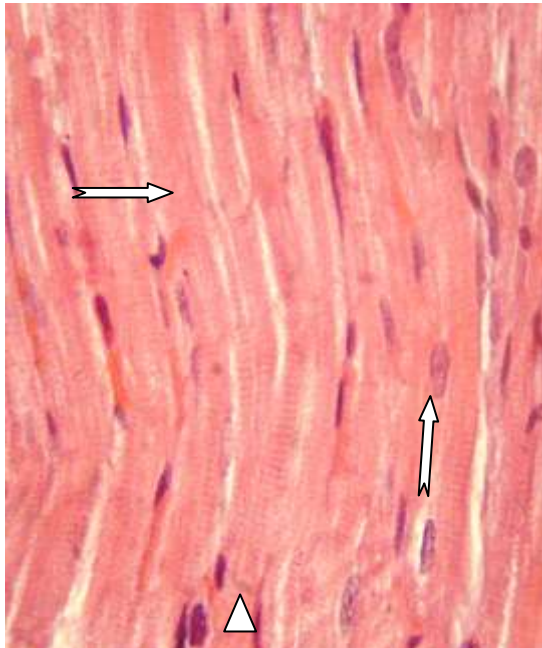


Fig. 1: A photomicrograph of cardiac muscle fibres of a rat of group (C) showing striated muscle fibres (transverse arrow) with single centrally located oval nuclei (vertical arrow) and intercalated (arrowhead) (Hx. & E, X 400).

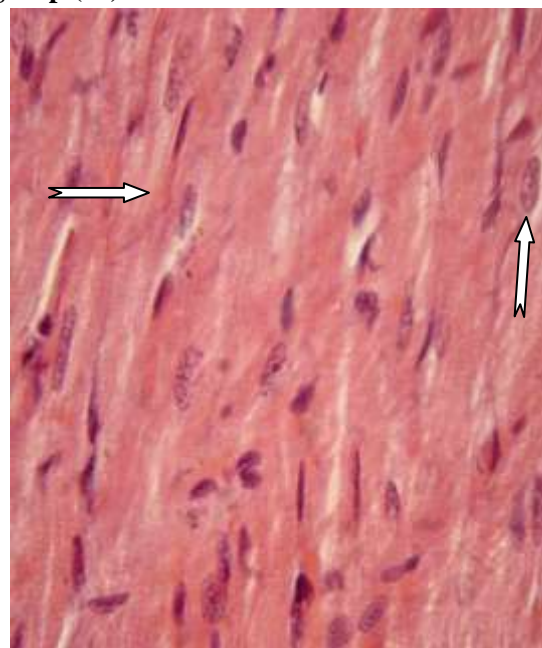


Fig. 2: A photomicrograph of cardiac muscle fibres of a rat of group (C) showing normal branching muscle fibres (transverse arrow) with single centrally located oval nuclei (vertical arrow) (Hx. & E stain, X 1000).

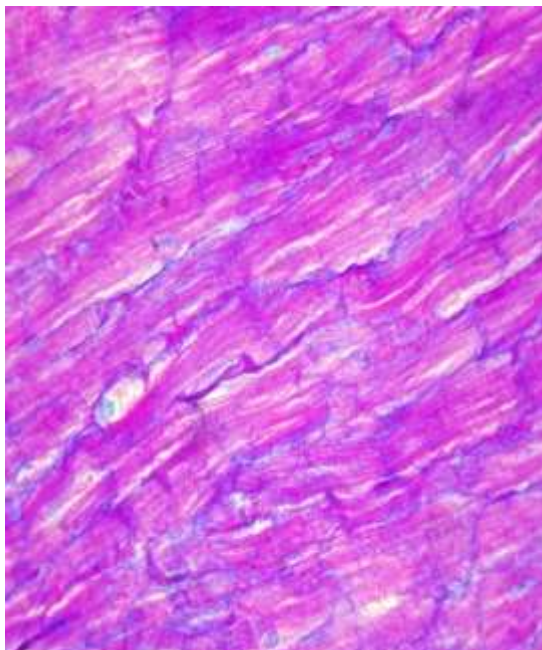


Fig. 3: A photomicrograph of cardiac muscle fibres of a rat of group (C) showing fine collagen fibres between the cardiac muscle fibres (Masson's trichrome stain X 400).

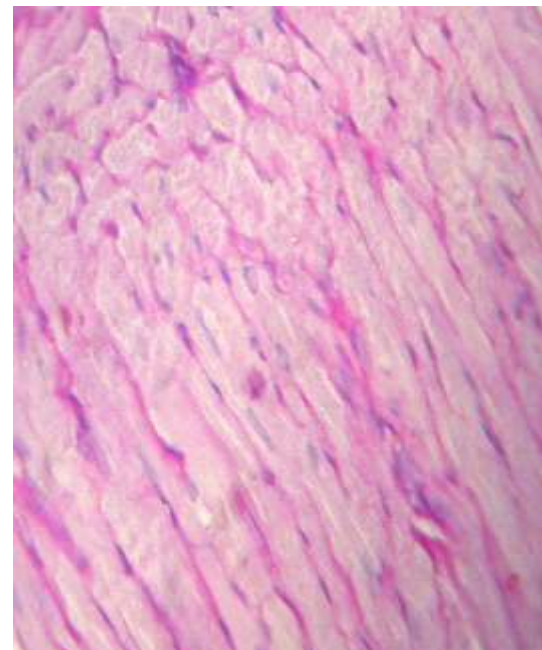


Fig. 4: A photomicrograph of cardiac muscle fibres of a rat of group (C) showing PAS +ve materials (magenta red) in sarcolemma of the cardiac muscle fibres (PAS reaction X 400).

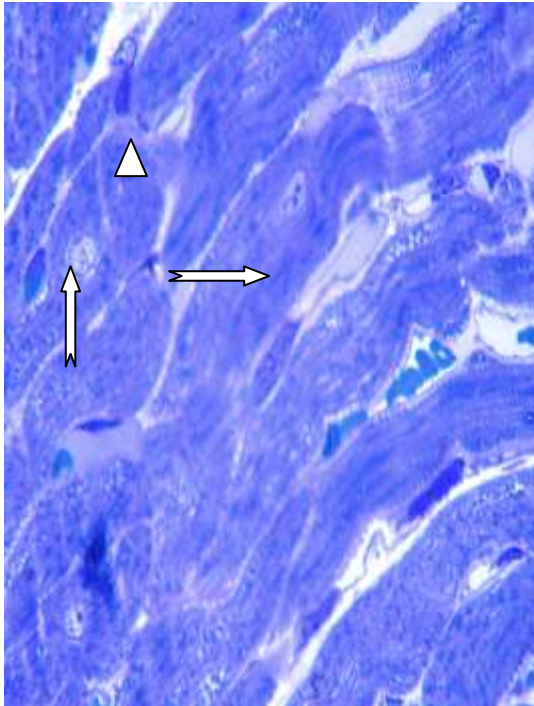


Fig. 5: A photomicrograph of cardiac muscle fibres of a rat of group(C) showing visible cross striations of muscle fibres (transverse arrow) and central vesicular nuclei surrounded by a clear perinuclear zone (vertical arrow) and intercalated discs (arrowhead) (semithin section, Toluidine blue stain X 1000).

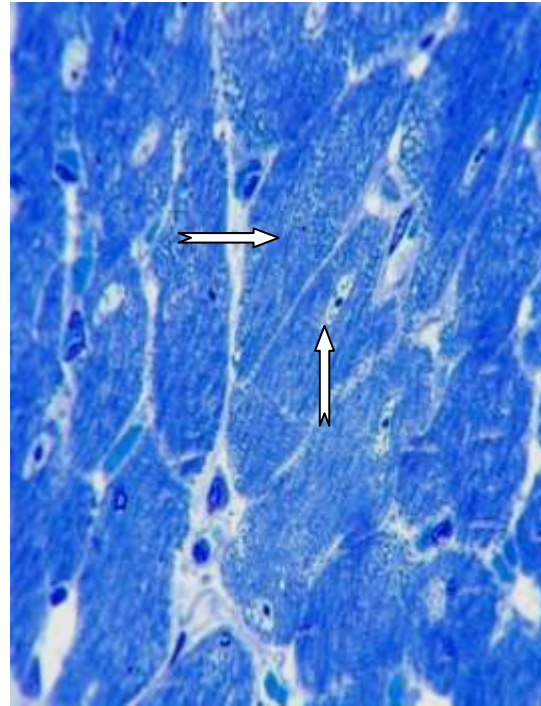


Fig 6: A photomicrograph of cardiac muscle fibres of a rat of group(C) showing fibres with visible striations (transverse arrow) and single central vesicular nuclei (vertical arrow) (semithin section Toluidine blue stain X 1000).

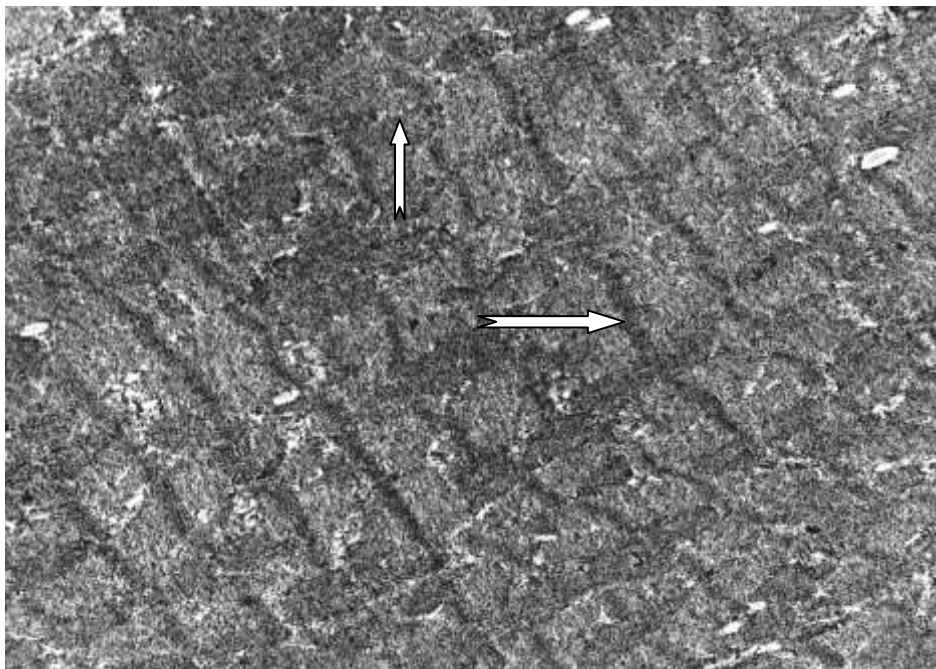


Fig. 7: An electron-micrograph of cardiac muscle fibres of a rat of group(C) showing normal structure of sarcomere (transverse arrow) and striation (vertical arrow) (X 10000).

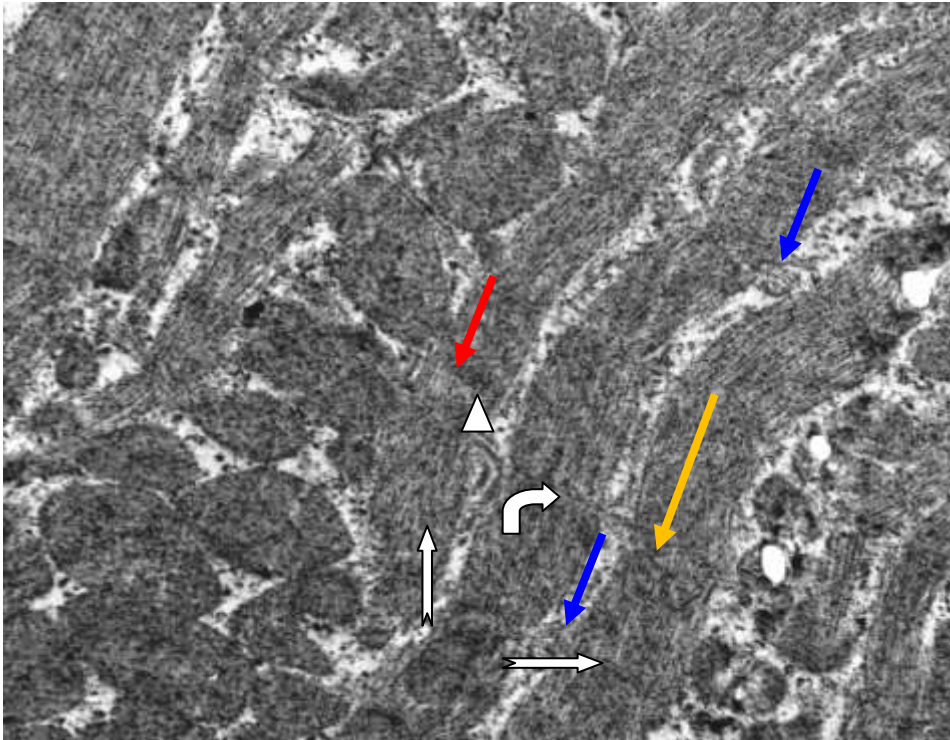


Fig. 8: An electron-micrograph of cardiac muscle fibres of a rat of group(C) showing cardiomyocytes which contain strands of myofibrils formed of light bands (transverse arrow), Z lines (curved arrow), dark bands(vertical arrow), H zone (arrow head), M line (red arrow) , sarcomere (yellow arrow) and mitochondria with normal organization of their cristae(blue arrow) (X6000).

2-Hyperlipidemic group (H)

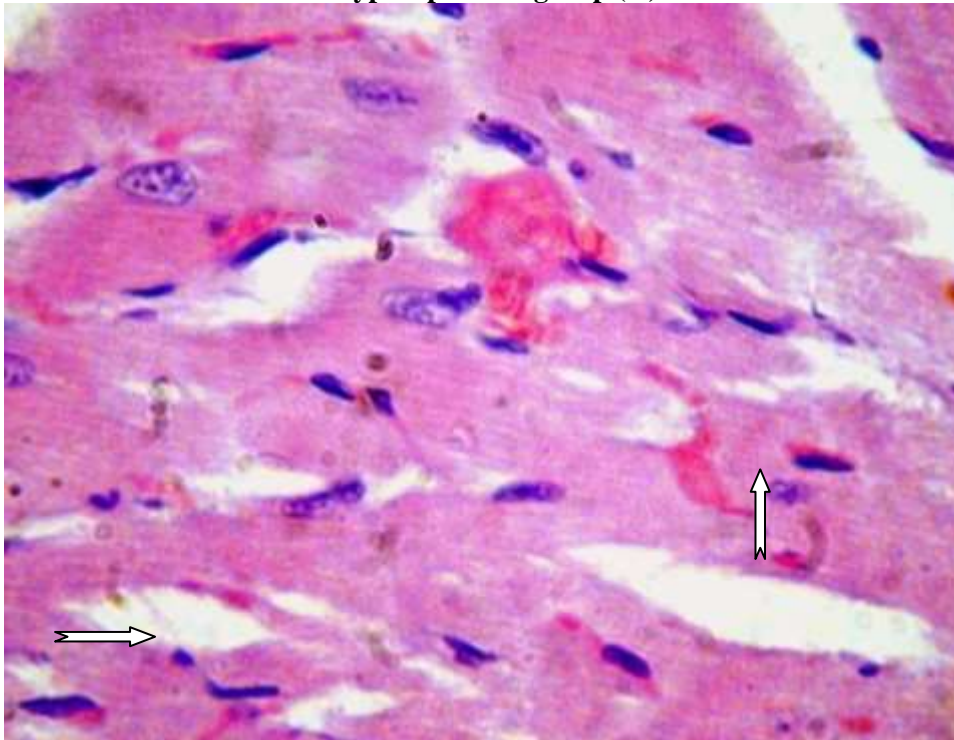


Fig. 9: A photomicrograph of cardiac muscle fibres of a rat of group (H) showing indistinct and distorted striation of muscle fibres(vertical arrow) with separation of them (transverse arrow) ((Hx & E ×1000).

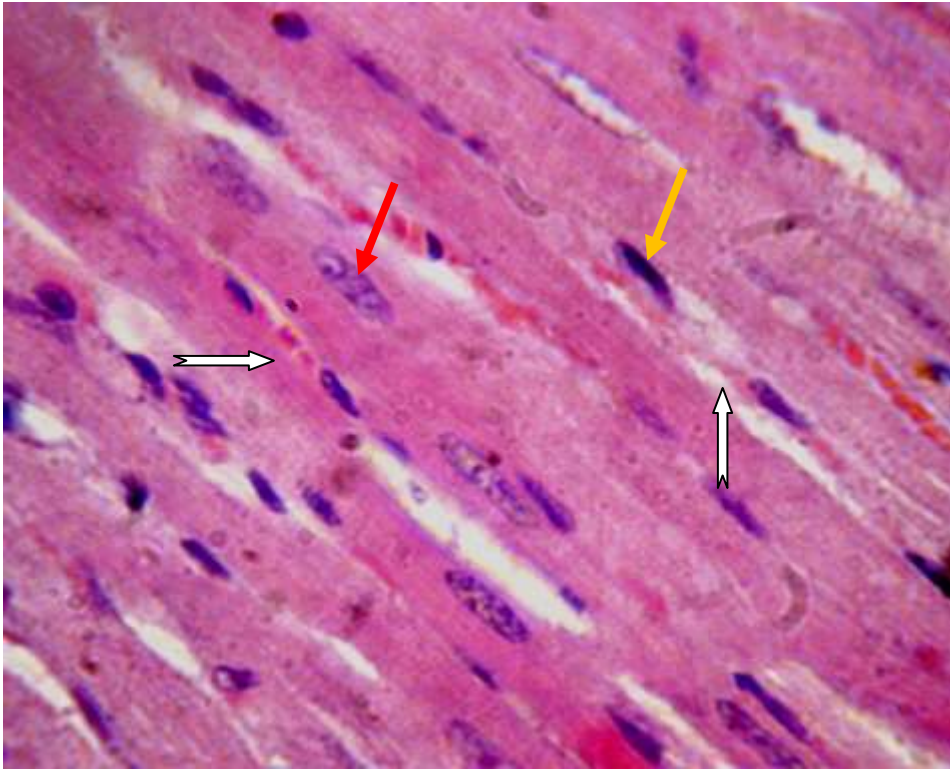


Fig. 10 : A photomicrograph of cardiac muscle fibres of a rat of group (H) showing patches of distorted striation(transverse arrow) ,separation of muscle fibres (vertical arrow) with vesicular nuclei(red arrow) and presence of myofibroblast (yellow arrow) (Hx & E \times 1000).

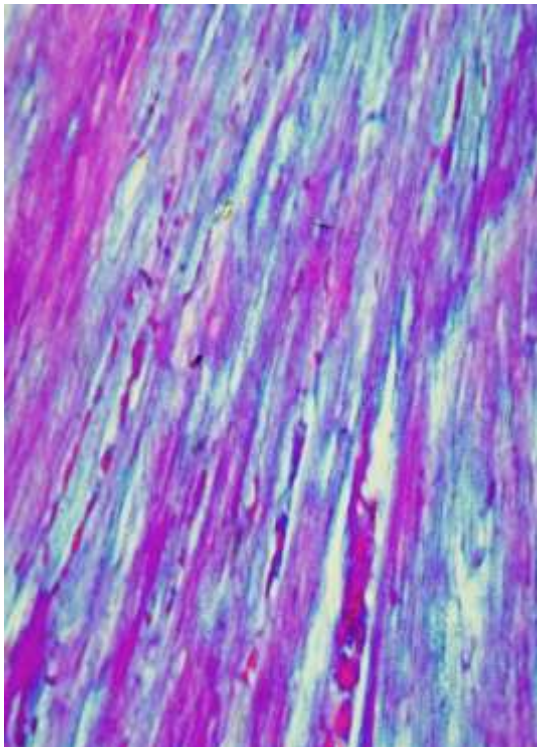


Fig.11:A photomicrograph of cardiac muscle fibres of a rat of group(H)showing mild to moderate increase in collagen fibres between the cardiac muscle fibres (Masson`s trichrome stain X 400)

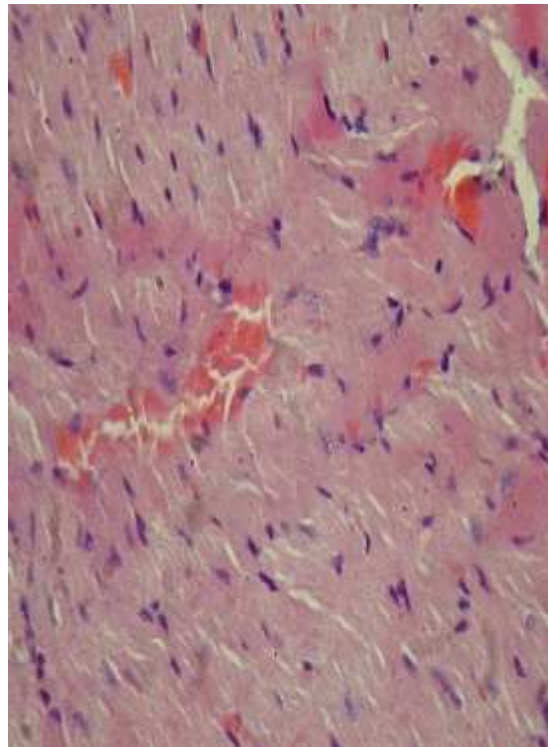


Fig.12: A photomicrograph of cardiac muscle fibres of a rat of group (H) showing decreased PAS+ ve materials in the cardiac muscle fibres .(PAS reaction x 400).

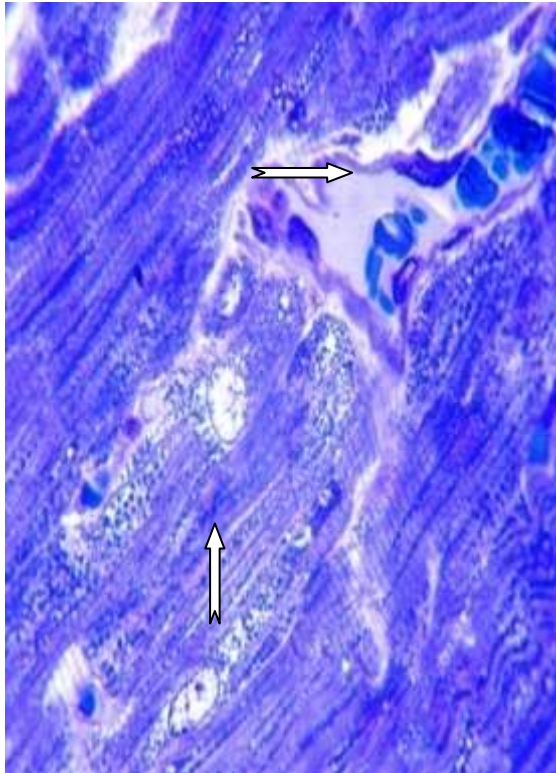


Fig.13:A photomicrograph of cardiac muscle fibres of a rat of group (H) showing distorted striation of muscle fibres (vertical arrow) with congested and dilated blood vessels (transverse arrow) (semithin section, Toluidine blue stain X 1000).

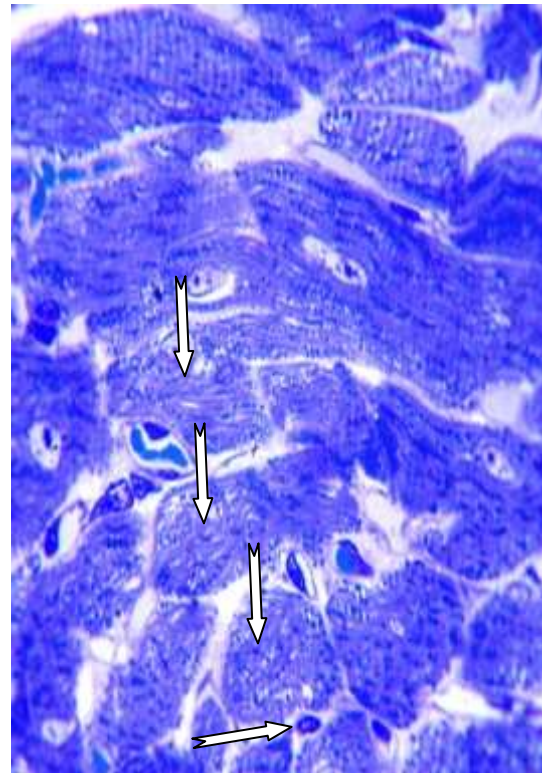


Fig.14: A photomicrograph of cardiac muscle fibres of a rat of group (H) showing cardiac muscle fibres with loss of some nuclei (vertical arrows) and presence of myofibroblasts (transverse arrow) (semithin section Toluidine blue stain X 1000).

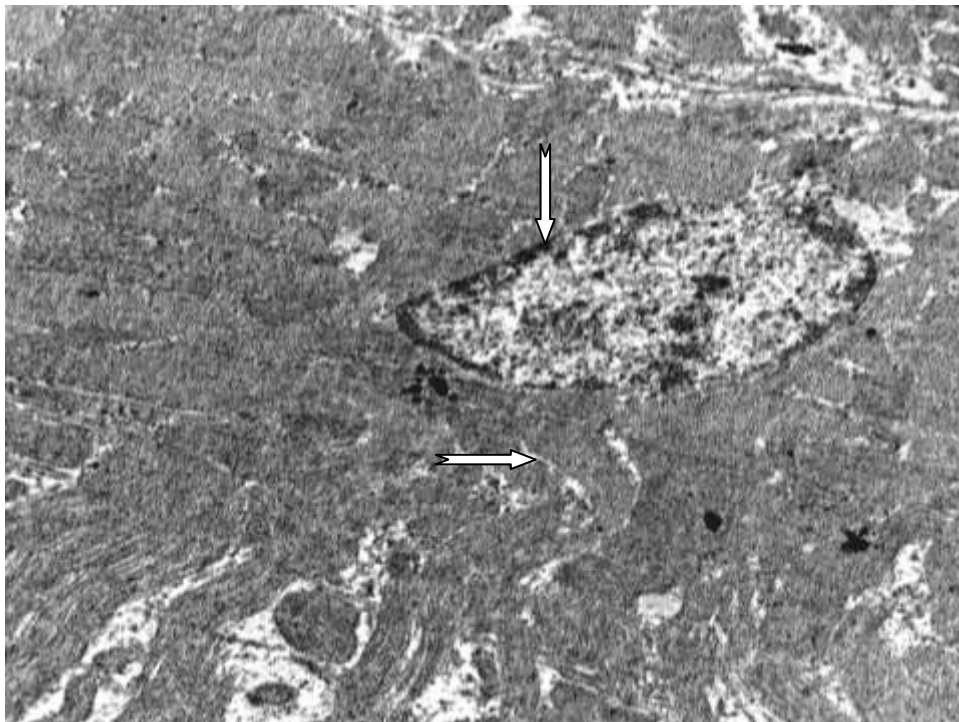


Fig. 15 :An electron-micrograph of cardiac muscle fibres of a rat of group (H) showing an area of loss of striation (transverse arrow) and vesicular nuclei (vertical arrow) (X12000).

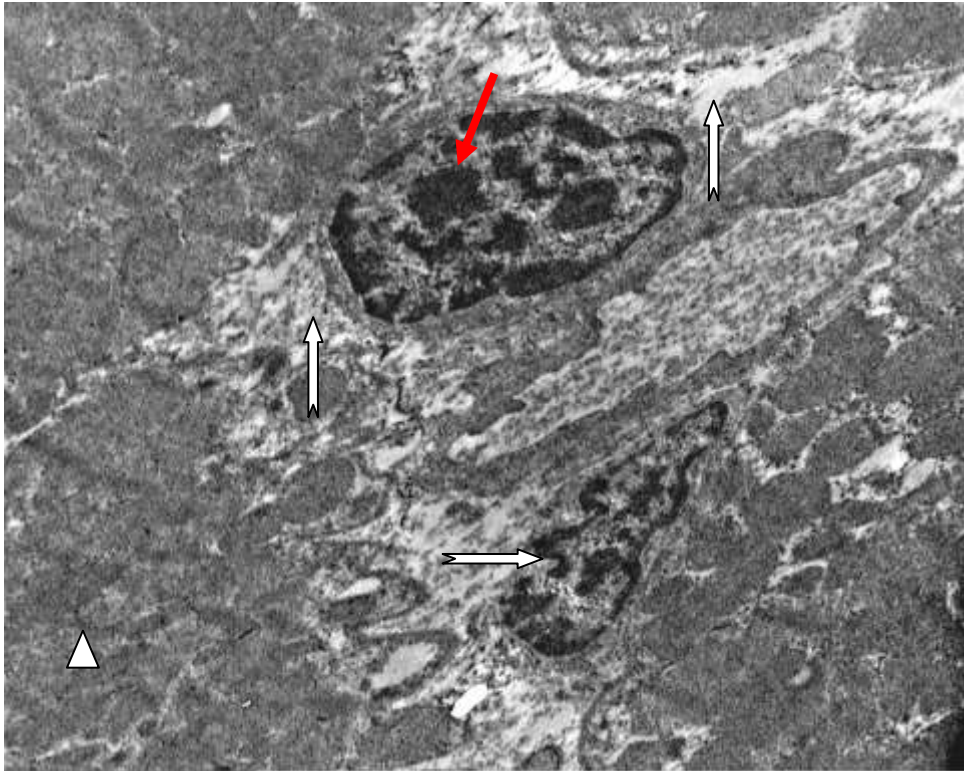


Fig. 16: An electron-micrograph of cardiac muscle fibres of a rat of group (H) showing nucleus of myofibroblast (transverse arrow) ,splitting of muscle fibres (vertical arrows) with condensation of nuclear chromatin(red arrow) and intercalated disc (arrow head) (X12000).

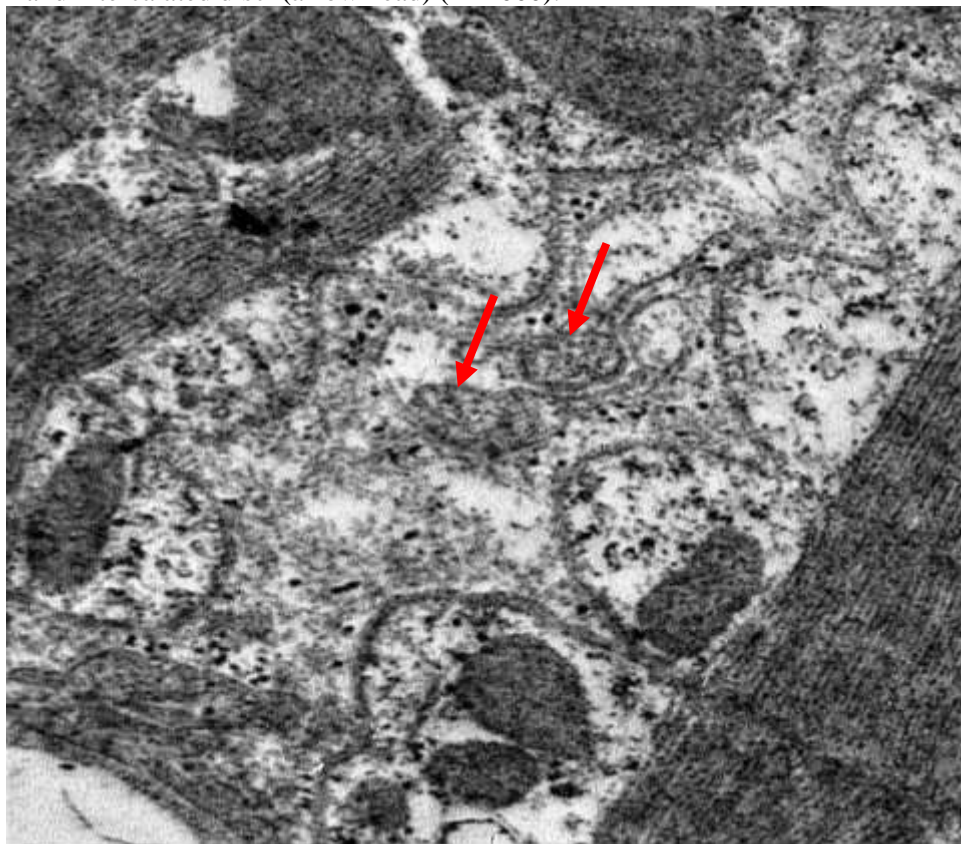


Fig. 17 :An electron-micrograph of cardiac muscle fibres of a rat of group (H) showing some normal mitochondria(red arrows) (X25000).

3-Treated group one(T1)

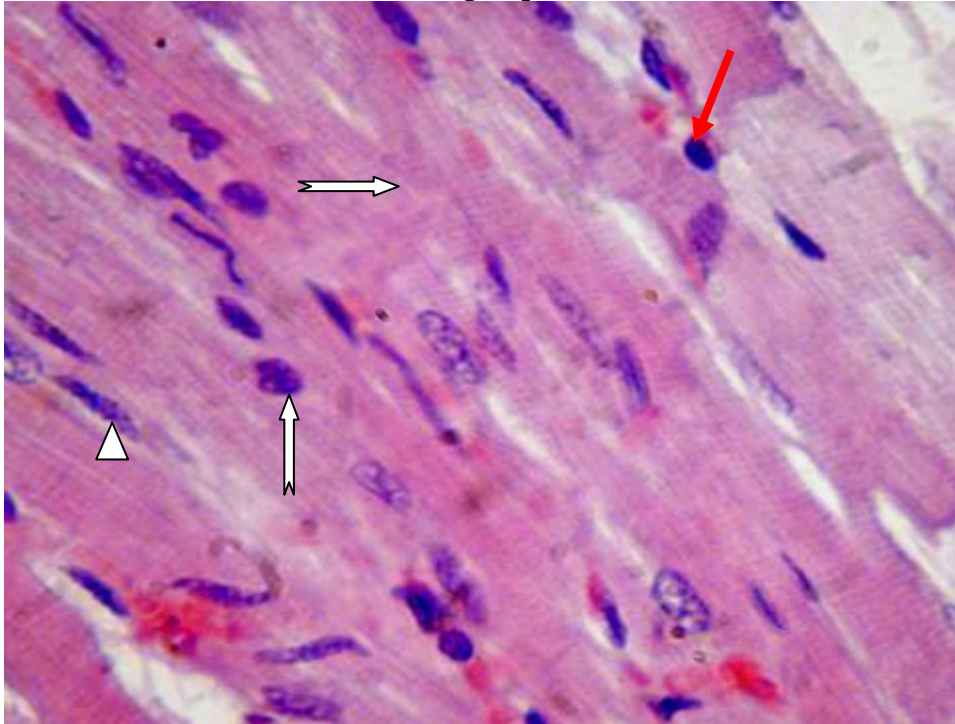


Fig. 18 : A photomicrograph of cardiac muscle fibres of a rat of group (T1) showing cardiac muscle fibres striation (transverse arrow) and pyknotic nuclei (vertical arrow) ,increased myofibroblast (arrow head) and lymphocytic infiltration (red arrow) (Hx & E x 1000).

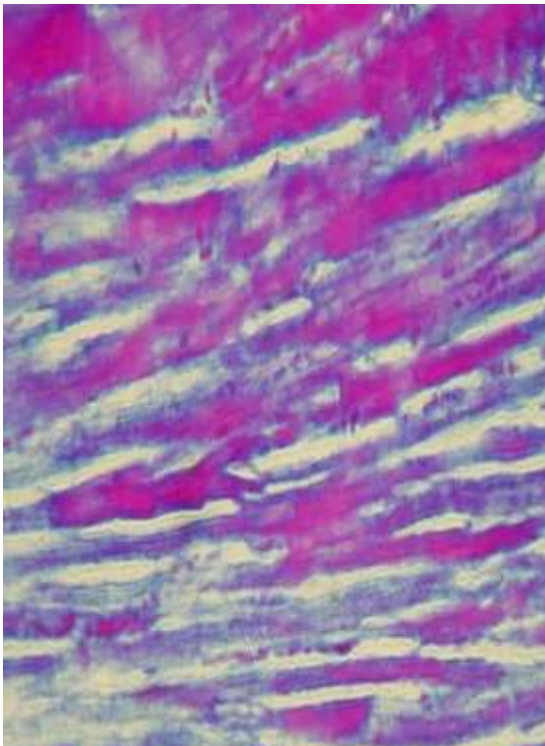


Fig.19: A photomicrograph of cardiac muscle fibres of a rat of group (T1) showing mild decrease in collagen fibres between the cardiac muscle fibres (Masson`s trichrome stain, x 400).

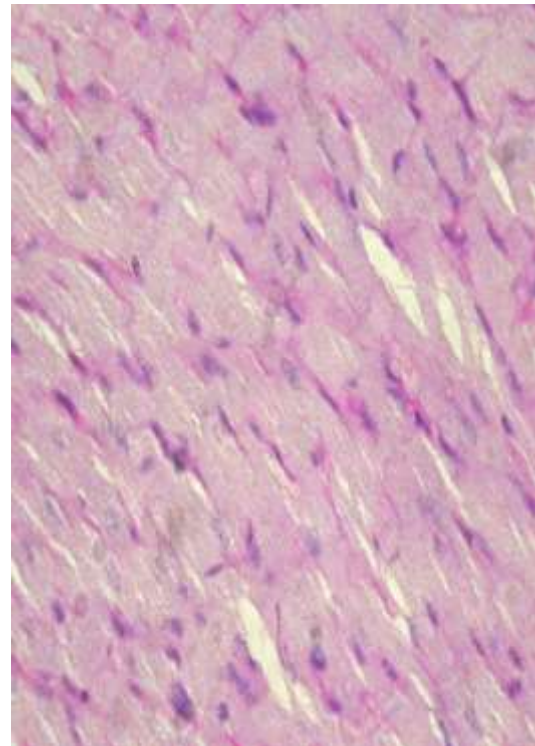


Fig .20: A photomicrograph of cardiac muscle fibres of a rat of group (T1) showing mild increase of PAS + ve materials (magenta red) in the cardiac muscle fibres (PAS reaction x 400).



Fig. 21: photomicrograph of cardiac muscle fibres of a rat of group (T1) showing restored striation(transverse arrow) myofibroblast nucleus(vertical arrow)and lymphocytic infiltration (semithin section (red arrow),Toluidine blue stain X 1000).

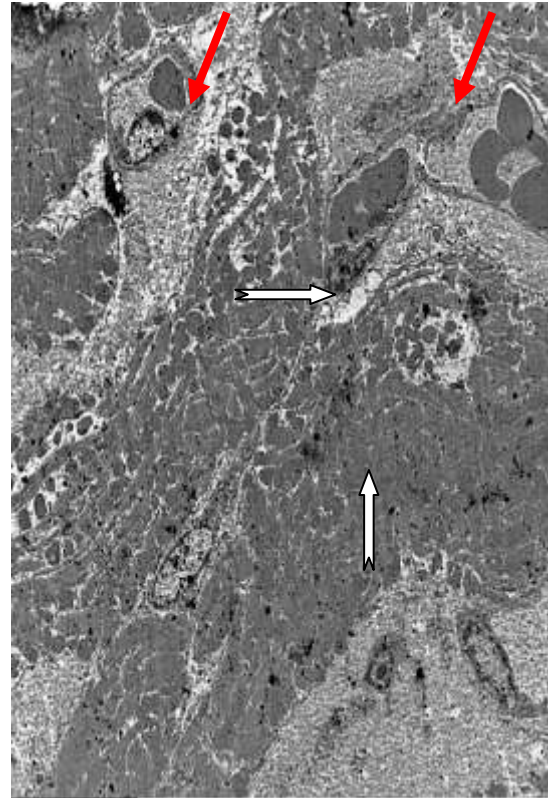


Fig. 22: An electron-micrograph of cardiac muscle fibres of a rat of group (T1) showing nucleus of myofibroblast(transverse arrow) ,congested blood vessels (red arrows) with areas of loss of striation(vertical arrow) (X6000).

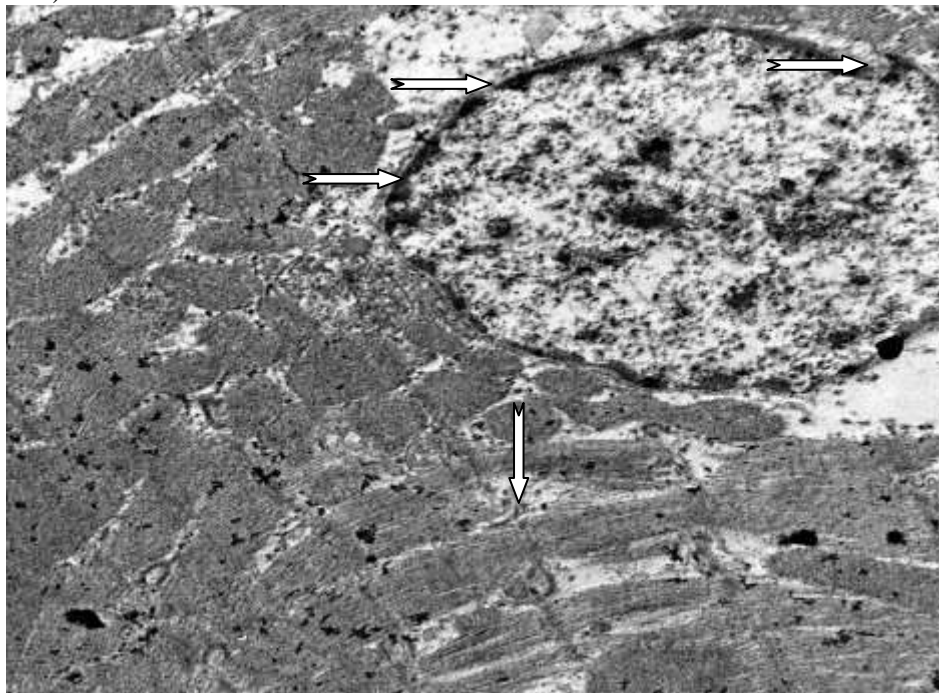


Fig. 23: An electron-micrograph of cardiac muscle fibres of a rat of group (T1) showing vascular nuclear and nuclear pore (transverse arrows) with separated and splitted myofibres(vertical arrow) (X15000).

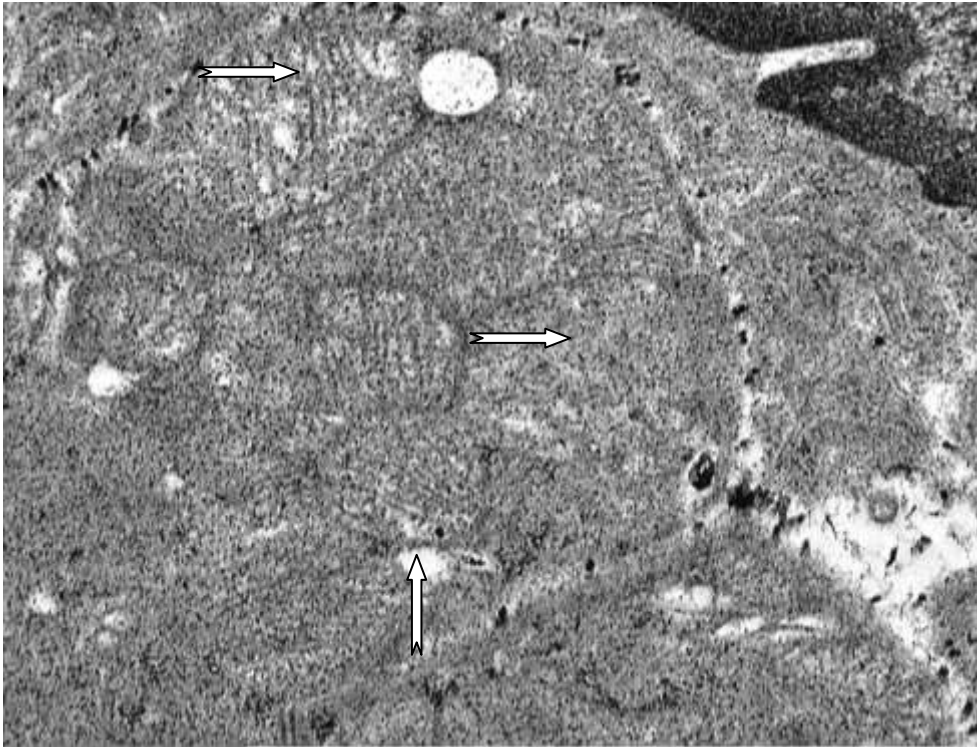


Fig. 24: An electron-micrograph of cardiac muscle fibres of a rat of group (T1) showing mitochondrial vacuolation (vertical arrow) with disorganization of their cristae (transverse arrow) (X30000).

4-Treated group two (T2)

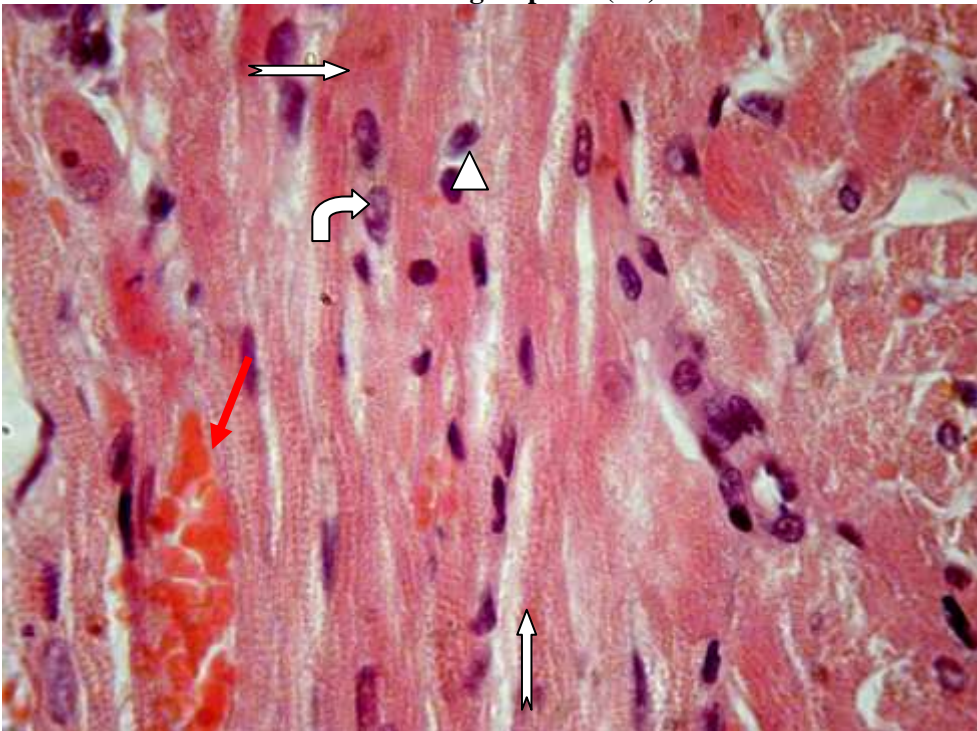


Fig. 25: A photomicrograph of cardiac muscle fibres of a rat of group (T2) showing patchy loss of striation(transverse arrow) with splitted muscle fibres(vertical arrow) , some nuclei become pyknotic(curved arrow) ,congested blood vessel (red arrow) and lymphocyticinfiltration(arrow head) (Hx & E \times 400).

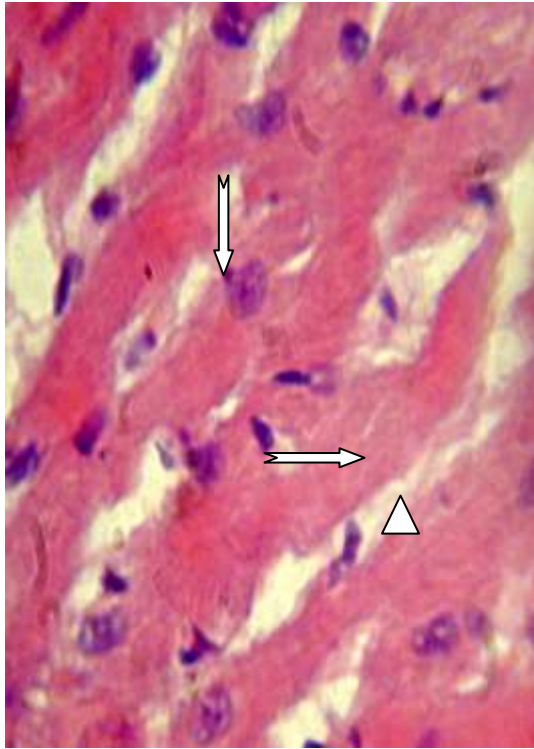


Fig. 26: A higher magnification of the previous section of group (T2) showing patchy loss of striation(transverse arrow) with splitted muscle fibres (arrow head) and pyknotic nucleus with margination of nuclear chromatin (vertical arrow) (Hx& E x 1000).

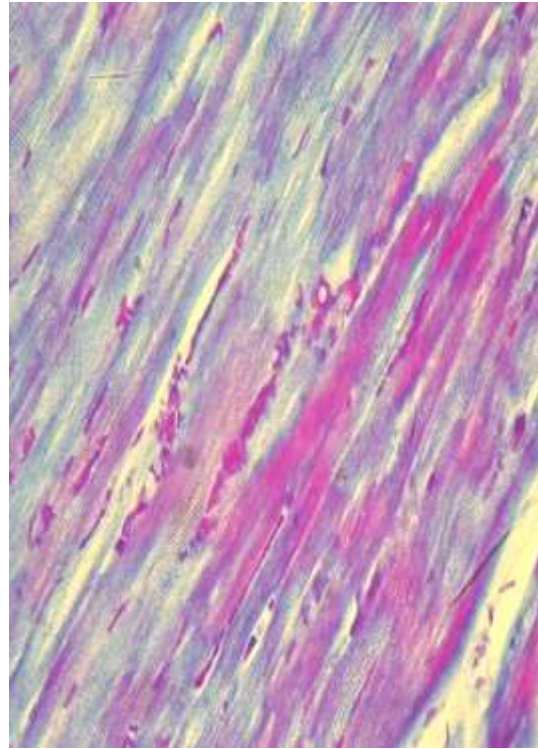


Fig.27:A photomicrograph of cardiac muscle fibres of a rat of group(T2) showing increased collagen fibres between the cardiac muscle fibres .Notice: focal areas of excessive collagen fibres deposition (Masson`s trichrome stain, x 400).

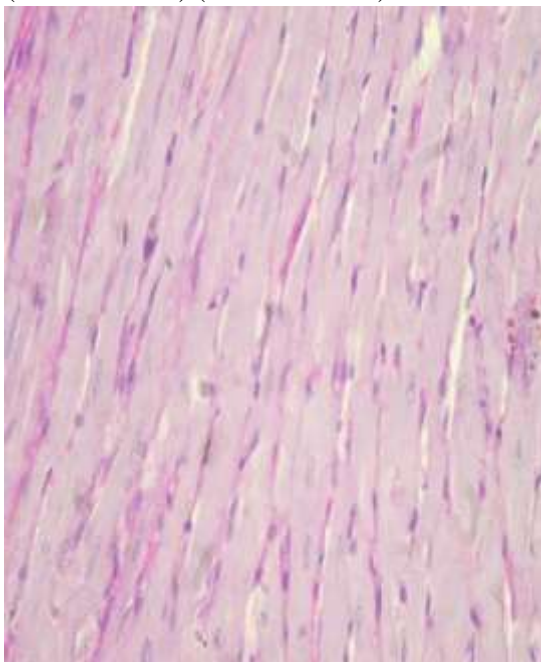


Fig. 28: A photomicrograph of cardiac muscle fibres of a rat of group (T2) showing decreased PAS+ ve materials (magenta red) in the cardiac muscle fibres (PAS reaction , x 400).

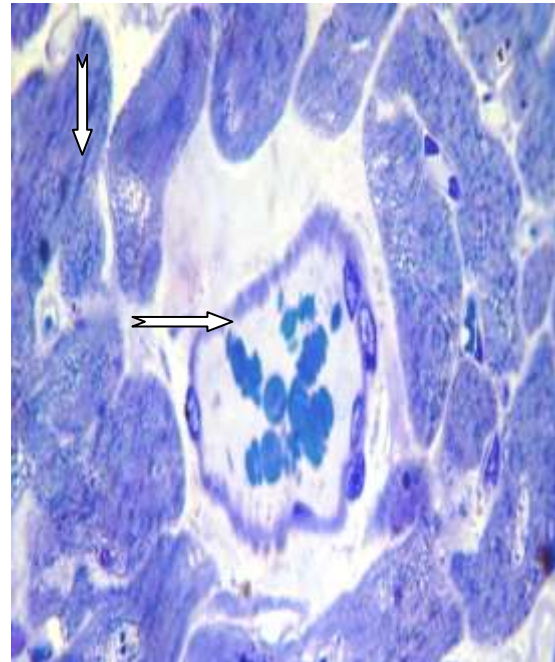


Fig. 29: A photomicrograph of cardiac muscle fibres of a rat of group (T2) showing loss of striation(vertical arrow) ,dilated and congested blood vessel(transverse arrow) (semithin section ,Toluidine blue stain x1000).

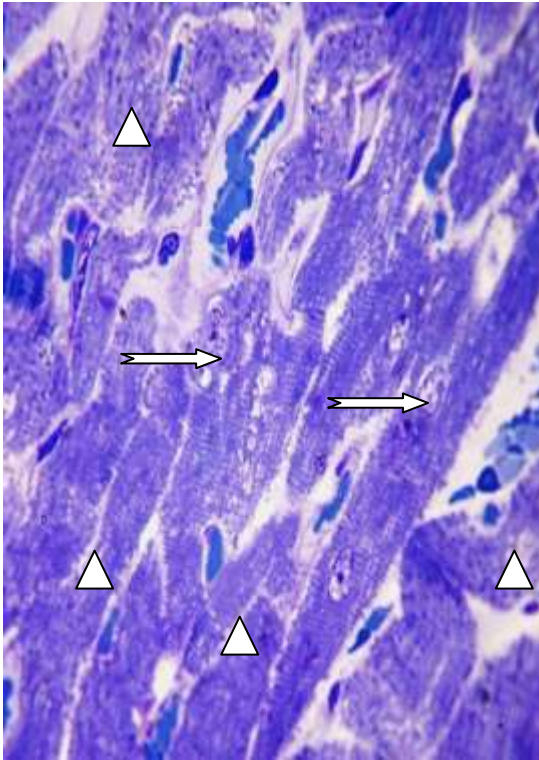


Fig. 30: A photomicrograph of cardiac muscle fibres of a rat of group (T2) showing loss of striation (arrow heads) with vacuolation and nuclear damage (transverse arrows), (semithin section, Toluidine blue stain, x 1000).



Fig. 31: An electron-micrograph of cardiac muscle fibres of a rat of group (T2) showing indented degenerated nucleus of cardiomyocytes (vertical arrows) (x15000).



Fig. 32: An electron-micrograph of cardiac muscle fibres of a rat of group (T2) showing pyknotic nucleus of cardiomyocyte (vertical arrow) with patchy loss of striation (transverse arrow) and disorganization of muscle fibril (arrowhead) (x 15000).

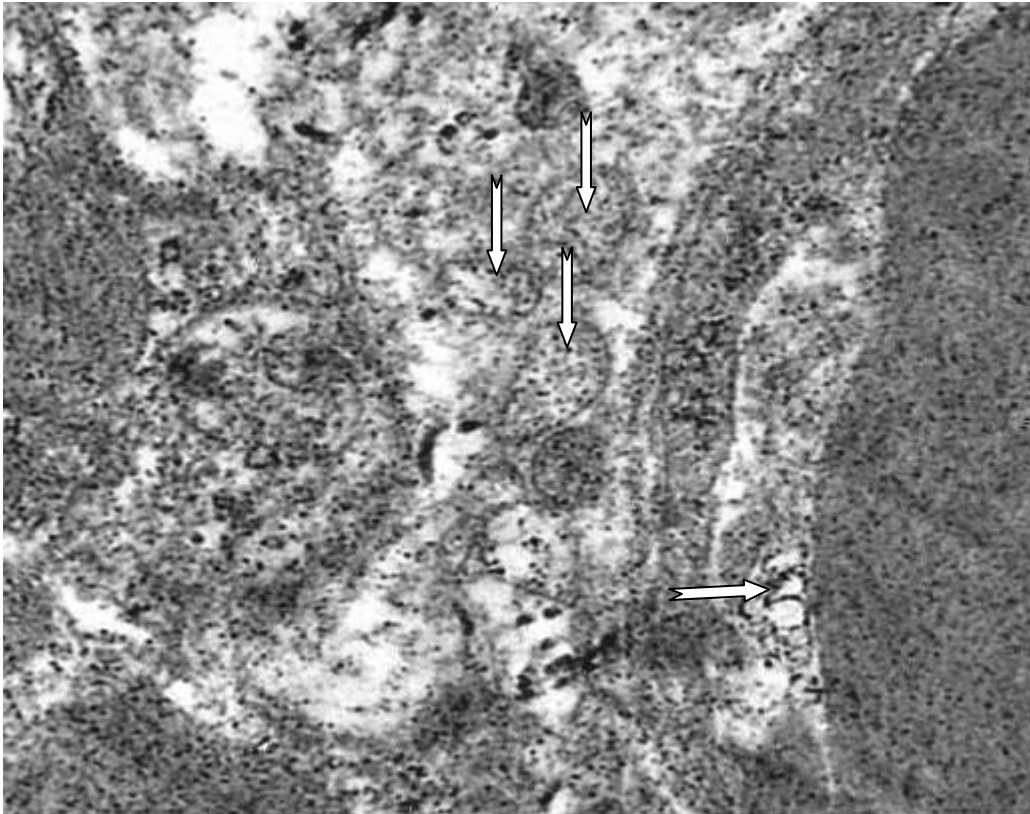


Fig. 33: An electron-micrograph of cardiac muscle fibres of a rat of group (T2) showing disorganization of cristae (vertical arrows) with swelling and destruction of mitochondria (transverse arrows) (X40000).

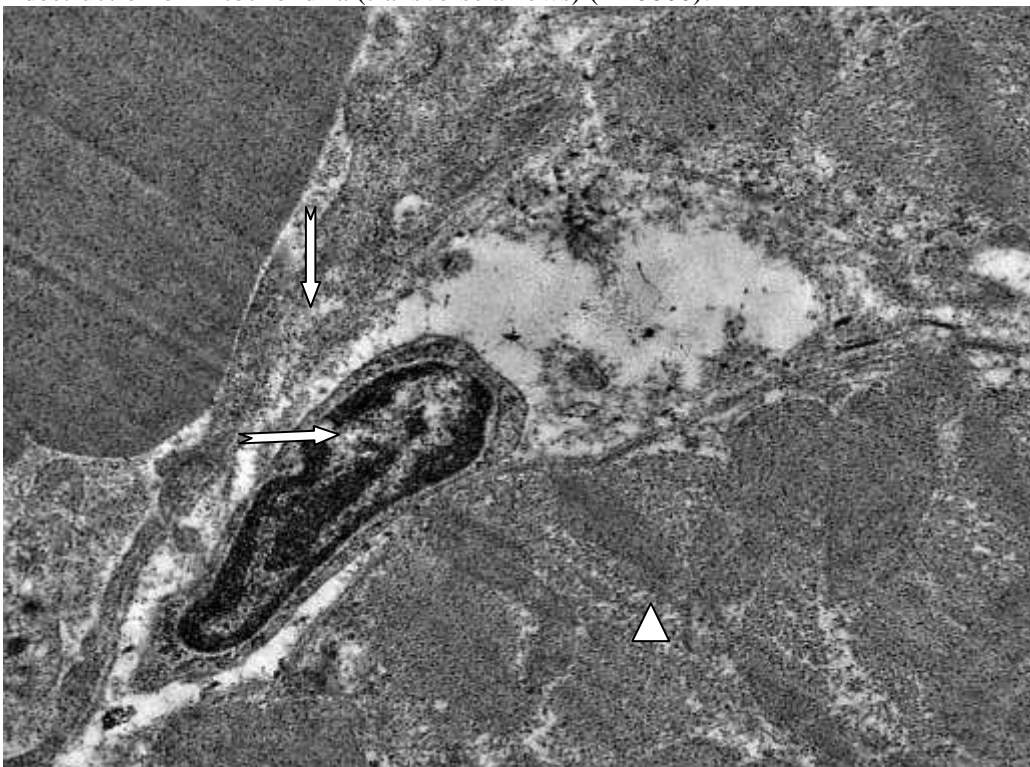


Fig. 34: An electron-micrograph of cardiac muscle fibres of a rat of group (T2) showing nucleus of myofibroblast (transverse arrow) with patchy loss of striation (arrow head) and highly destructed mitochondria with disorganization of their cristae (vertical arrow) (x 20000).

5-Recovery group one (R1)

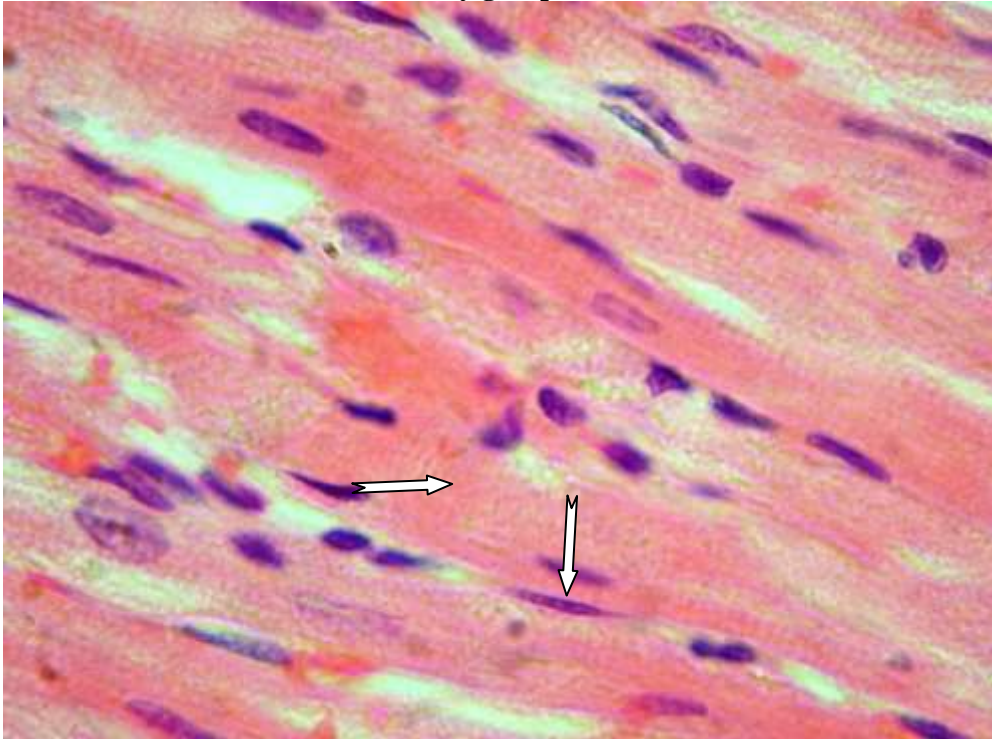


Fig.35 :A photomicrograph of cardiac muscle fibres of a rat of group **(R1)** showing restored striation(transverse arrow) and myofibroblast(vertical arrow) (H & E stain $\times 400$).

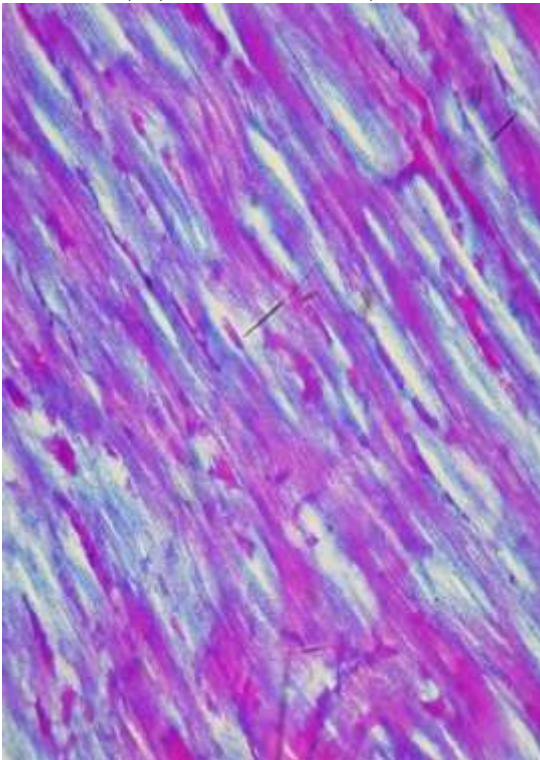


Fig.36: A photomicrograph of cardiac muscle fibres of a rat of group **(R1)** showing decreased collagen fibres between the cardiac muscle fibres (Masson`s trichrome stain , x 400).

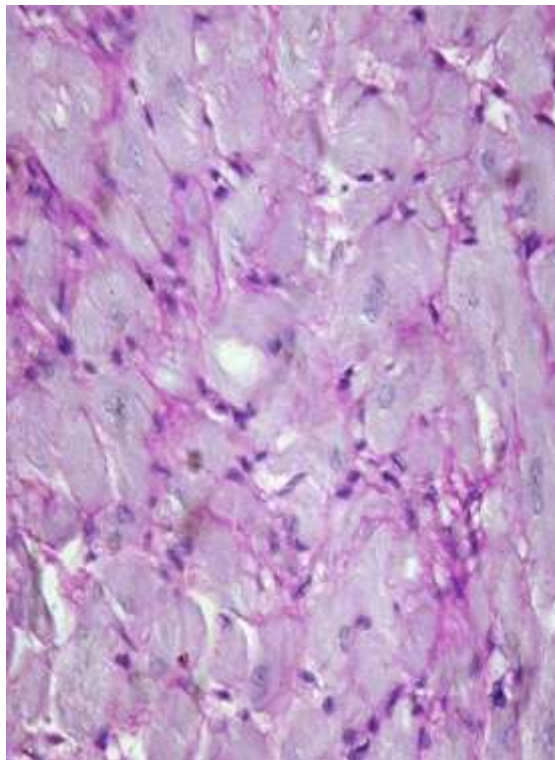


Fig. 37: A photomicrograph of cardiac muscle fibres of a rat of group **(R1)** showing increased PAS + ve materials (magenta red) in the cardiac fibres (PAS reaction x 400).

6-Recovery group two (R2)

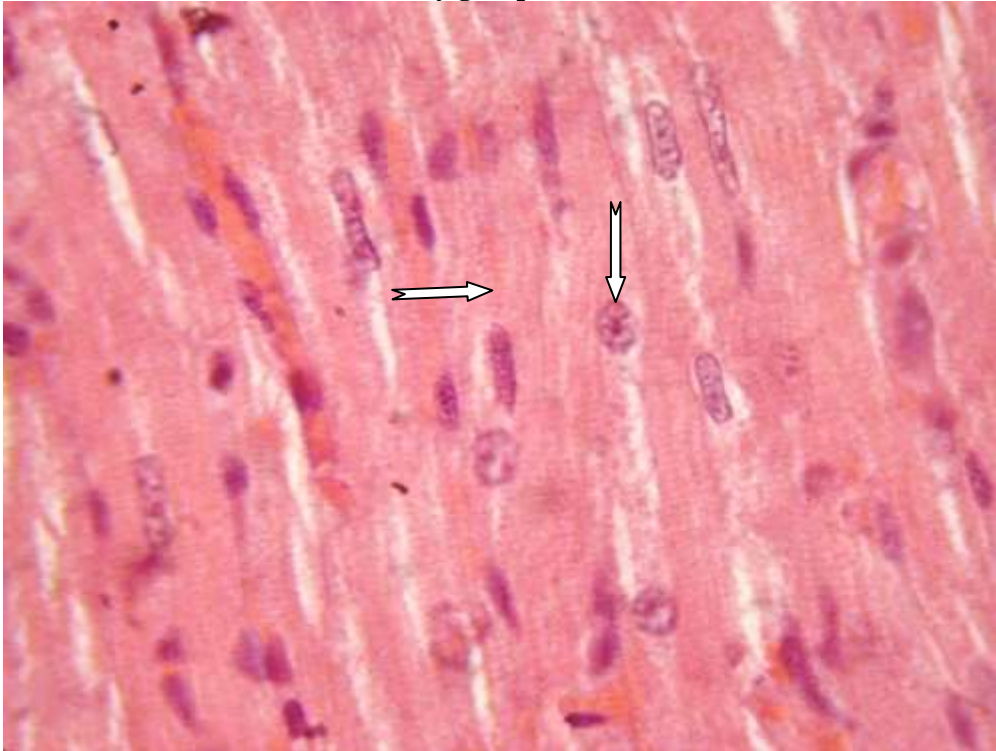


Fig.38: A photomicrograph of cardiac muscle fibres of a rat of group (R 2) showing restored of striation(transverse arrow) and central vesicular nuclei (vertical arrow) of the cardiac muscle fibres(Hx& E \times 1000).

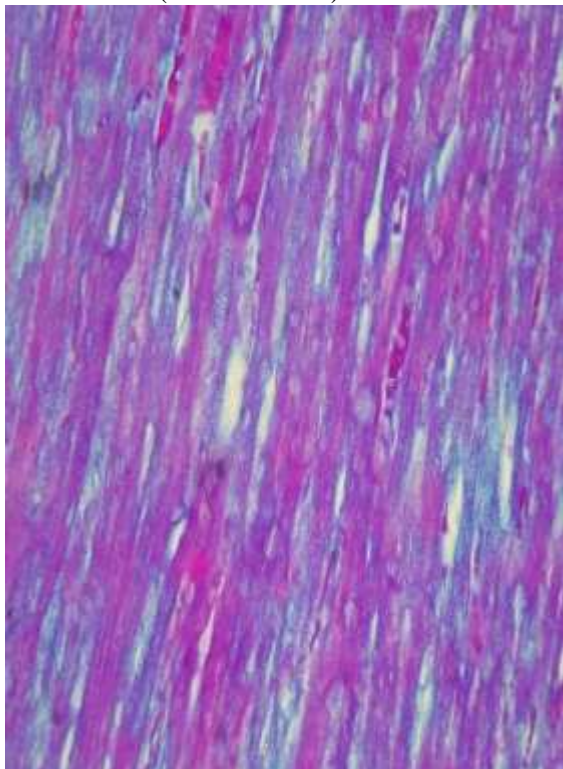


Fig.39: A photomicrograph of cardiac muscle fibres of a rat of group (R 2) showing decreased collagen fibres between the cardiac muscle fibres (Masson's trichrome stain, x 400).

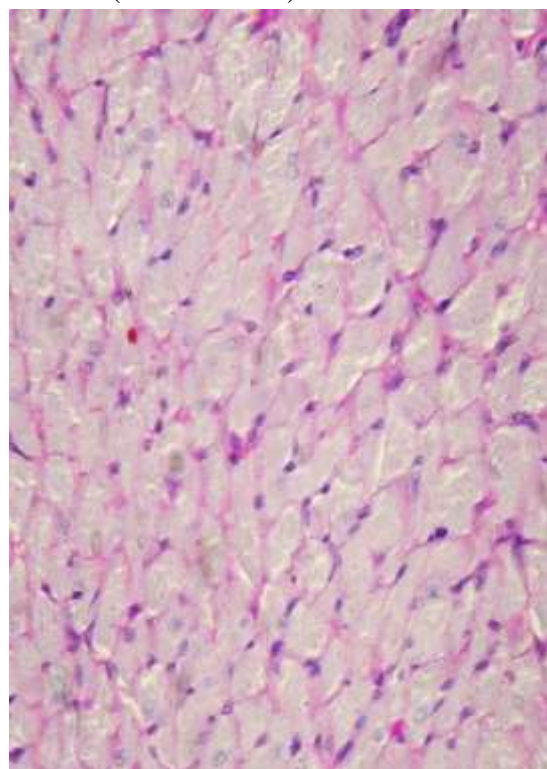


Fig.40: A photomicrograph of cardiac muscle fibres of a rat of group (R 2) showing increased PAS+ ve materials in the cardiac muscle fibers (PAS reaction x X 400).

Table. 1: percentage of collagen fibres.

	Group I	Group II	Group III	Group IV	Group V	Group VI
	control	Hyperlipidemic	Treated 1	Treated 2	Recovery 1	Recovery 2
Mean	0.91	1.81	1.52	1.98	1.35	1.21
N	10	10	10	10	10	10
SD±	0.12	0.34	0.23	0.45	0.19	0.16
Significance		p<0.05 Sig.	p<0.05 Sig.	p<0.05 Sig.	p<0.05 Sig.	p<0.05 Sig.

Table .2: Optical density of PAS reaction.

	Group I	Group II	Group III	Group IV	Group V	Group VI
	control	Hyperlipidemic	Treated 1	Treated 2	Recovery 1	Recovery2
Mean	1.04	0.69	0.81	0.78	0.89	0.98
N	10	10	10	10	10	10
SD ±	0.16	0.13	0.18	0.17	0.15	0.14
Significance		p<0.05 Sig.	p<0.05 Sig.	p<0.05 Sig.	p<0.05 Sig.	p<0.05 Sig.

Table. 3: Cholesterol level.

	Group I	Group II	Group III	Group IV	Group V	Group VI
	control	Hyperlipidemic	Treated 1	Treated 2	Recovery 1	Recovery2
Mean	84.37	149.45	121.76	102.51	98.68	95.72
N	5	5	5	5	5	5
SD ±	2.91	3.02	3.91	2.04	1.66	1.03
Significance		p<0.05 Sig.	p<0.05 Sig.	p<0.05 Sig.	p<0.05 Sig.	p<0.05 Sig.

Table. 4:Triglycerides level.

	Group I	Group II	Group III	Group IV	Group V	Group VI
	control	Hyperlipidemic	Treated 1	Treated 2	Recovery 1	Recovery2
Mean	90.62	158.85	134.20	115.10	105.73	98.73
N	5	5	5	5	5	5
SD ±	1.83	4.81	3.18	2.59	2.67	1.98
Significance		p<0.05 Sig.	p<0.05 Sig.	p<0.05 Sig.	p<0.05 Sig.	p<0.05 Sig.

Table. 5: LDL-C level.

	Group I	Group II	Group III	Group IV	Group V	Group VI
	control	Hyperlipidemic	Treated 1	Treated 2	Recovery 1	Recovery2
Mean	38.33	99.03	72.77	55.90	52.81	49.70
N	5	5	5	5	5	5
SD ±	0.05	1.28	1.12	1.03	0.97	0.78
Significance		p<0.05 Sig.	p<0.05 Sig.	p<0.05 Sig.	p<0.05 Sig.	p<0.05 Sig.

Table. 6: HDL-C level.

	Group I	Group II	Group III	Group IV	Group V	Group VI
	control	Hyperlipidemic	Treated 1	Treated 2	Recovery 1	Recovery2
Mean	27.91	18.65	21.90	23	24.73	26.29
N	5	5	5	5	5	5
SD ±	0.99	1.14	1.08	0.78	0.54	0.75
Significance		p<0.05 Sig.	p<0.05 Sig.	p<0.05 Sig.	p<0.05 Sig.	p<0.05 Sig.

Table .7 : VLDL

	Group I	Group II	Group III	Group IV	Group V	Group VI
	control	Hyperlipidemic	Treated 1	Treated 2	Recovery 1	Recovery2
Mean	18.12	31.77	26.84	23.02	21.14	19.74
N	5	5	5	5	5	5
SD ±	0.86	1.18	1.20	0.99	0.93	0.9
Significance		p<0.05 Sig.	p<0.05 Sig.	p<0.05 Sig.	p<0.05 Sig.	p<0.05 Sig.

Table .8 :Atherogenic index (AI)

	Group I	Group II	Group III	Group IV	Group V	Group VI
	control	Hyperlipidemic	Treated 1	Treated 2	Recovery 1	Recovery2
Mean	3.24	8.51	6.12	5	4.27	3.64
N	5	5	5	5	5	5
SD ±	0.61	1.25	0.93	1.58	0.79	0.72
Significance		p<0.05 Sig.	p<0.05 Sig.	p<0.05 Sig.	p<0.05 Sig.	p<0.05 Sig.