



## The effect of Diabetes mellitus (DM) induction on the Structure of Skeletal Muscle in experimental rats and the protective role of arginine on (DM) complications (Ultrastructural Study )

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### ABSTRACT

**Objective:** The aim of this study was detect the ultrastructural alterations of gastrocnemius skeletal muscle related to diabetic rats and control was studies , role of arginine as protective agent against (DM) complication was clarified at each period (15,30,45) days post induction.

**Methods:** Thirty rats divided into two groups diabetic and nondiabetic group , the diabetic group are subdivided to two groups , the first given tap water , the second given tap water containing L-arginine dissolved in the drinking water.

**Results:** Ultrathin sections from skeletal muscles biopsies showed myofibrils with sever contraction , atrophied and less regular , disorganized Z-line , variable degree of axonal atrophy , congested capillaries, post(30)days, abnormal myofibrils, changes in mitochondrial position and showed subsarcolemma location, large lipid droplets , distortion of sarcoplasmic reticulum , lamellar formation of collagen, also post (45) day, revealed to irregular T-tubules , absence of Z-line , degenerated myofibrils , muscle fibers fragments , fibers splitting . The study determined the effect of arginine on skeletal muscle related to diabetes rats at each period (15,30,45 ) days , results recorded condensed myofibrils,more regular,well developed, regular Z-line and clear lamellar structure of collagen , the axoplasm filled with mitochondria , normal schwann cell nuclei and deposition of glycogen granules in the interstitial space, oriented Z-line , myotube with normal myonuclei and the arrangement of T-tubules more obvious , the results showed that the arginine resulted in minimized the histological changes and reduced STZ-induced ( DM) and its toxic effect that seen in rats with (DM).

**Conclusion:** Strong association between ultrastructural changes of diabetic skeletal muscles and their innervation was established and the results referred to arginine as protective, bioactive agent reduced myopathy , neuropathy of diabetic muscles.

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## INTRODUCTION

This disease causes disorder with metabolism of proteins, carbohydrates and fats due to functional deficiency of insulin, which can be characterized by an elevation of blood glucose, this causes abnormal metabolism lead to hyperglycemia, resulting in a high risk of complications in kidney and neuropathy, state that (DM) is used to describe a condition characterized by chronic hyperglycaemia and other disorders of carbohydrate, fat and protein metabolism<sup>1,2</sup>.

Many factors caused muscles atrophy, including prolonged disuse, ageing and chronic disease such as (T2DM), muscle atrophy was result of imbalance between the rate of contractile protein synthesis and degradation, in catabolic conditions muscle atrophy in combination with inactivity can decrease the capacity to perform activities of daily living, quality of life and subsequently increase mortality<sup>3,4</sup>.

Myopathies complications were also found and even though less than the vascular complications, compromises the quality of patients life, it is a clinical condition commonly characterized by a smaller muscle mass (atrophied), weakness and reduced physical capacity<sup>5</sup>.

Diabetes has been associated with impaired angiogenesis in skeletal muscles, reduction in capillary diameter and number also reduced capillary diffusing capacity in animal models of diabetes, in addition regression of the capillary network in skeletal muscle with diabetes, e.g., decreased diameter, tortuosity and volume, therefore regression of the capillary network in skeletal muscle can negatively impact O<sub>2</sub> exchange, and consequently may play a role in diabetic complications<sup>6,7</sup>.

Diabetic polyneuropathy (DPN) was common complication of (DM) associated with changes in the neuromuscular system and motor dysfunction, as a result of DPN can manifest broadly as muscle atrophy, weakness, and increased susceptibility to fatigue, these changes regarded to a complication of DM induced alterations to the motor neuron, neuromuscular junction and skeletal muscle fibers<sup>8,9,10</sup>.

DM is characterized by peripheral neuropathy of sensory and motor nerves, peripheral nerves and muscles dysfunctions have been demonstrated in both humans and rodents, the morphological destabilization of (NMJ) in diabetes such as axonal degeneration, axonal atrophy and demyelination has been explained<sup>11</sup>.

Amino acids are important organic compounds, in mammals amino acids can be divided into essential, semi essential and non-essential amino acids, arginine is classified as a semi essential or conditionally essential amino acid, depending on the developmental stage and health status of the individuals<sup>12</sup>.

Arginine is the sole precursor of nitric oxide (NO), a signal molecule, among others, involved in immune responses, angiogenesis, epithelialization and formation of granulation tissue, the amino acid arginine has been identified as an important mediator in wound healing<sup>13,14</sup>. Moreover nitric oxide synthases (NOS) are

enzymes which produce nitric oxide (NO) from L-Arginine, three major isoforms of this enzyme have been isolated and endothelial (eNOS), inducible (iNOS) and neuronal (nNOS), the latter occurs in several cell types, including neurons and skeletal muscles, both nNOS and eNOS are Ca<sup>2+</sup>-dependent and constitutively expressed, whereas iNOS is Ca<sup>2+</sup>-independent and expressed abundantly in response to immunological challenges<sup>15,16</sup>.

## MATERIALS AND METHODS

### Experimental animals

Thirty healthy adult virgin females Wistar albino rats (*Rattus norvegicus*) age (10 -12) weeks and the average weight (225±25) gm, which are breed at the animal house of the Science college, Al-Basrah University. The animals are housed under controlled standard conditions in a temperature (20 -23) °C, controlled room on a (12: 12) Light:Dark cycle, they are randomly isolated in plastic cages with hygienic bed and were fed on standard laboratory food. The animals divided into two group (non diabetic and diabetic group) with about 24 female in diabetic group and 6 female nondiabetic group (control group), The diabetic group are subdivided into two groups 12 of each one, both of them are single injected intraperitoneally with single dose of streptozotocin (STZ) (60 mg/ kg of body weight), with mean 10 rats females for each period (15, 30, and 45) days post induction of DM. The first subdivided group( diabetic group ) were given tap water but the second subdivided group (diabetic treated with arginine ) were given tap water containing L-arginine nitro-L-arginine methyl ester dissolved in the drinking water at 10 mg/L, 3 days after (STZ) injection<sup>17</sup>.

### Sacrificed the experimental rats

Fasting overnight experimental rats from each group (treated and control) were randomly sacrificed after being anaesthetized with overdose of chloroform and Sacrificed on ( 15 - 30 - 45 ) day post diabetic induction, then samples were collected included hind limbs skeletal muscle (gastrocnemius )Specimen's from rats muscles were exiced and cut to small pieces, then prepared for ultrastructural study by ( TEM )<sup>18</sup>.

## RESULTS

Results on ultrathin sections from control rat gastrocnemius muscles showed that the fibers consist of regularly, rounded myofibrils completely separated from each other with spaces from sarcoplasm and appeared with variable width, the muscle fibers surrounded with sarcolemma that it is continued within the skeletal muscle fibers as numerous transverse tubules which appeared as invaginations among the myofibrils, there was regular distribution of the myofibrils in each cross-section, normal mitochondria near the myofibrils or sometimes wrap around the fibrils and numerous located near or deep of the sarcolemma and fine bands extend to separate between myofibrils referred to endomysium, moreover the nuclei oval shaped, periphery with one or more nucleoli, and some organelles appeared as rim

around the nucleus, satellite cell easily observed also capillaries around fibrils and within interfibrillar space, well developed sarcoplasmic reticulum (SR) and vesicles from it found between some myofibrils (Fig. 1,2,3).

Ultrathin sections from diabetes rats gastrocnemius after 15 days revealed to damaged myofibrils, most sarcomeres had distorted (Z) line and other absent from Z-line, clear atrophied myofibrils, swollen and irregular mitochondria appeared with less cristae, fewer in number than the control, smaller mitochondria with crescent shape surrounded with focal area was also noticed, absent of T-tubules within the myofibrils, more lipid droplets usually in juxtaposition to the mitochondria, or out of the mitochondria under the sarcolemma, oval myonuclei under the sarcolemma with their heterochromatin distributed along the inner surface of the nuclear envelope showed discontinuity at some points, Irregular boundaries, surrounded with degenerated zone and amorphous material most of glycogen granules, variable condensed of myofibrils and different shaped, some of circular and smaller, other arranged longitudinally showed changes with sarcolemma (Fig. 4,5).

Ultrastructural changes on motor terminal revealed to Schwann cell surrounded the axon terminal, dense mitochondria and glycogen, vesicles and granules deposited, obvious changes were appeared that the sarcolemma with folded and degenerated in the region and form poor folds near motor terminal ending in addition disorganized Z line (Fig. 6,7,8).

Photograph in ultrathin sections from gastrocnemius muscle showed degenerated area with irregular bands of myofibrils and these fibrils appeared with different shape and size, changes with axon morphology of myelinated nerve fibers as early as 15 days after the injection of (STZ) was observed and variable degree of axonal atrophy seen, and demyelination figures, Dilated, congested capillaries with increased thickness of basal lamina with clear degenerated pericytes observed (Fig. 9,10,11,12).

Variable changes on ultrastructural features post 30 days of diabetes like abnormal myofibrils, changes in mitochondrial position to a subsarcolemmal location, severe atrophy and contracted myofibrils, large lipid droplets, distortion of (SR), changes with myonucleus hyperchromatin and irregular boundaries (Fig. 13,14,15), degenerating capillaries and thickening in basal lamina observed, there was accumulation of glycogen granules, some myofibers exhibited pyknotic nucleus whereas others show focal area of degeneration, in addition collagen, fibrin and reticulin fibers were seen between the completely degenerated myofibrils, pyknotic nucleus of myofibroblast and collapsed of endoneurial vessels, also changes in ultra structural of matrix (ground substance) was observed reveals to granular material fill the spaces between collagen and elastic fibers, developing fibre consist of small microfibrils composed mainly from fibrin, Lamellar structures as parallel tubules with undulating borders found close to nucleus and near the plasma membrane (Fig. 16,17,18).

Micrograph on diabetes gastrocnemius post 45 days of STZ-induced showed absence of Z-line, distortion (SR), irregular arrangement of T-tubules and degenerated myofibrils, satellite cell observed beneath the sarcolemma, variable size of muscle fiber fragments, fibers splitting and clusters with the same internal structure, sometimes more than one showed more advanced degenerative changes and may be separated from the origin fibers (parent) or connect by narrow cytoplasmic bridge and others connect with the muscle fiber (Fig.19,20).

The basement membrane formed redundant processes, projection and loops around highly atrophic myofibrils show with small fragments and pyknotic nuclei, some splitting myofibrils different myelin structures with variable size and shape associated with degenerated myofibrils, lipid droplets with different size in close to SR. and near the mitochondria, there was endothelial hyperplasia of most endoneurial vessels surrounded with pericyte and focal degeneration around nucleus, the myelin fibers with atrophied axon also changes occur within intramuscular nerve (Fig. 21,22).

The study clarified the role of arginine on ultrastructural changes of gastrocnemius in diabetes rats at 15 days of STZ-induced, the micrograph showed that the myofibrils more regular, condensed, some arranged longitudinally with still others appeared degenerated, the intercellular spaces normal with accumulation of glycogen granules, well developed (SR), regular Z-line and lamellar structure of collagen appeared (Fig. 23,24). Primary motor terminal showed, the axoplasm filled with mitochondria, secretory vesicles and the schwann cell nuclei surrounded it, other myelinated figures appeared and heavy deposition of glycogen in the interstitial space (Fig. 25,26).

Electron micrograph from gastrocnemius post 30 days of STZ-induced and treated with (arg) revealed to normal nuclei beneath the subsarcolemmal space (Sb) with normal boundaries and peripherally hyperchromatic rim, the (Sb) filled with glycogen, dense granules and amorphous material, the arrangement of invagination regarded to T-tubules shown and the myelin figures more obvious, some of small remyelinated fibers, sometimes the surface of sarcolemma showed with filamentous processes towards the interstitial space between myofibrils, number of satellite cells and the Z line was obvious, moreover normal capillaries appeared with less basement membrane thickness, the myelin fibers with less atrophied axons and moderate distribution of lipid droplets among the sarcomere (Fig. 27,28,29).

Ultrathin sections from gastrocnemius at 45 days post STZ-induced diabetes and treated with arginine indicated to variable changes, giant mitochondria, oriented Z-lines in some regions, at the same time the sarcomere appeared more regular, more number of mitochondria, the active satellite cells extended between myofibrils, myotubes like cells with well normal myonuclei was noticed, the arrangement of T-tubules more obvious and clear, some of atrophied, rounded

myofibrils still found accompanied the normal myofibrils (Fig. 30, 31, 32, 33).

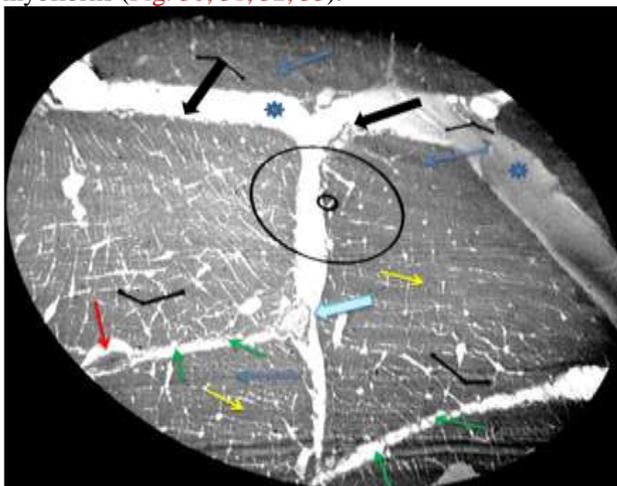


Figure 1: Electron micrograph of transverse section of gastrocnemius muscle related to control rat showed regular muscle fibers (→), the muscle fibre surrounded with sarcolemma (→), separated from each other with clear space (\*), regular and condensed myofibrils (→), numerous (T) tubules (→), satellite cells (→) with blood vessel (→) noticed. (1650 X).

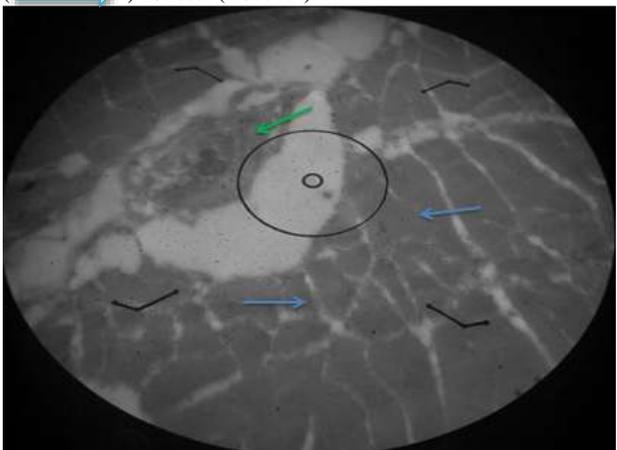


Figure 2: Electron micrograph on transverse section from control gastrocnemius muscle showed condensed, regular myofibrils (→), peripheral euchromatic nuclei (→) (16000 X).

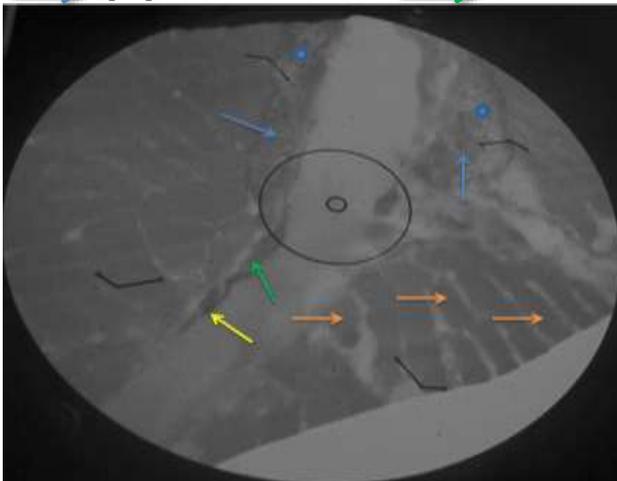


Figure 3: Photomicrograph on skeletal gastrocnemius muscle from rat showed peripheral oval nuclei (→), with granular chromatin (\*), dark (→) bands, one satellite cell (→), with long process (→) (8700 X).

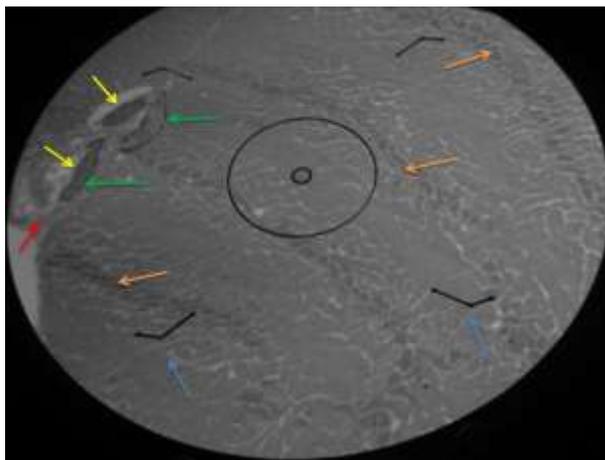


Figure 4: Electron micrograph on skeletal gastrocnemius muscle after 15 day of diabetes induction showed atrophied myofibrils (→), disorganized Z line (→), degenerated myonuclei (→), focal degeneration area (→), and amorphous material (→) (8700 X).

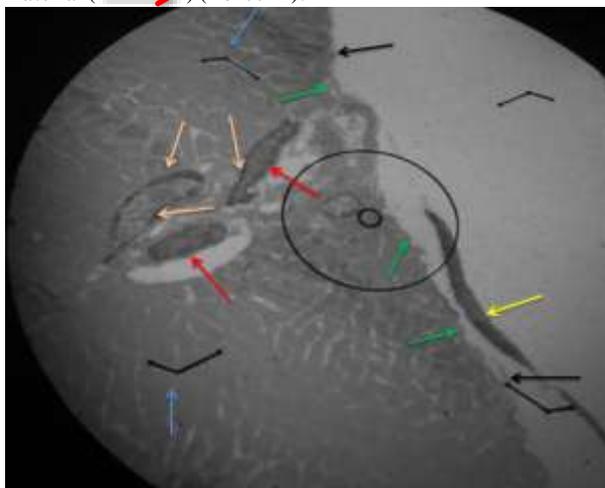


Figure 5: Photomicrograph on gastrocnemius muscle after 15 day of diabetes induction showed two muscle fiber (→), condensed irregular myofibrils (→), satellite cell (→), above the sarcolemma (→) which appeared irregular and folded, the boundaries of myonuclei (→) showed irregularity with discontinuity of nuclear envelope (→) (12500 X).

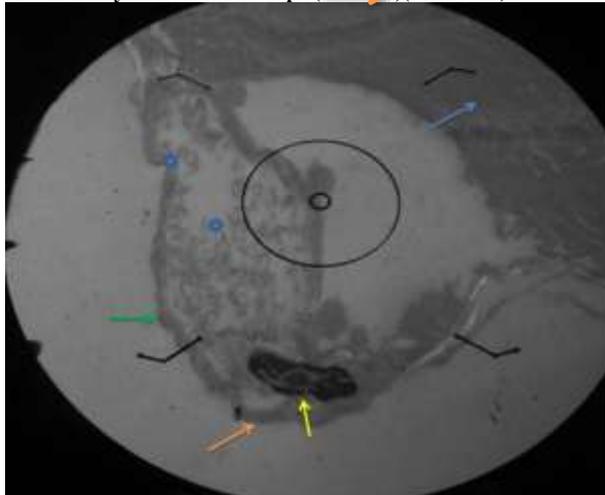


Figure 6: Photomicrograph of transverse section of gastrocnemius muscle related to diabetes rats at (15) day of diabetes induction showed degenerated myofibrils (→), axon terminal (\*) with vesicles, surrounded by schwann cell (→) with obvious nucleus (→), thickening of axolemma (→) (4400 X).

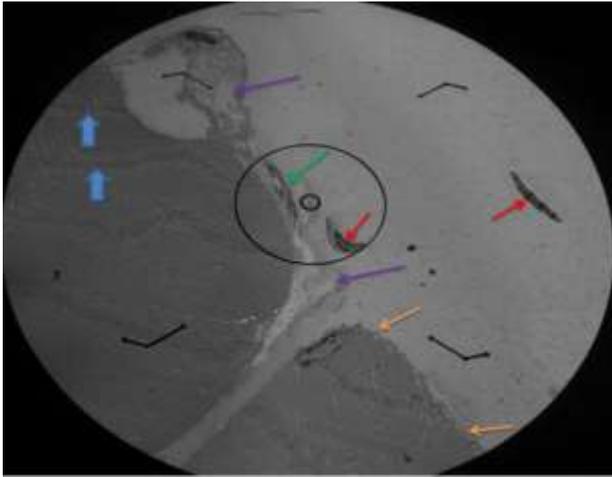


Figure 7: Photomicrograph of transverse section of gastrocnemius muscle related to diabetes rats at 15 day of diabetes induction showed two neighboring myofibrils(→), folded, irregular sarcolemma (→), beneath the sarcolemma, small blood vessels (→), satellite cell (→) above the sarcolemma, primary motor ending (→), and irregular Z line(→) ( 4400 X).

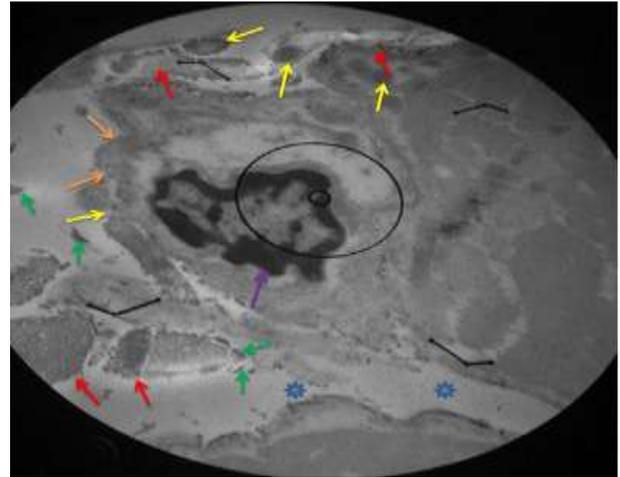


Figure 10: High power on electron micrograph of gastrocnemius muscle related to diabetes rats at 15 day of diabetes induction showed, thick, folded axolemma(→), dense glycogen granules (→), destructed mitochondria (→), cellular debris (→)within the intercellular space (→) and heterochromatin nucleus (→) of schwann cell ( 31000X ).

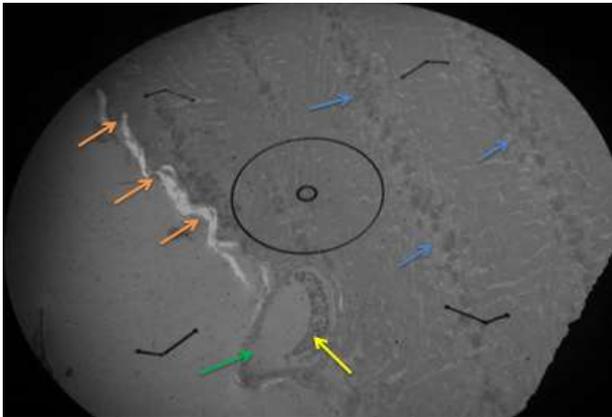


Figure 8: Photomicrograph section of gastrocnemius muscle related to diabetes rats at (15) day of diabetes induction showed disorganized Z line (→),folded sarcolemma form folds (→) and large capillary (→) with pericyte (→)( 6200 X).

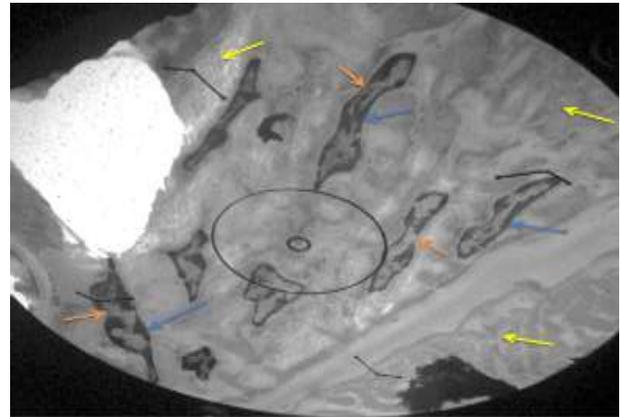


Figure 11: Electron micrograph of gastrocnemius muscle related to diabetes rats at 15 day of diabetes induction showed variable number of large nerve fibers(→) with atrophied axon, discontinuity of myelin sheath (→) and degenerated myofibrils (→) (12500 X).

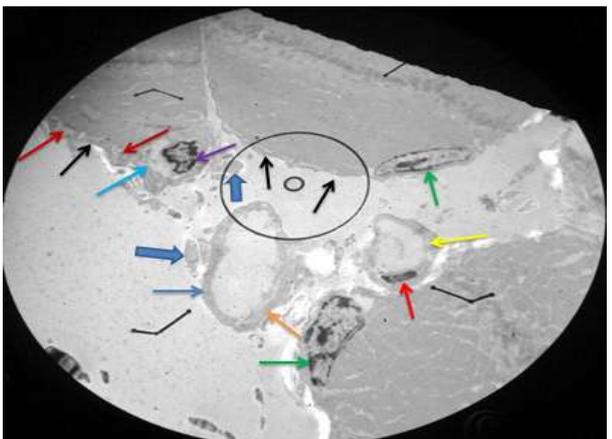


Figure 9: Photomicrograph section of gastrocnemius muscle related to diabetes rats at 15 day of diabetes induction showed dilated capillary (→)with thickening of basal lamina (→), other congested capillary (→)with obvious pericyte (→), most myonuclei showed irregular boundaries (→), axon terminal (→)surrounded with schwann cell (→), folded sarcolemma (→)and dense vesicles (→), destroyed mitochondria (→)was also seen ( 6200 X).

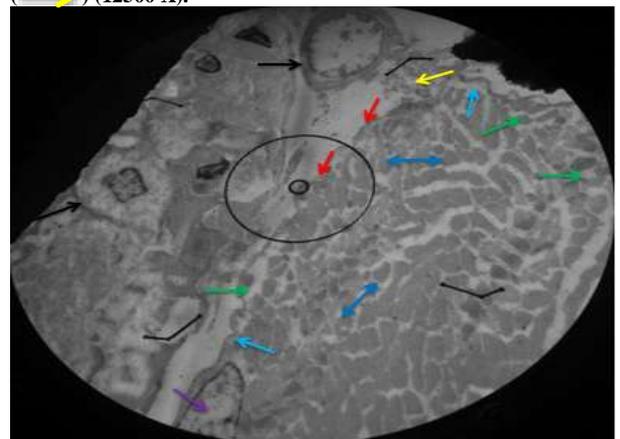


Figure 12:Photomicrograph of gastrocnemius muscle related to diabetes rats at 15 day of diabetes induction showed large nerve fibers with loose myelin sheath (→), glycogen granules deposition (→), degenerative mitochondria (→), folded sarcolemma (→) in some regions and degenerated at other region (→)and most myofibrils was circular and disorganized (→), degenerated myonuclei (→) with loss the nucleoli (→) ( 8700 X).

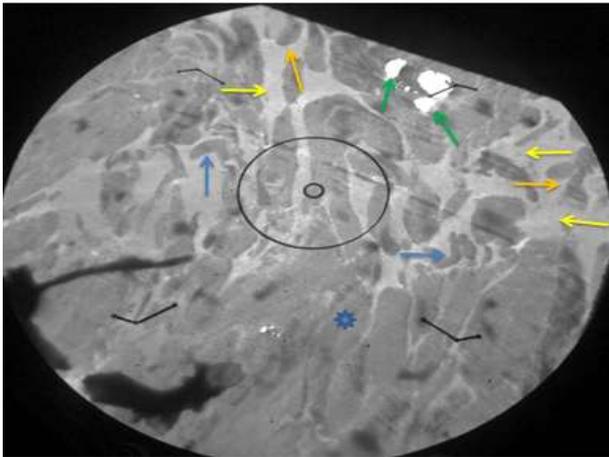


Figure 13: Electron micrograph on gastrocnemius muscle related to diabetes rats at (30) day of diabetes induction showed sever atrophied myofibrils (→) disorganized, other A band noticed (★), dilated intercellular spaces (→), distortion sarcoplasmic reticulum cisternae (→) and swollen, degenerative mitochondria (→) (2400X).

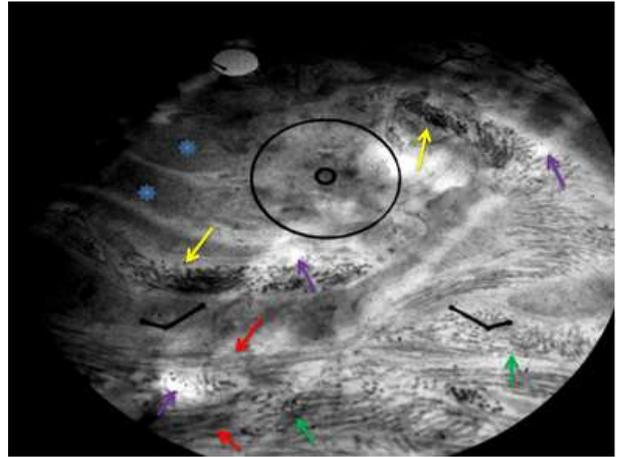


Figure 16: Electron micrograph on gastrocnemius muscle related to diabetes rats at (30) day of diabetes induction showed irregular, distortion sarcoplasmic reticulum (→), A - band (★) noticed, the collagen fibers (→), reticulin (→) and other lamellar fibers (→) noticed (31000 X).

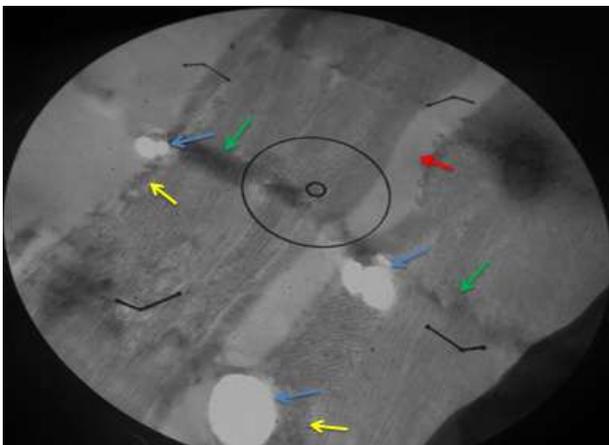


Figure 14: Longitudinal section of gastrocnemius muscle related to diabetes rats at (30) day of diabetes induction showed number of lipid droplets (→), disorientation Z line (→), electron-dense granules (→) and dilated intercellular spaces (→) (16000 X).

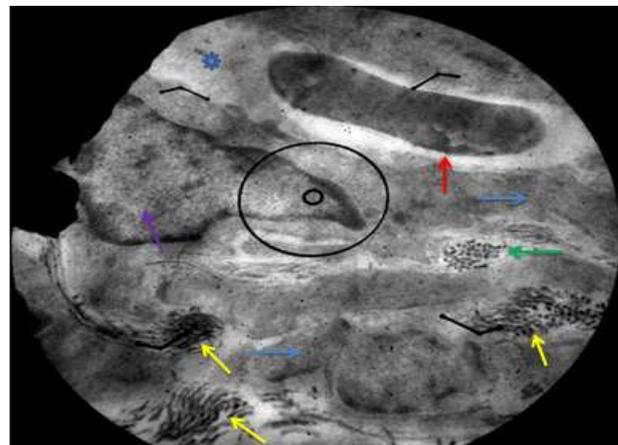


Figure 17: Electron micrograph on gastrocnemius muscle related to diabetes rats at 30 day of diabetes induction showed degenerated myofibrils (→), dense glycogen granules (→), degenerated myonuclei (→) with irregular boundaries, accumulation of fibrin (→) among the myofibrils and condensed myonuclei (→) with dilated interfibrillar space (★) (16000 X).

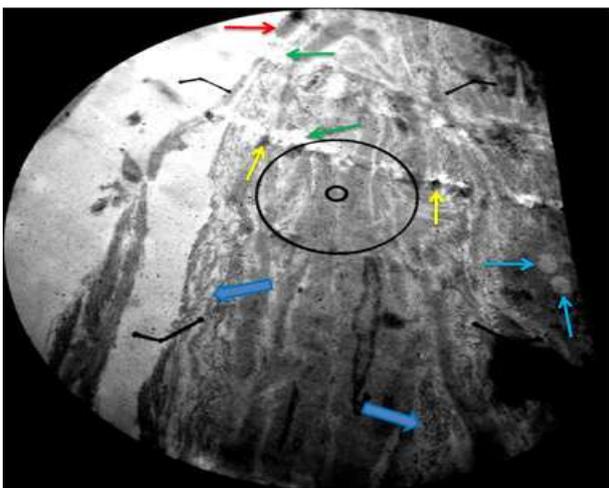


Figure 15: Longitudinal section of gastrocnemius muscle related to diabetes rats at (30) day of diabetes induction showed irregular myofibrils (→), distortion of sarcoplasmic reticulum (→), reduced T- tubules (→), mitochondria (→) located subsarcolemma, and other mitochondria (→) loss their cristae, collagen fibers deposition (→) (8700X).

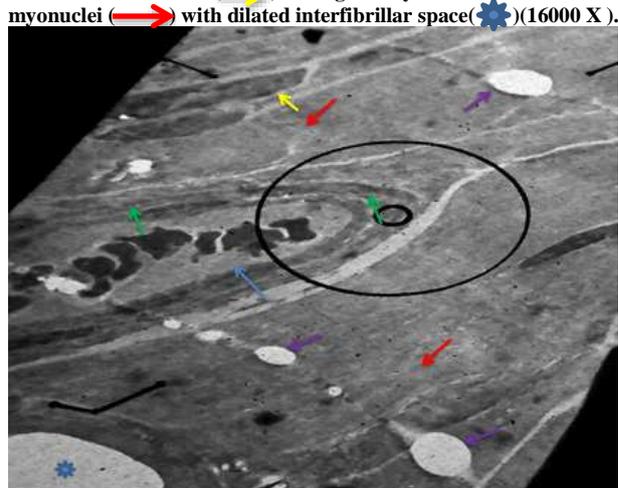


Figure 18: Electron micrograph on gastrocnemius muscle related to diabetes rats at 30 day of diabetes induction showed congested endonural blood vessels (→), thickening of wall (→), increased lipid droplets (→), pyknotic myonuclei (→), large myelin nerve fiber (★) the myofibrils loss their normal structure (→) (1650 X).

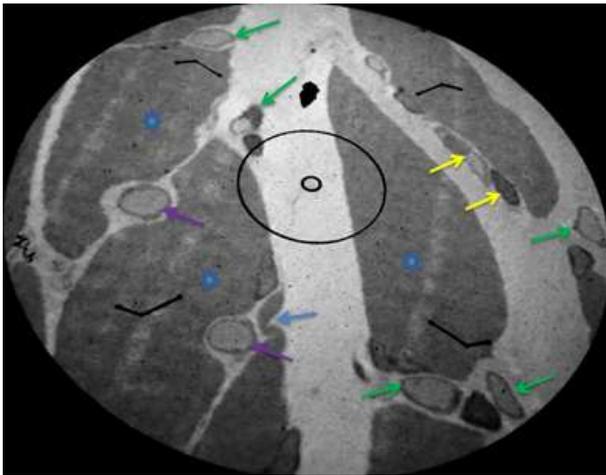


Figure 19: Transverse section on gastrocnemius muscle related to diabetes rats at 45 day of diabetes induction showed damaged myofibrils(★), splitting of ending(→), absent of intact sarcolemma, variable size and shape of muscle fibers fragments (→), collapsed capillaries(→) and degenerated myonuclei (→) (2400 X).

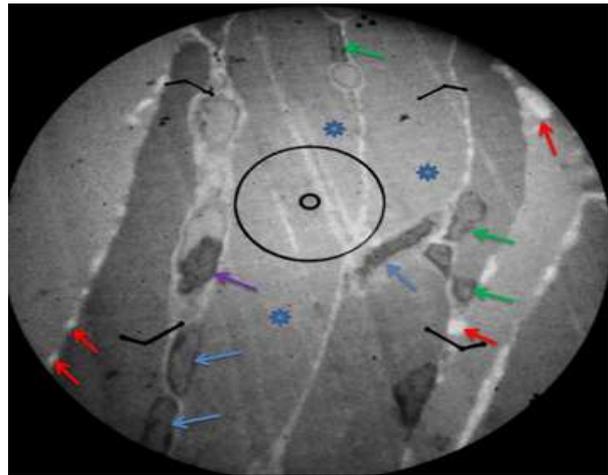


Figure 22: Electron micrograph on gastrocnemius muscle related to diabetes rats at 45 day of diabetes induction showed atrophied axon (→) with demyelination, muscle fragments (→), obvious degenerated myofibrils (★) and irregular, hyperchromatic nuclei (→) with irregular boundaries, lipid droplets (→) (3400 X).

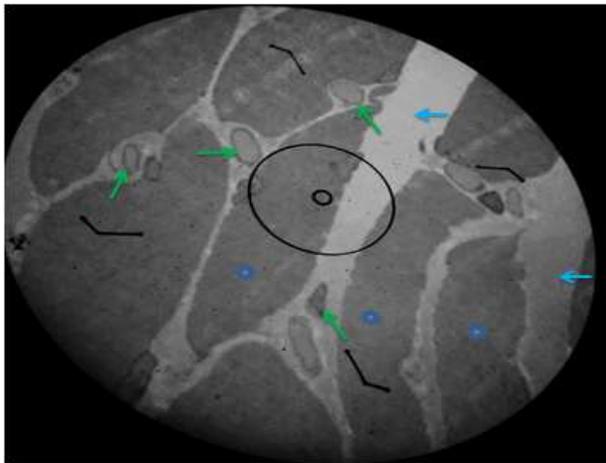


Figure 20: Electron micrograph on gastrocnemius muscle related to diabetes rats at 45 day of diabetes induction showed more degenerative myofibrils(★) dilated interfibrillar space(→), more muscle fragments (→) (2400 X).

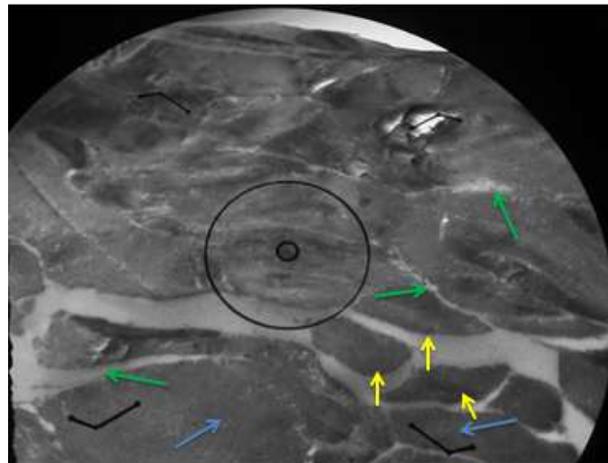


Figure 23: Electron micrograph on gastrocnemius muscle after 15 day of diabetes induction and treated with arginine showed the myofibrils more condensed(→), regular and less focal degeneration, less dilated interfibrillar space (→), still some hypercontracted myofibrils (→) (6200 X).

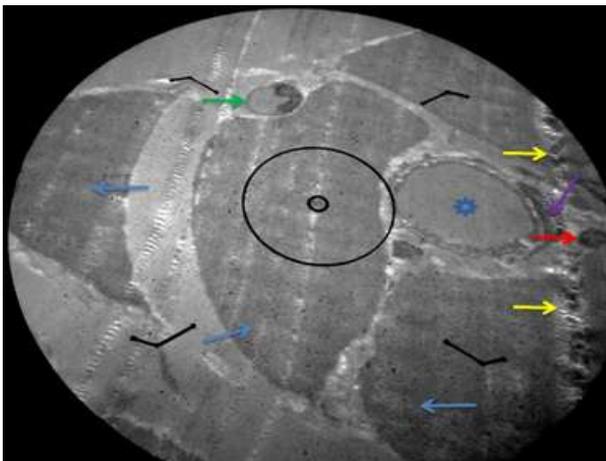


Figure 21: Electron micrograph on gastrocnemius muscle related to diabetes rats at 45 day of diabetes induction showed damaged myofibrils (→), muscle fragments (→), deformation of sarcoplasmic reticulum(→), mitochondria (→), collapsed capillary (★) surrounded with pericyte (→) (3400 X).

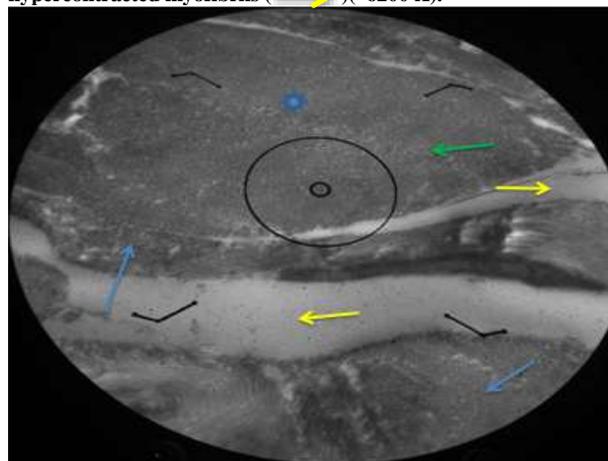


Figure 24: Electron micrograph on gastrocnemius muscle after 15 day of diabetes induction and treated with arginine showed the fibrin (→), collagen fibers (→), normal interfibrillar space (→), more condensed myofibrils (★) (16000 X).

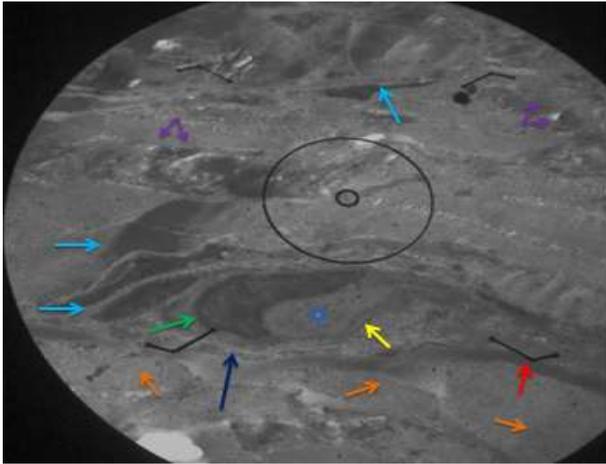


Figure 25: Electron micrograph on gastrocnemius muscle after (15) day of diabetes induction and treated with arginine showed axon terminal (→) formed motor ending, axoplasm (★), surrounded with schwann cell (→), dense vesicles (→), swollen mitochondria (→), glycogen deposition (→) and some normal myofibrils (→), myelin structures (→) (6200 X).

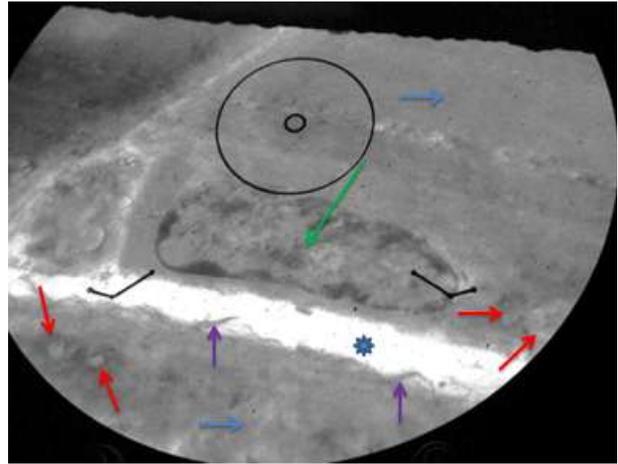


Figure 28: Photomicrograph on gastrocnemius muscle after 30 day of diabetes induction and treated with arginine showed more regular myofibrils(→), normal myonuclei(→) with regular boundaries , filamentous surface (→) of sarcolemma , and normal interstitial space(★),and more normal T-tubules (→) (8700 X).

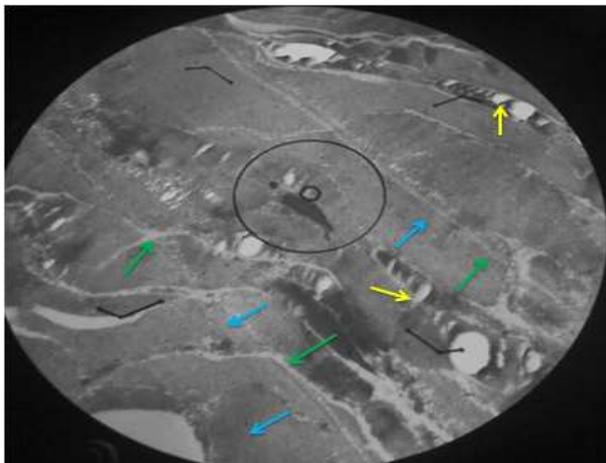


Figure 26: Electron micrograph on gastrocnemius muscle after 15 day of diabetes induction and treated with arginine showed normal myofibrils (→), separated by regular space (→), well-developed SR(→) (4400 X).

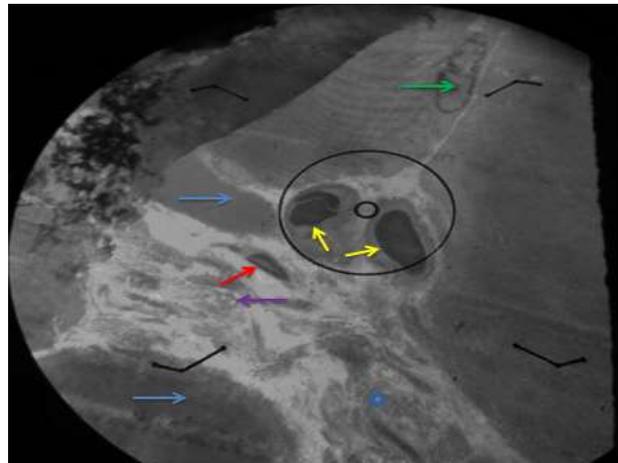


Figure 29: Electron micrograph on gastrocnemius muscle after 30 day of diabetes induction and treated with arginine showed normal myofibrils(→), other appeared hypercontracted(★), normal myonuclei(→),the interfibrillar space filled with amorphous material(→), normal endoneurial blood vessels (→) and small axon (→) (4400 X).

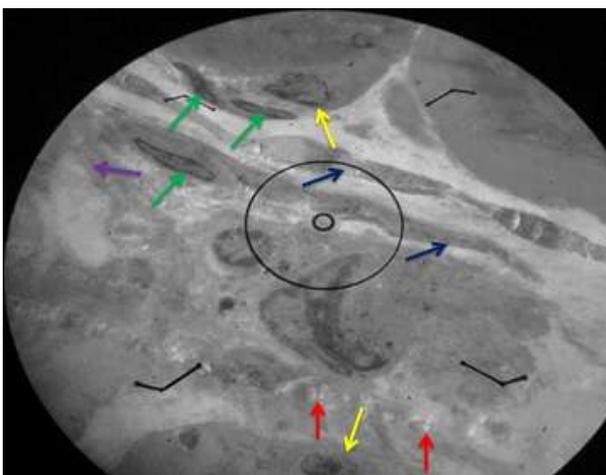


Figure 27: Electron micrograph on gastrocnemius muscle after 30 day of diabetes induction and treated with arginine showed myofibrils extend longitudinally (→), mild glycogen deposition (→), more myelinated axons (→), normal nuclei (→), more T- tubules (→) (4400 X).

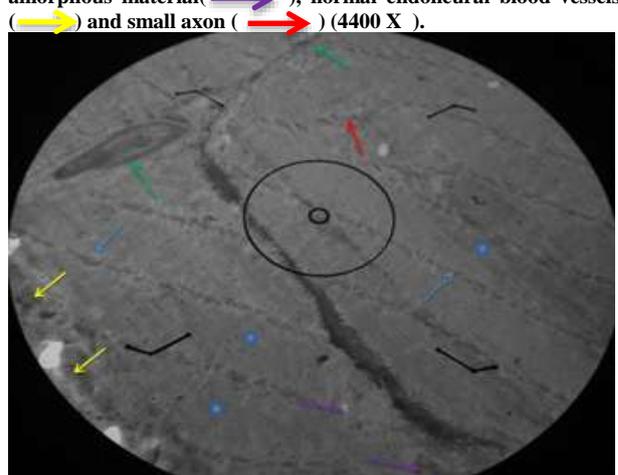


Figure 30: Electron micrograph on gastrocnemius muscle after 45 day of diabetes induction and treated with arginine showed regular Z - line (→), regular sarcomere (★), satellite cells (→),number of mitochondria (→), normal nuclei (→), well developed T-tubules (→) (4400 X).

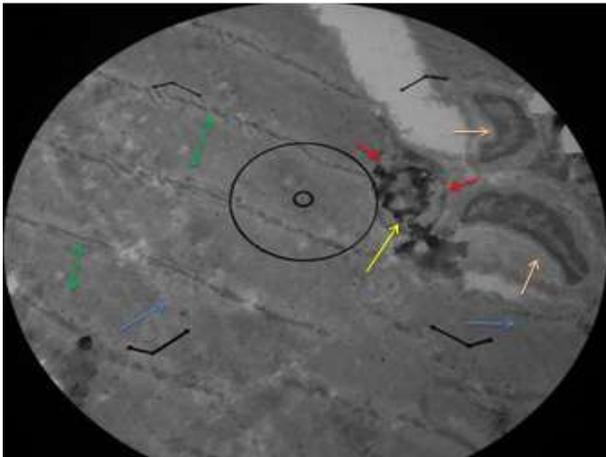


Figure 31: Electron micrograph on gastrocnemius muscle after 45 day of diabetes induction and treated with arginine showed normal myofibrils ( → ), regular Z- line ( → ), normal myonuclei ( → ) with regular boundaries ( → ), and satellite cells ( → ) (8700 X).

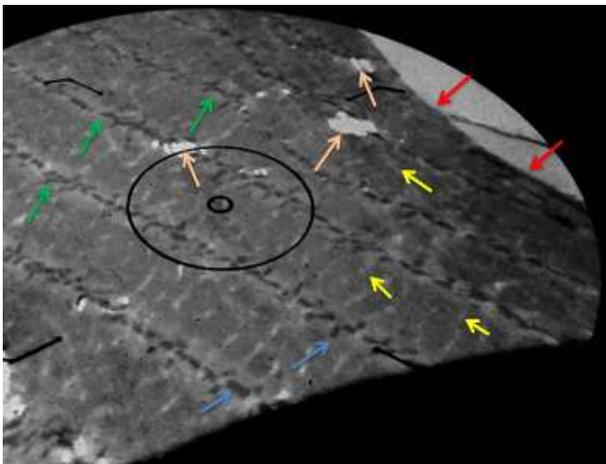


Figure 32: Electron micrograph on gastrocnemius muscle after (45) day of diabetes induction and treated with arginine showed the structure of T-tubules ( → ), clear Z-line ( → ), regular A-band ( → ), normal sarcolemma ( → ) and vesicle of (SR) was shown ( → ) ( 4400 X).

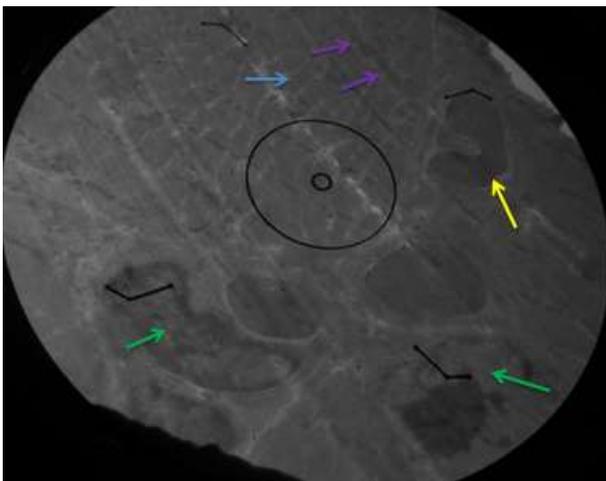


Figure 33: Electron micrograph on gastrocnemius muscle after 45 day of diabetes induction and treated with arginine showed normal structure of myofibrils , obvious A-band ( → ), normal myonuclei ( → ), regular Z-line ( → ), myelinated fiber ( → ) ( 8700 X).

## Discussion

In recent study the diabetes skeletal muscles showed variable changes in structural features at each period (15, 30, 45) days post (DM) induction by (STZ), most myofibrils was damaged, absent Z-line, atrophied myofibrils, swollen irregular mitochondria, more lipid droplets, degenerated myonuclei with irregular boundaries and showed hyperchromatic, the most features noticed at 30 days post (DM) induction was hypercontracted myofibrils, congested capillaries with thickening in basal lamina, pyknotic nucleus, focal degeneration, in addition to collagen, fibrin and reticulin fibers deposition , while at 45 days post (DM) the figures showed more sever alteration, degenerated myofibrils, absent Z-line, splitting of myofibrils and formation of muscle fibers fragments, demyelination, atrophied axons and myelin figures formed, all these marked distortion and destruction of the myofibrils related to hyperglycemia and hypoinsulinemia, caused by insufficient secretion of insulin, to metabolic disorder in glucose level and glycogen storage specially within skeletal muscles or to the changes in muscles morphometric and structure, these results was obtained by others investigators who related myopathies to (DM) complications, such as neuromuscular junction abnormalities, damaged myofibrils, degenerative axons terminals and motor end plates, swollen mitochondria and reduced vascular supply may be an important factor caused changes in skeletal muscles<sup>8,19,20</sup> .

Figures revealed to disorientation of T-tubular system, increased number of lipid droplets and glycogen deposition, in addition to the changes of myonuclei and rapid degeneration of mitochondria these results related to the cytotoxic effect of (STZ) on skeletal muscles , its role on metabolic compound, the (DM) effect on muscle enzymes , these results in agreement with other studies referred that morphologically,  $Ca^{+2}$  ions overload has also been shown to induced rapid swelling and disruption of mitochondria<sup>21,22</sup> .

Also increased lipid droplets was observed , this may be related to the lipolysis that associated with (DM) and this also discussed by other researchers who reported that principal enzymes involved in the metabolism of triglycerides (TG) was inhibited during (DM) and increased the lipolysis caused by lack of insulin and lead to an increae with ( TG), chylomicrons and fatty acids plasma levels also greater loss in oxidative potential on the fast –twitch fibers population that the slow twitch fibers<sup>23,24</sup> .

Results revealed to changes in some capillaries , arteries showed endothelial cells hyperplasia, other vessels showed degenerated pericytes and endothelial layer, most peripheral nerves appeared patchy, atrophied, demyelinated, heavy collagen deposition and this related to the degenerative changes, necrosis, inflammatory response, hypoinsulinemia, the results of present study confirmed the previous studies on skeletal muscles changes, innervation and vascularization, light and electron microscope showed variable changes, both study similar, but some ultrastructural changes like

intima hyperplasia, extensive deposition of collagen fibers, folded sarcolemma, most axon was atrophied and myelination figures specially at (30 , 45 ) days post(DM) induction.

Many studies described the microscopic findings of endothelial cells, platelets adhesion, narrow lumen with deposition of fibrin, degenerated cells with pyknotic nuclei, thickening with basement membrane and collapsed of endoneural vascular which regarded it the most primary changes associated with neuropathy<sup>25,26,27</sup>. Recent study clarified the role of arginine (arg) as protective agent sections from gastrocnemius muscle related to diabetes rats treated with this amino acids showed more regular myofibrils, condensed, obvious Z-line, well developed (SR) and still some degenerated myofibrils, secretory vesicles, more glycogen deposition and some myelinated figures appeared , the results on 30 days post (DM) induction reported normal myonuclei beneath the sarcolemma, regular T-tubules, more obvious myelin figures with less atrophied axons, the endoneural vascular vessels and capillaries appeared normal with less basal lamina thickness, also the results at 45 days showed oriented Z-lines, activated satellite cells, the sarcomeres regain their normal structure, normal sarcolemma, normal most of capillaries, increased myelin figures and deposition of glycogen within intercellular space, reduced muscle fragments and regular sarcomere, these results related to the role of arginine as factor reduced the complications of diabetes, may be act on glucose homeostasis, increased secretion of insulin, regulated the metabolism of glucose and fatty acids, these results was mentioned and reported by other investigators who clarified that the arginine was improved the structural changes but not completely through it effect on hyperglycemia, dyslipidemia and stimulate protein synthesis, improve circulation especially in tissue with thin capillaries and prevent dangerous circulatory disturbances caused by blood sugar level<sup>28</sup>.

Also arginine was a semi-essential amino acids that is required during periods of sever stress, injury and growth, it was the substrate for protein synthesis and modulate cellular biologically active compounds such as ( NO), polyamine and creatine<sup>29</sup>.

## Conclusions

Our study showed strong association between ultrastructural changes of diabetic skeletal muscles and their innervation was established and the results referred to arginine as protective, bioactive agent reduced myopathy , neuropathy of diabetic muscles.

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