Study on Skeletal muscles during myotoxicity induced by (Bupivacaine) in experimental rats (Rattus norvegicus).

Mohammed k. Hassani and Maha K. Ibrahim Assistant Lecturer :

Dept. of Science ,College of Basic Education ,Missan University

Dept. of Biology ,College of Science ,Basrah University

Summary

Recent study was aimed to identified the effect of local anaesthetic drugs , Bupivacaine (Bpvc) injection into the skeletal muscles to induced myotoxicity , So muscles degeneration and latter the regeneration of the damaged muscles was established , also histopathological changes on (2h , 2d , 5d , 10d , 15d) post injection was clarified in Triceps brachii and Gastronemius muscles , in addition biochemical parameters which associated with muscles toxicity was evaluated.

Total number (90) rats male (Rattus norvegicus) was used in this study as experimental animals , all animals with weight (250 – 300) gm and (12 -14) weeks old . The rats divided into two group , first group regarded as control which injected intramuscularly with (0.6) ml of normal saline into each triceps and gastronemius muscle,second group injected with (0.6) ml of (0.5% Bpvc) mixed with same volume of normal saline to induced myotoxicity .

Rats were anaesthetic and muscles were excised from each rat at (2h ,2d , 5d, 10d , 15d) post injection , these muscles prepared for light microscopy exam, Blood Samples were collected and serum separated ,stored for determined the biochemical tests (Protein content , Calcium concentration , Alkaline phosphatase level) in both control and treated rats.

Histopathological changes after (2h) in muscles injected with (Bpvc) included degeneration ,necrosis ,hypercontracted flber ,the damage extend to the deeper layer while normal nerves and blood vessel's were noticed . After (2d) post injection the histological exam showed myonecrosis , dense inflammatory cells most of macrophages and polymorphonuclear cells (PMN) , the muscle

fiber with barrely surface and comparative with (5d) post injection there was variable changes like hypercontracted ,vacuolated fibers with condensed myofibrils increased with myofibroblasts and also at the same time appeared of regenerated myotubes as distinct units .

The period (10d) post injection referred to more regenerated fibers with still some myonecrosis fibers , the muscle fiber also still tapers elongated and bifurcated endings , Also (15 d) post injection showed regenerated of most fibers , differentiated of epimysium and connective tissue contents with few damaged fibers .

Comparative with section from control skeletal muscles injected with normal saline which showed interistitial spaces dilation with edema of connective tissue septa , there was no signs of damage or myonecrosis .

Depending on biochemical analysis with significant on total differences protein content , calcium concentration and Alkaline phosphatase (ALp) level at (2h , 2d , 5d) post injection with significant difference at ($P \leq 0.01$) compared to control group . while not recorded any significant increase with these parameters at (10 d , 15d) post injection compared to control group at ($P \geq 0.01$) .

Introduction:

Local anaesthetic drugs are myotoxic agents at clinical concentration and the potency of toxicity is drug specific and dose dependent (cherng et al.,2010).

Skeletal myotoxicity regarded as one of the un commone side effects of locally anaesthetic drugs and regular injection with it lead to disturbances and morphological abnormalities with histological changes in striated muscular tissues (Zink et al.,2003).

Bupivacaine (Bpvc) is considered to be the most myotoxic agent induced myopathy in an animals models has been established to study skeletal muscles degeneration (Platet et al.,2005).

Myotoxicity were not observed in skeletal muscles after the infusion of other local anaesthetic drugs like prilocaine and lidocaine , there were no complication cases of anesthesia , So these drugs suggested to be applied safely by infusion(Ragbetli et al.,2009).

Muscle damaged by (Bpvc) provides a suitable models for studying muscle degeneration and then regeneration when injected into skeletal muscles that it was caused disrupting intracellular Ca+2 homeostasis (Irwin et al.,2002).

Absorption of the local anaesthetic drugs depend on variable factors like particular local anaesthetic ,the patient hemodynamic condition , the site of administration , the concentration and dose in addition to vascularity of administration site also role of vasoactive drug such as epinephrine (Astrazeneca,2008)

Local anaesthetic drugs was blocked the sensory and motor functions through disturbance nerve cells membrane to diffuse sodium ions which it was important at action potential depolarization , this action of these drugs also against else ions like potassium and calcium (Kinder& yost,2005 ; Heavner,2007)

Rapid degeneration and regeneration changes observed after the (Bpvc) had been applied to the surface of the muscles and histological examination at daily intervals showed rapid series of degeneration changes (Couteaux et al., 1988).

Anumber of amphiphilic and lipid soluble drugs of heterogenous pharmacological properties , when injected into skeletal muscles induced acute muscle fibers necrosis and the pathogenesis of this suggested to involved direct damage to cell membranes by these drugs (Manor & Sadeh, 1989).

Bpvc disturbs sarcoplasmic reticulum (SR) by inhibiting Ca+2 uptake this may result in elevation of Ca+2 concentration in the sarcoplasm , causing not only myofibrillar hypercontracted but also activation of Ca+2 activated neutral proteases which are known to causes deterioration of specific proteins (Nonaka et.al.,1983; Rosenblatt& woods,1992).

Change in serum enzyme activities after injection of (Bpvc) into skeletal muscles was established by Nonaka (1996) , and it appeared that increases in these enzymes reflected muscle damage , the changes occurred in the early stage of myonecrosis .

Zhang et al. (2010) illustrated that (Bpvc) injection on extraocular muscle in rabbit caused extensive myonecrosis followed with regeneration at the site injection.

Many observations referred that all skeletal muscles with atrophy showed variable levels of abnormal concentrations with muscle enzymes when compared with normal skeletal muscles (Reddy et.al.,1992 ; Punk et. al.,1999).

Alkaline phosphatase (Alp) connect with myopatheis diseases and high concentration of enzyme recorded in muscle atrophy of experimental mice (Neymark et. al.,1980).

The mechanisms that involved in (Bpvc) induced myotoxicity are still unclear ,but increased with Ca+2 concentration has been proposed to play an important role in the pathogenesis of (Bpvc) induced myotoxicity (7 – 8) .

Therefore, the current study was conducted to obtain more informations about the effect of (Bpvc) injection on skeletal muscles and its capacity to induced myotoxicity and regeneration at different periods post injection with this dependent dose which clinically relevant setting .

2-Materials and methods

2-1 Experimental animals

Thirty male rats (Rattus norvegicus) weighting (250 -300) gm and (12-14) weeks old were used in this experiment , the animals were housed in cages at room temperature (22°c) in a 12 :12 (light – dark) cycle and provided food and water ad libitum . The animals divided into two group(20 treated and 10 control) , first group regarded as control which injected intramuscularly with (0.6) ml of normal saline into each triceps and Gastronemius muscles ,second group injected with adose of (0.6) ml from (0.5 %) Bupivacaine mixed with the same volume of normal saline to induced the myotoxicity , the injection done under mild anaesthesia with a (25) gauge needle which inserted along the muscle and the (Bpvc) administered intramuscularly during the withdrawl of the needle (Hall- Graggs,1974) .

2-2 Rats dissection

All animals were anaesthesized with chloroform , and dissected at (2h , 2d , 5d, 10d , 15d) post injection , then blood and muscles collected and treated as follow .

2-2-1 Blood samples collection

About (4 – 5) ml of venous blood was collected from heart directly by puncture with disposable syringes and serum separated , transferred to sterilized tubes and stored at (-20°c) for analysis of (Ca+2) concentration ,(Alp) level and protein content with analyzed spectrophotometrically by using variable kits from (Bio-lab- SA) according to (Tietz ,1999 ; Clark,1975; Belfield & Goldberg,1971).

All the values expressed as mean ± SD.

2- 2- 2 Tissue processing for light microscopy

Both left and right Triceps brachii and Gastronemius muscles regarded to treated group and control were dissected out , these muscles were taken at the same time of blood collection and after (2h , 2d , 5d, 10d , 15d) post injection .

All the muscles were cut to suitable pieces, washed with normal saline , stretched on pieces of cardboard ,immersed into (10%) formaline solution and prepared according to (Luna,1968) for light microscopy exam .

Transverse and longitudinal section about (5-7) µm thickness were cut , stained with (H & E) stain , examined and photographed.

2- 3 Statistical analysis

Comparison was used to measured the differences among and within the groups (control and treated) for the serum parameters activities .

All the results analysed with Analysis of variances (ANOVA) and the means compared with (SPSS) at least significant differences (LSD) on ($P \leq 0.01$).

3- Results

3-1 Determination of biochemical parameters

3-1-1 Total protein level determination

Results showed an increased significantly with total protein content at ($P \leq 0.01$) after (Bpvc) injection(2h , 2d , 5d) post injection comparative to control group that the protein level reached to (7.58± 0.16 , 8.02± 0.27 , 6.29±0.08 , 5.7±0.15 g /dl) respectively (fig 1) .

There was no significant differences at ($P \leq 0.01$) on total protein content between treated group and control at (10 d , 15 d) post injection (fig 1).

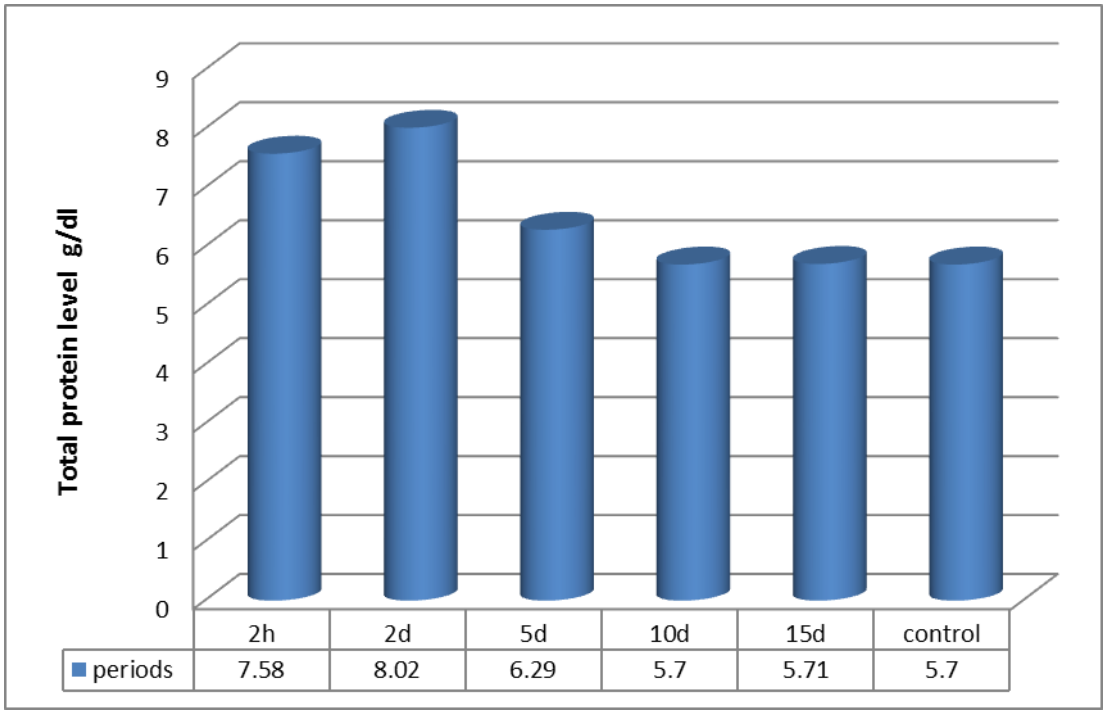


Fig (1)

Illustrate effect of local anaesthetic on total protein level of treated rats at different periods comparative to control group . All the values expressed as (mean ± SD.).

3- 1-2 calcium ion concentration

Data from biochemical test referred to significant differences at (P≤ 0.01) with (ca) ion concentration in serum of rats treated with (Bpvc) at (2h , 2d , 5d post injection compared to control group , the mean concentration was (11.35±0.15 , 12.24±0.05 , 9.59±0.31 ,8.5±0.19 mg /dl) respectively (fig2).

While there was no significant changes with (ca) ion concentration at (10d ,15d) post injection at (P ≤ 0.01) between treated and control group (fig 2) .

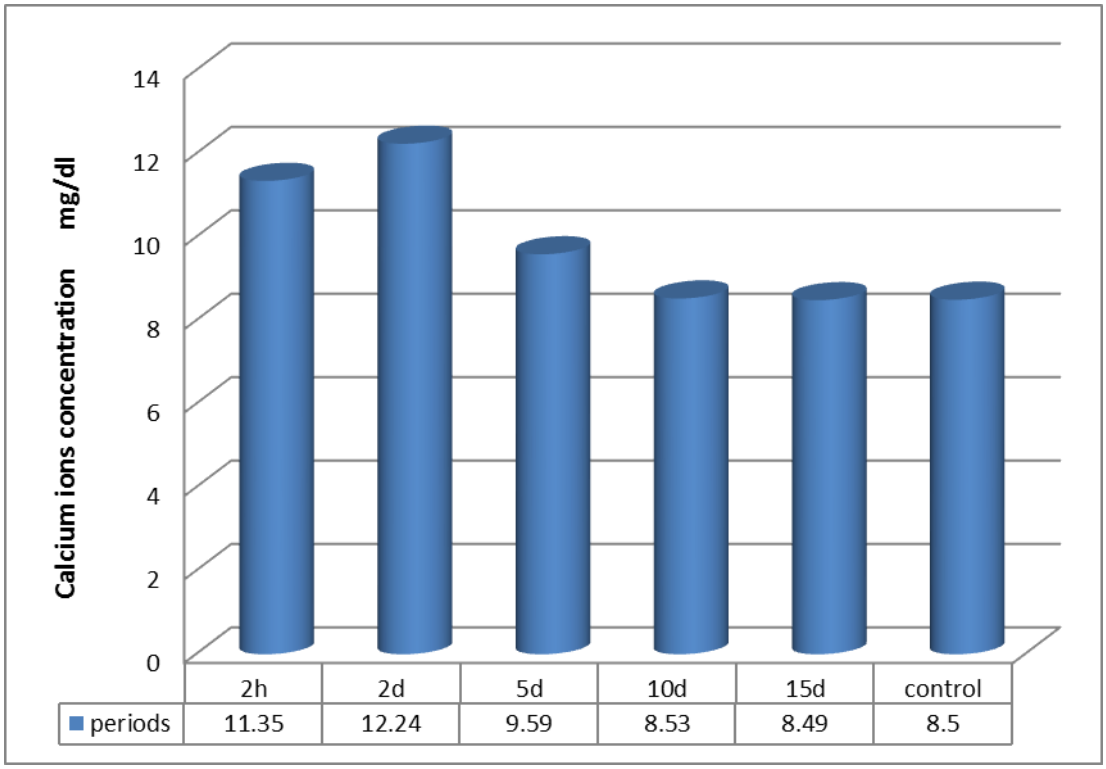


Fig (2)

Illustrate the effect of local anaesthetic on calcium ion concentration of treated group rats at different periods comparative to control group. All the values expressed as (mean ± SD.).

3- 1 -3 Alkaline phosphatase (Alp) enzyme concentration

Recent study indicated to an increased significant at ($P \leq 0.01$) on (Alp) concentration on rat injected with (Bpvc) at (2h , 2d , 5d) post injection compared to control group , the mean concentration was (20.32 ±0.34 , 40.3±2.04 , 28.47±0.07 , 11.67±0.15 IU/100ml) respectively (fig 3) .

The results did not recorded any changes with significant at ($P \leq 0.01$) at (10d ,15d) post injection on (Alp) level between treated and control group (fig 3) .

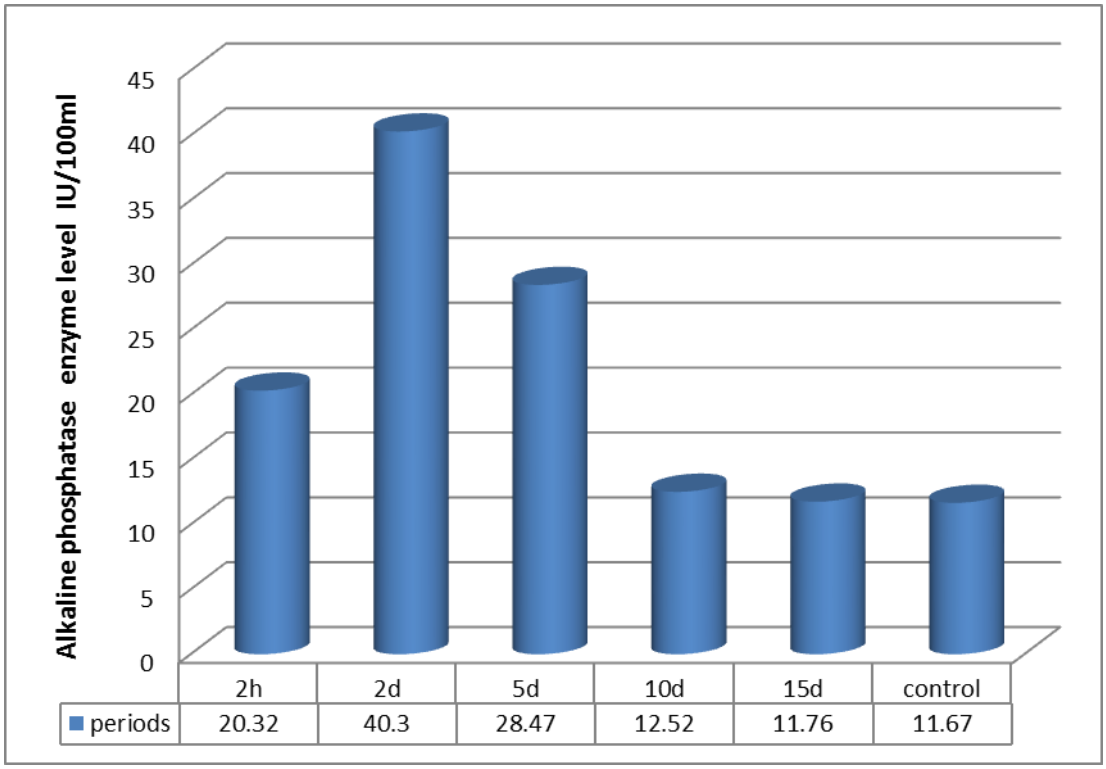


Fig (3)

Illustrate the effect of local anaesthetic on Alkaline phosphatase enzyme level of treated group rats at different periods comparative to control group. All the values expressed as (mean ± SD.).

3-2 Histological study

Microscopical observation on section from Triceps brachii and Gastronemius muscles injected with normal saline showed some hypertrophied fibers , dilation, edema with interistitial spaces between muscle fibers and connective tissue septa , most fibers appeared normal with peripheral nuclei and obvious striations pic.(1,2,3,) .

Effect of (Bpvc) on skeletal muscles was assessed and the results showed that injection of Bpvc post (2h) most muscle fibers hypercontracted , disorganized , the changes extend to the deep layers , in addition necrosis of fibers , no obvious myofibrilles with red blood cells (RBCs) has been extravasation from blood vessels and heavy an inflammatory cells (pic. 4,5,6), while nerve fibers ,large blood vessels within perimysium appeared intact (pic. 7) .

Histological changes on (2d) post injection with Bpvc ranged from vacuolated ,swelling fibers on both Triceps and Gastronemius muscles to complete degenerated and myonecrosis fibers with severe infiltration of inflammatory cells most of macrophages and polymorphonuclear (PMN) , other fibers noticed

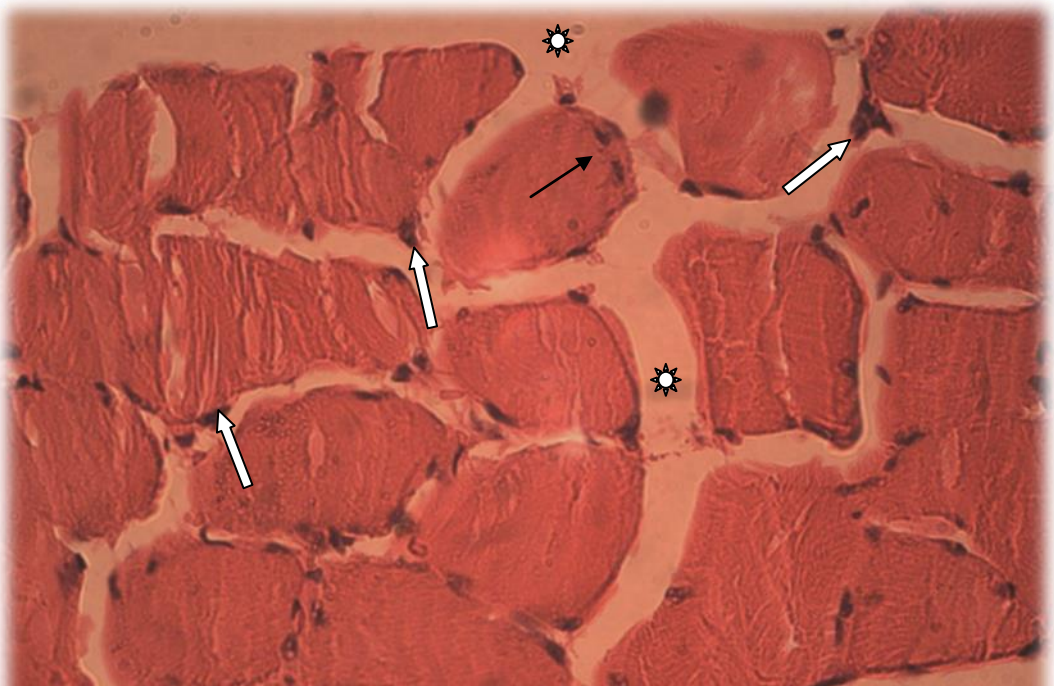
with pyknotic nuclei and hyalinized myoplasm , sometimes circular fibers with central nuclei appeared also (pic. 8,9,10) . Also variation with fibers shape and diameter appeared , that some fibers with reduced size and tapering endings with still other fibers normal , moreover vascular and connective tissue were visible with no changes (pic.11 ,12) , the most characteristic feature at this period was the barely shaped muscle fibers (pic. 13) .

Microscopic exam on sections from triceps and Gastronemius muscles at (5d post injection referred to hypercontracted fibers , degenerated fibers with condensed myofibrils in addition there was circular fibers with edema of interistitial spaces and myoseptal pic. (14 ,15.16) .

At this period there was reduction in inflammatory cells specially the macrophages while there was an increased with myofibroblasts within the connective tissue and near the regenerated myotubes which appeared as distinct units extend among the damaged fibers pic. (17 , 18 ,19) .

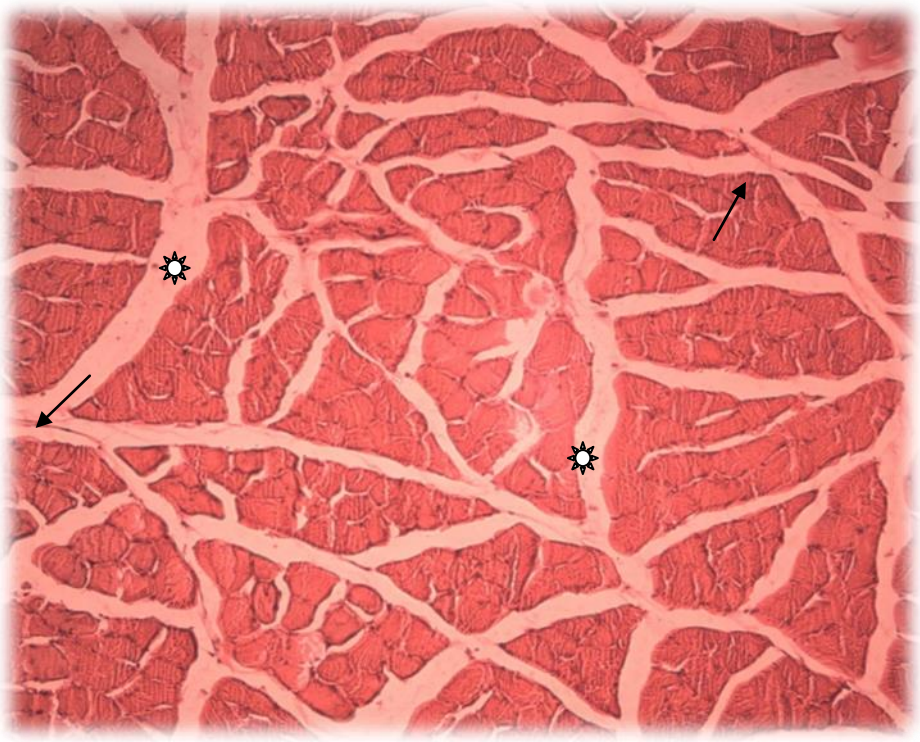
Results indicated that post (10d) of injection there was more regenerated fibers than other periods, with reduction with inflammatory cells , most nuclei located peripherally , moreover myonecrosis fiber still found pic. (20,21). At this stage the results clarified barely fibers , tapering endings of other fibers and irregular external margins pic. (22 , 23) .

Pictures also showed that post (15d) of Bpvc injection most fibers were regenerated with differentiation of epimysium and perimysium , blood vessels with large nerve fibers more regularly , the most regenerated fibers nearest to vasculature with normal diameter and parallel to each other pic.(24 , 25) .



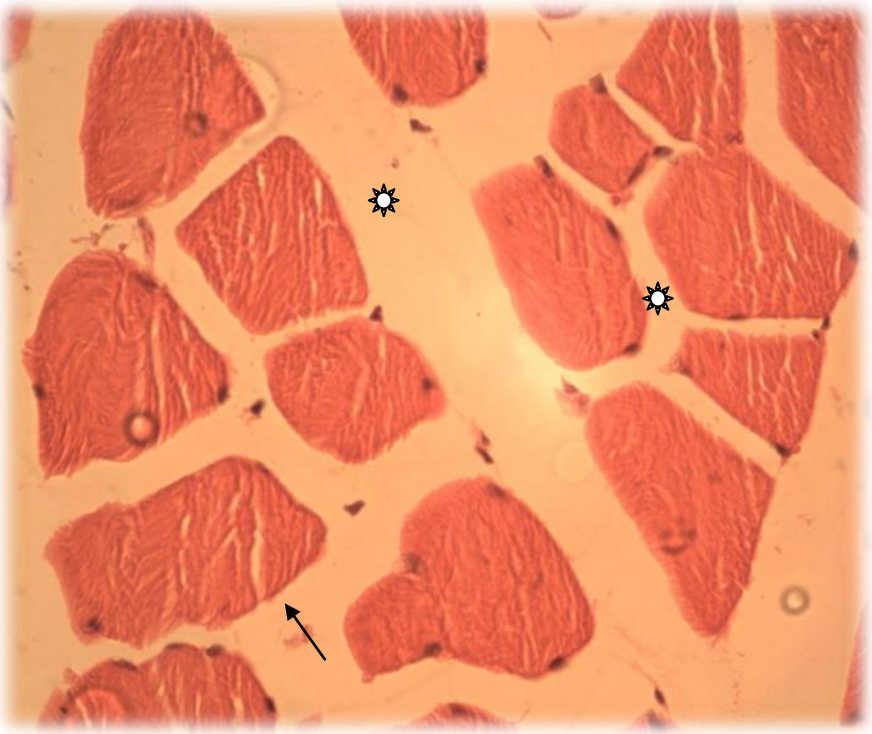
Pic. 1

Transverse section on control triceps brachii showed bundles of muscle fibers with peripheral nuclei (⟶),interistitial spaces (☼) with blood capillaries (⟹). (H & E , 540 x).



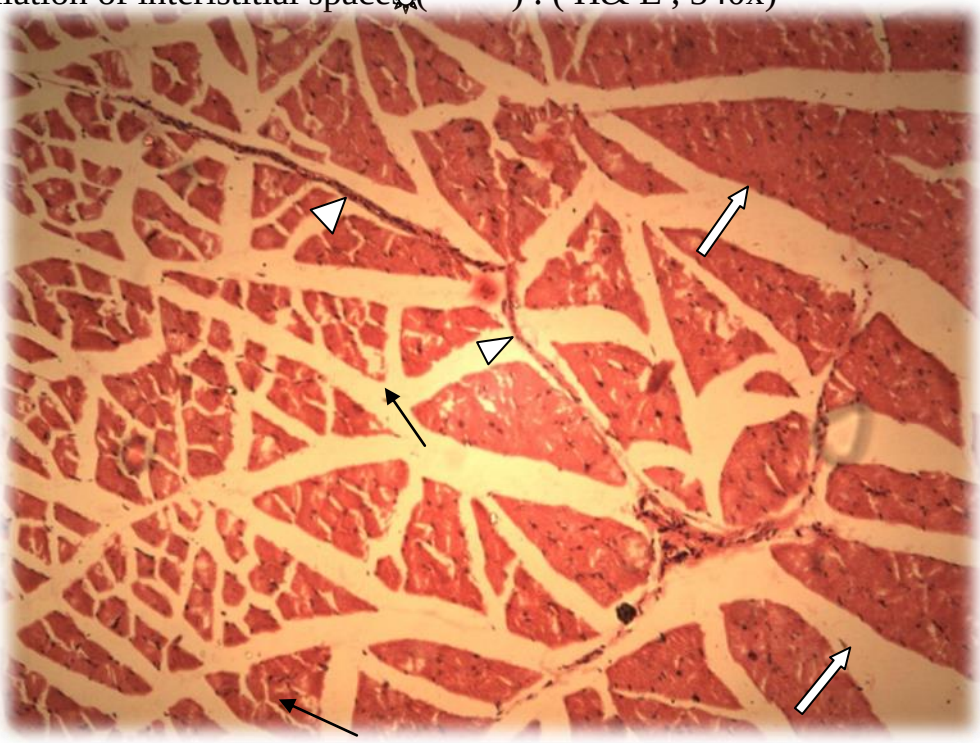
Pic. 2

Transverse section on control Triceps showed dilation of spaces (✡) with connective tissue myosepta (⟶).(H & E ,200 x) .



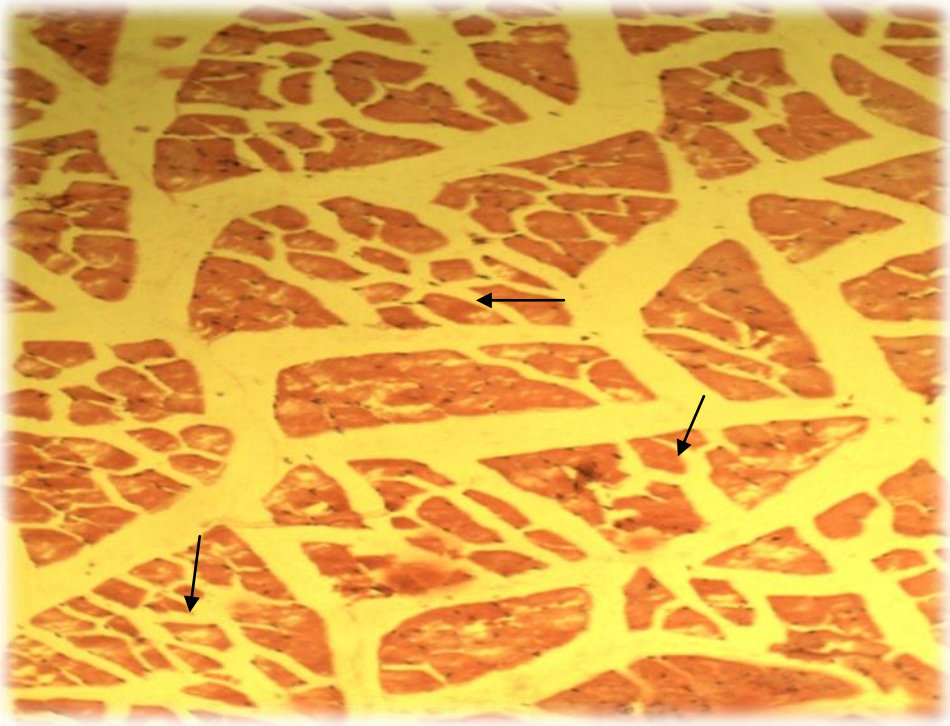
Pic.3

Transverse section on control Gastronemius showed muscle fibers (⟶) and dilation of interistitial spaces(✡) . (H& E , 540x)



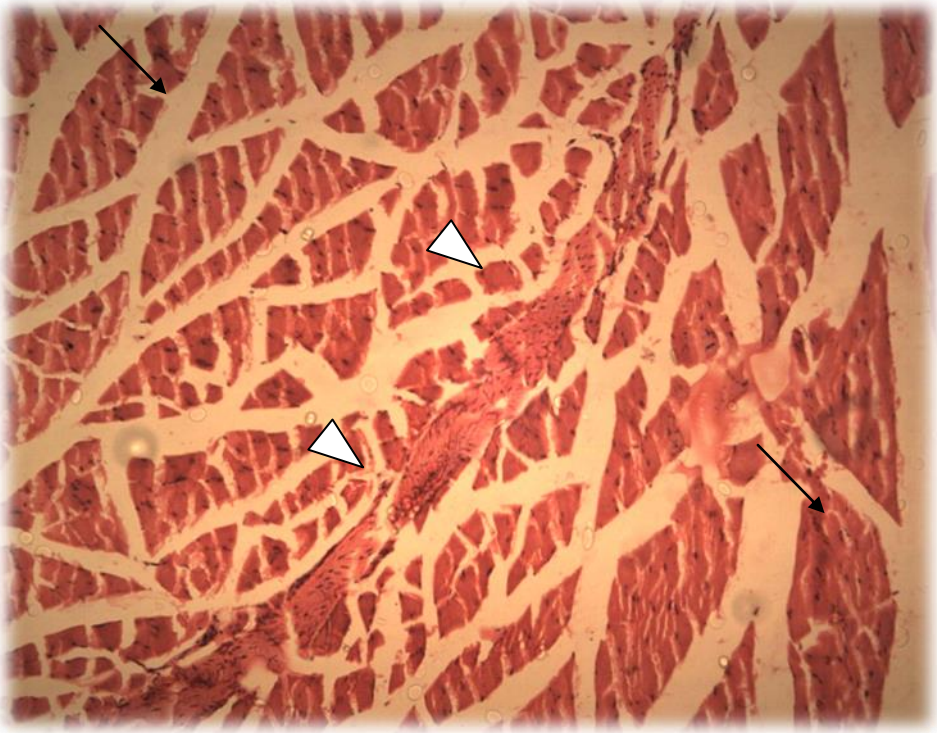
Pic. 4

Transverse section on Triceps muscle treated with (Bpvc) at (2h) post injection showed irregular degenerated muscle fibers (⟶) extend among normal muscle fibers (⟹)with strands of connective tissue (▷). (H & E , 280x)



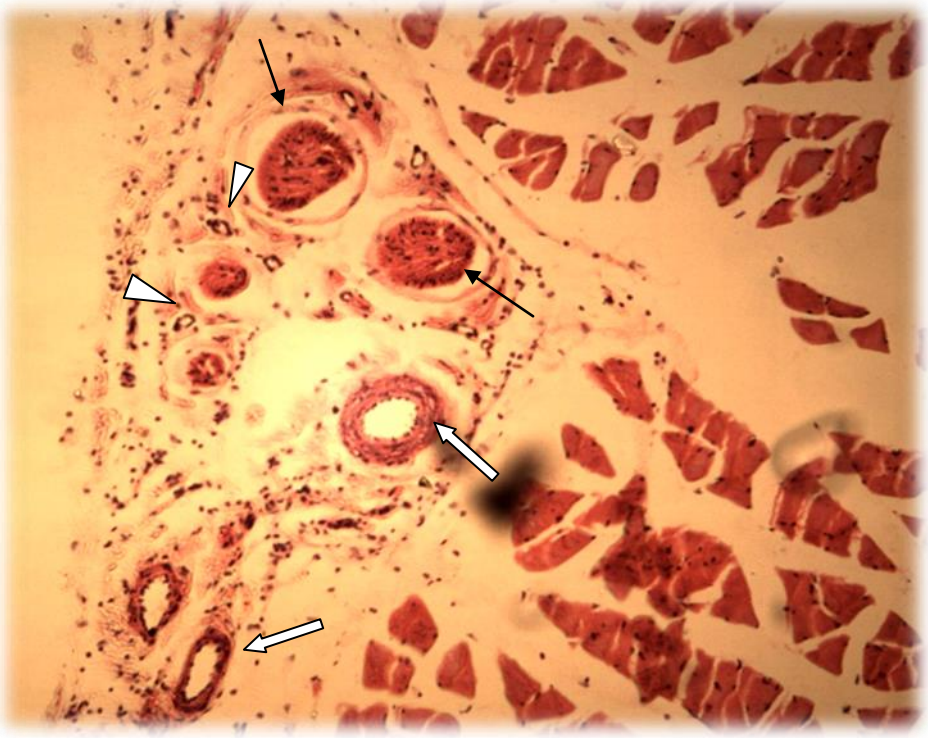
Pic. 5

Transverse section on triceps muscle belly treated with (Bpvc) at (2h) post injection showed degeneration of most muscle fibers and nucleus (⟶) in variation in size and diameter of fibers also noticed . (H & E ,280x).



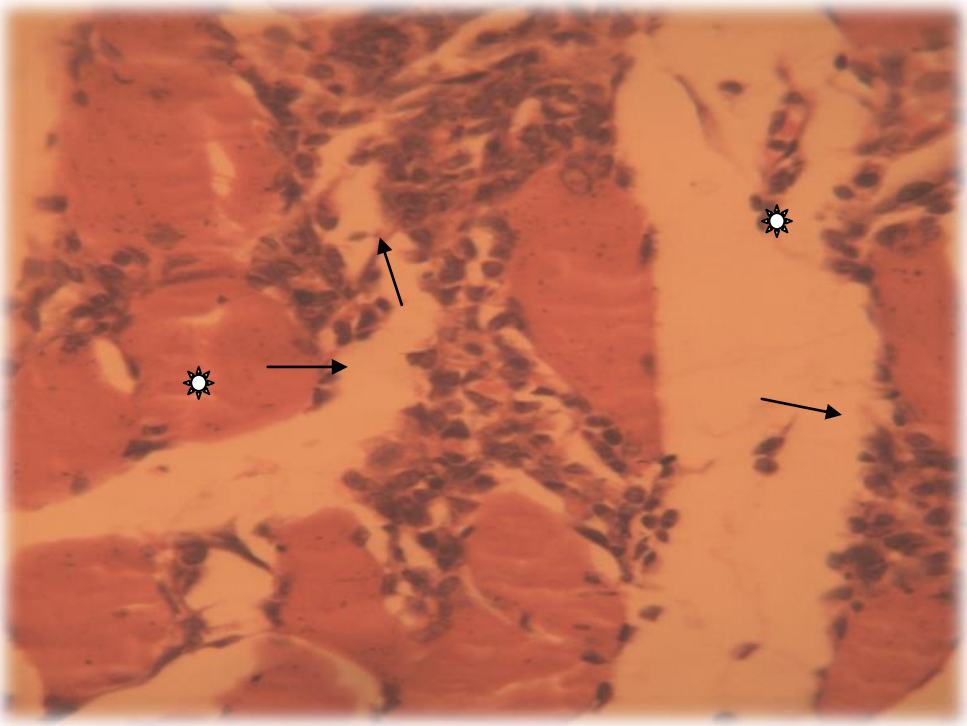
Pic.6

Section on Gastronemius at (2h) post injection with (Bpvc) showed extend of damaged from surface layer (⟶) to deep layer of muscl (▷).
(H & E ,280x) .



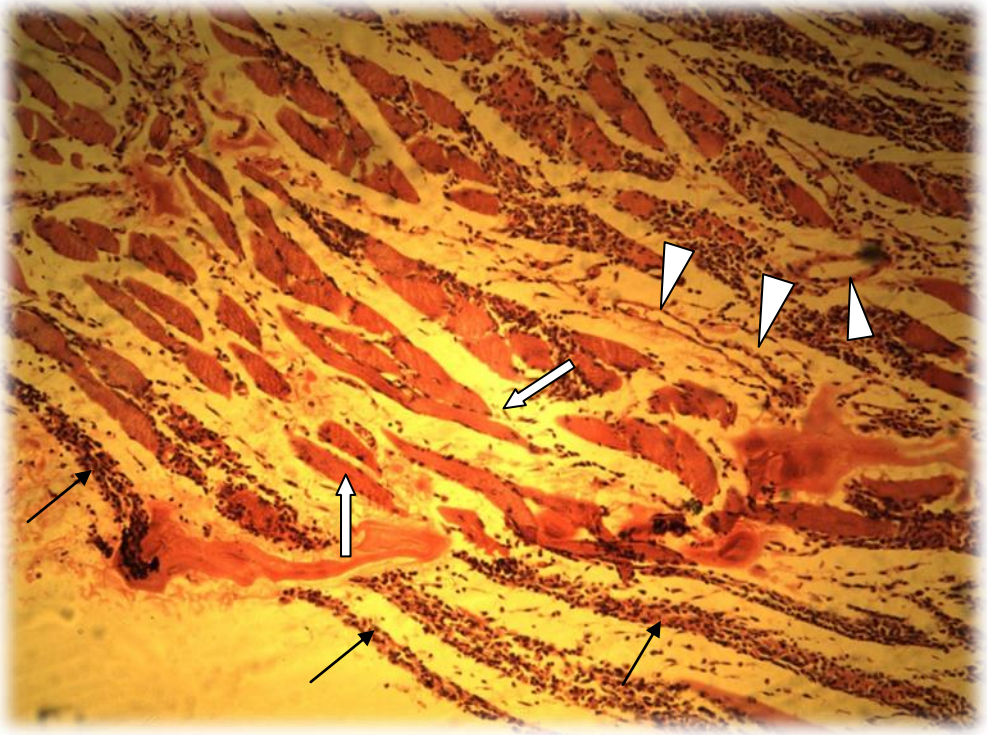
Pic. 7

Section on Gastronemius treated with (Bpvc) at (2h) post injection showed heavy infiltration of inflammatory cells , normal large nerves (⟶) , intact blood vessels (⟹) and capillaries (▷). (H & E , 280x) .



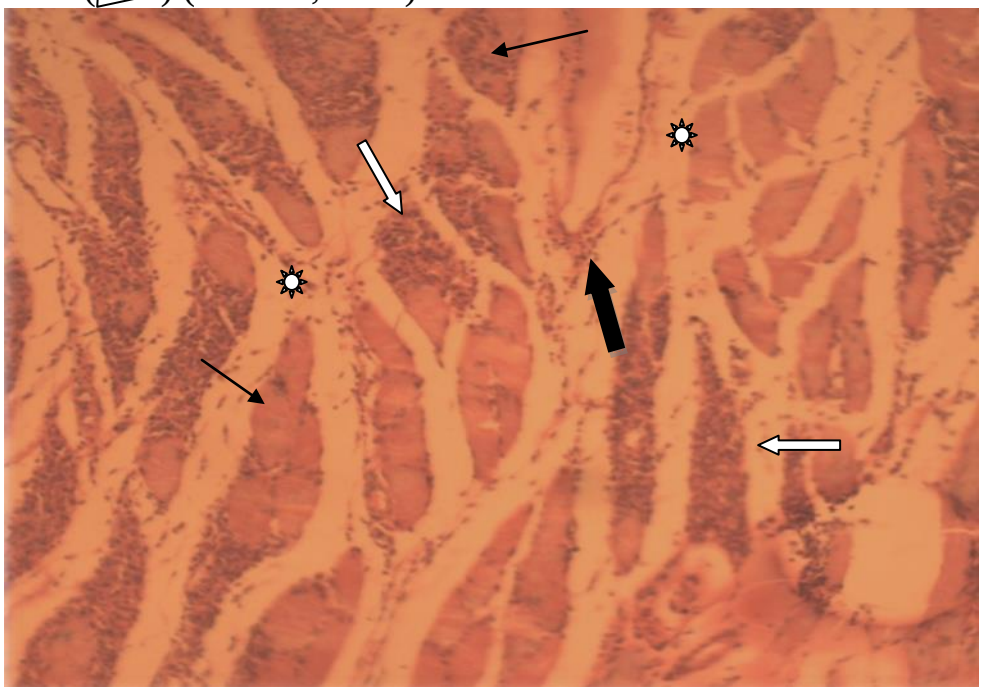
Pic. 8

Transverse section on treated Gastronemius at (2d) post injection with (Bpvc) illustrate necrosis , irregular fibers with dense an inflammatory cells (⟶) , dilation of interistitial spaces (☼).(H & E , 540x) .



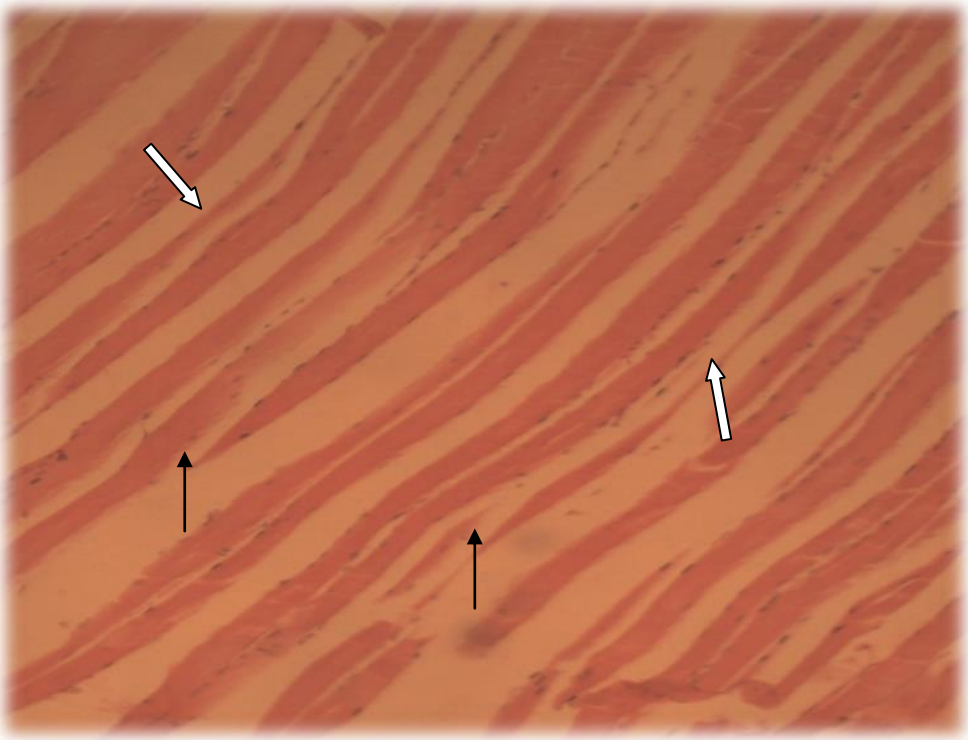
Pic. 9

Oblique section on gastronemius treated with (Bpvc) after (2d) illustrated complete degeneration (⟶) extend with normal muscle fibers (⟹) and capillaries (▷).(H & E , 280x).



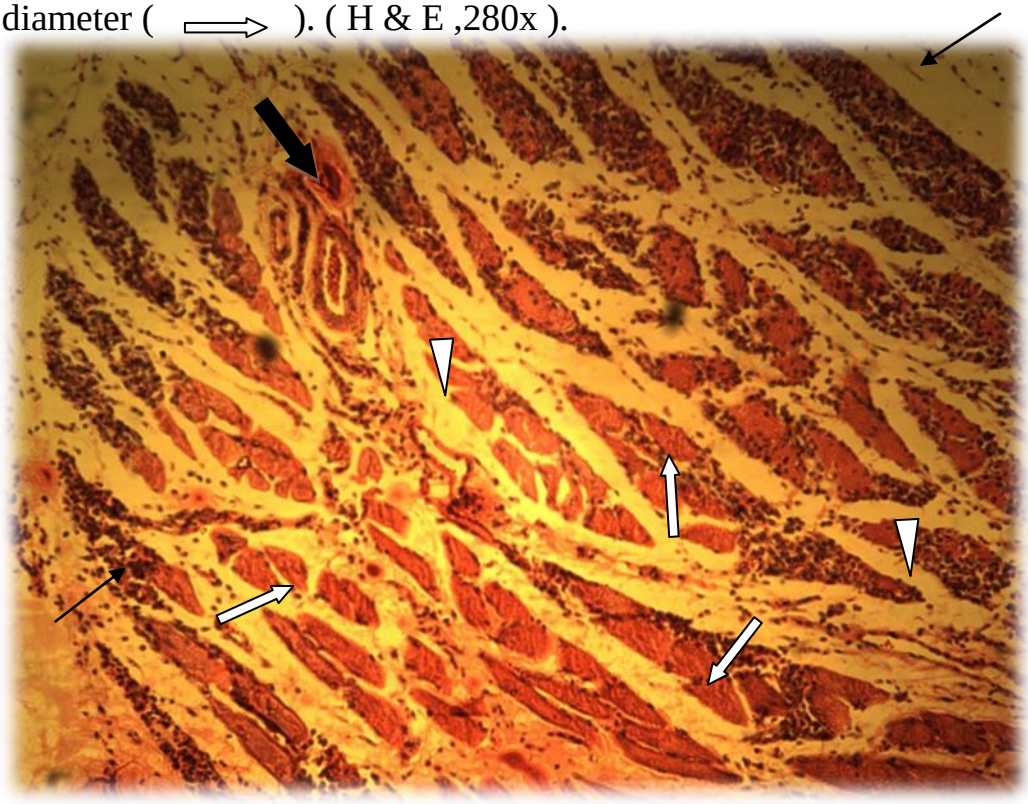
Pic.10

Oblique section on gastronemius treated with (Bpvc) after (2d) showed hyalinized fibers (⟶) , degeneration muscle fibers (⟹) and dilation of spaces (☼) with inflammatory cells (➡).(H & E ,280x).



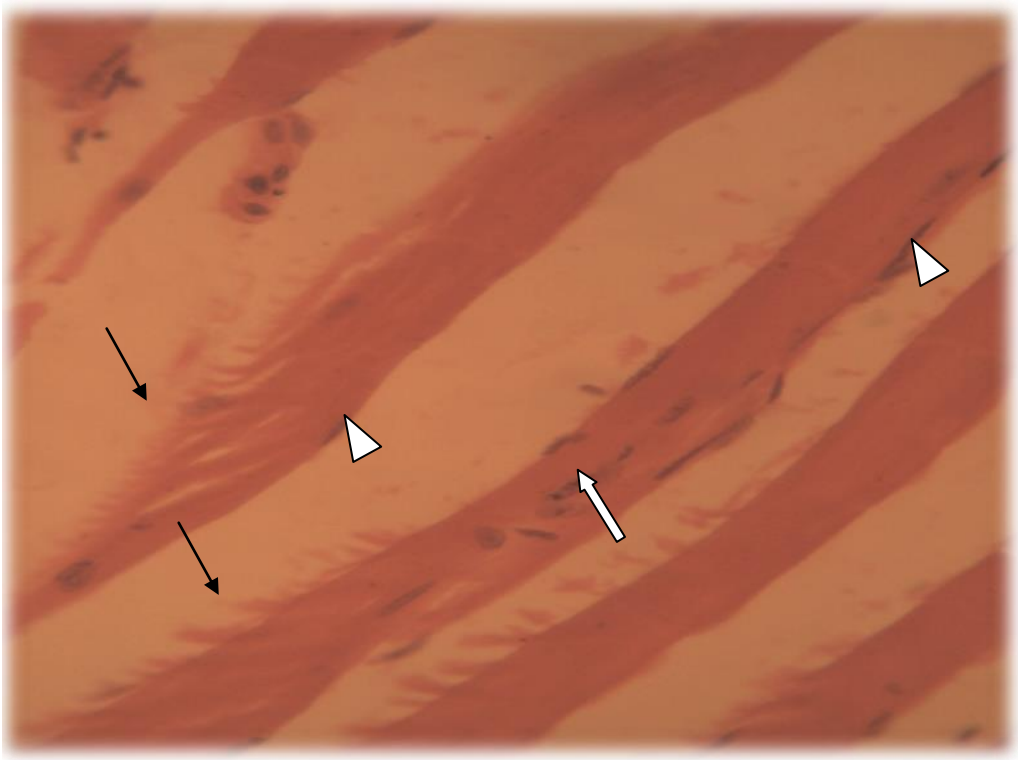
Pic.11

Longitudinal section on triceps treated with (Bpvc) after (2d) of injection showed bifurcated , tapering endings (⟶) with severe reduced in muscle fibers diameter (⟹). (H & E ,280x).



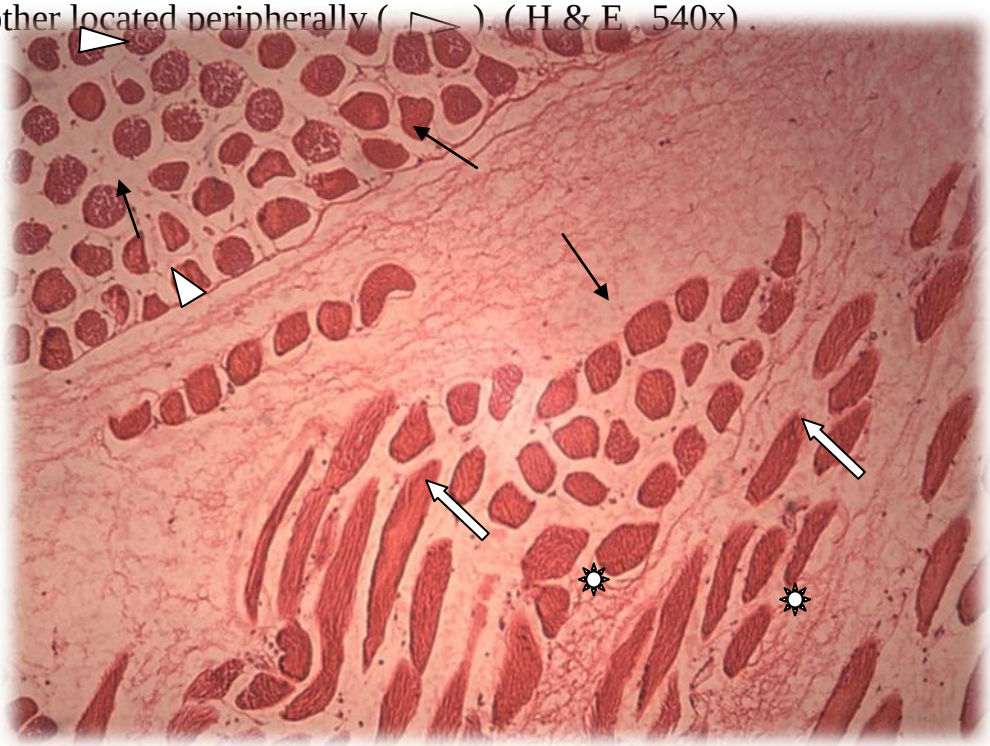
Pic. 12

Oblique section on Gastronemius at (2d) post injection with (Bpvc) showed myonecrosis (⟶) with still normal fibers (⟹),branches of blood vessels (➡) and small vessels (▷).(H & E , 280x) .



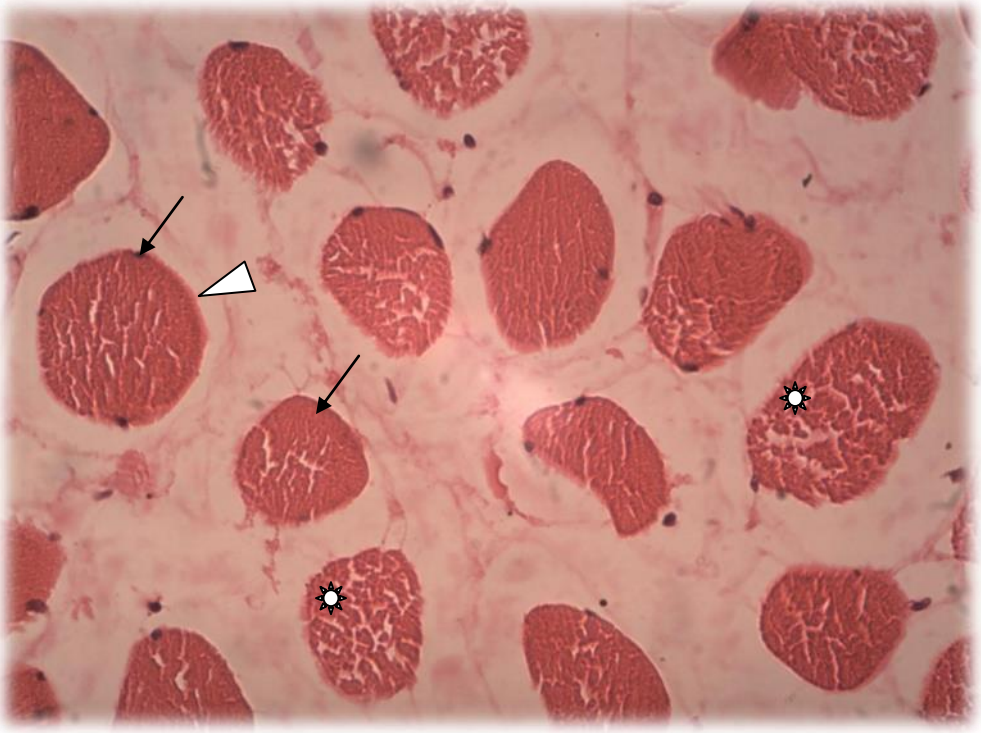
Pic. 13

Longitudinal section on triceps treated with (Bpvc) after (2d) of injection illustrated barely surface shape (⟶) of muscle fibers , central nuclei (⟹ and other located peripherally (▷). (H & E , 540x) .



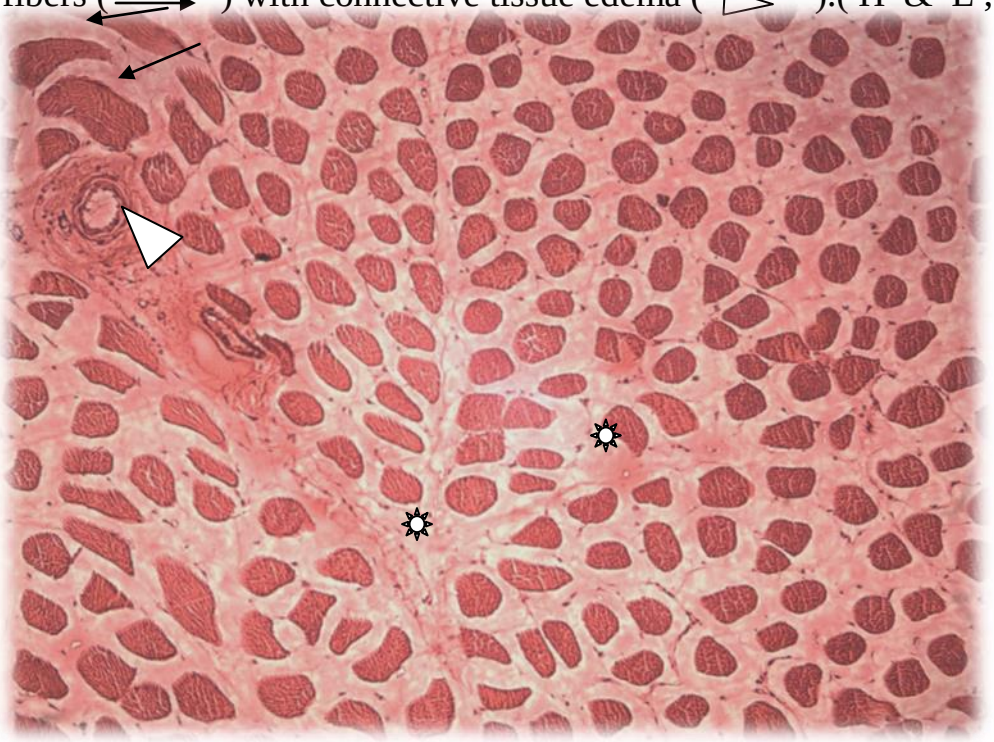
Pic. 14

Oblique section on treated gastronemius at (5d) post injection showed circular fibers (⟶) , interistitial edema (✡), regenerated myotubes (⟹) other fibers appeared hypercontracted (▷) . (H & E , 160 x) .



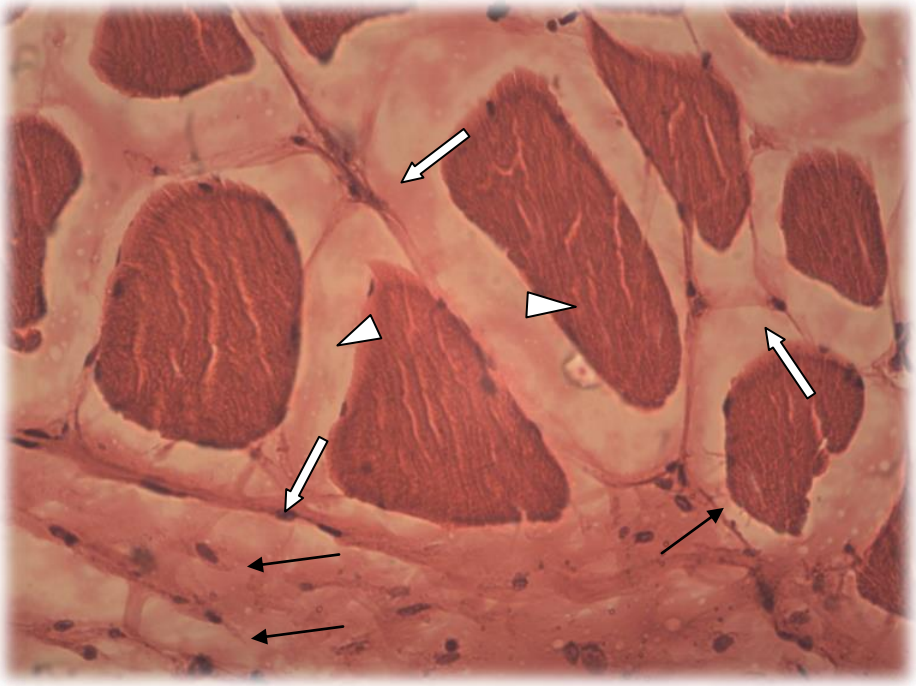
Pic. 15

High magnification on gastronemius on muscle treated with (Bpvc) on (5d) post injection illustrated degenerated fibers with condensed myofibrils (✡) circular fibers (⟶) with connective tissue edema (▷).(H & E , 540x).



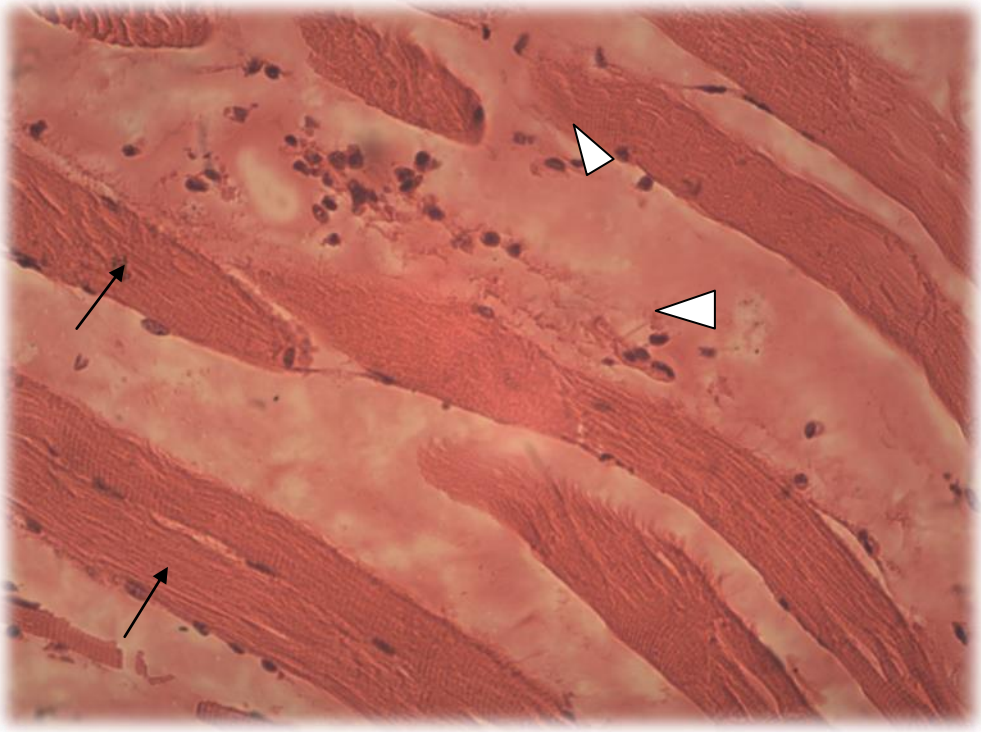
Pic. 16

Transverse section on treated triceps with (Bpvc) after (5d) showed regenerated myotubes (⟶) near the large blood vessels (▷) ,dense connective tissue (✡).(H & E ,160x).



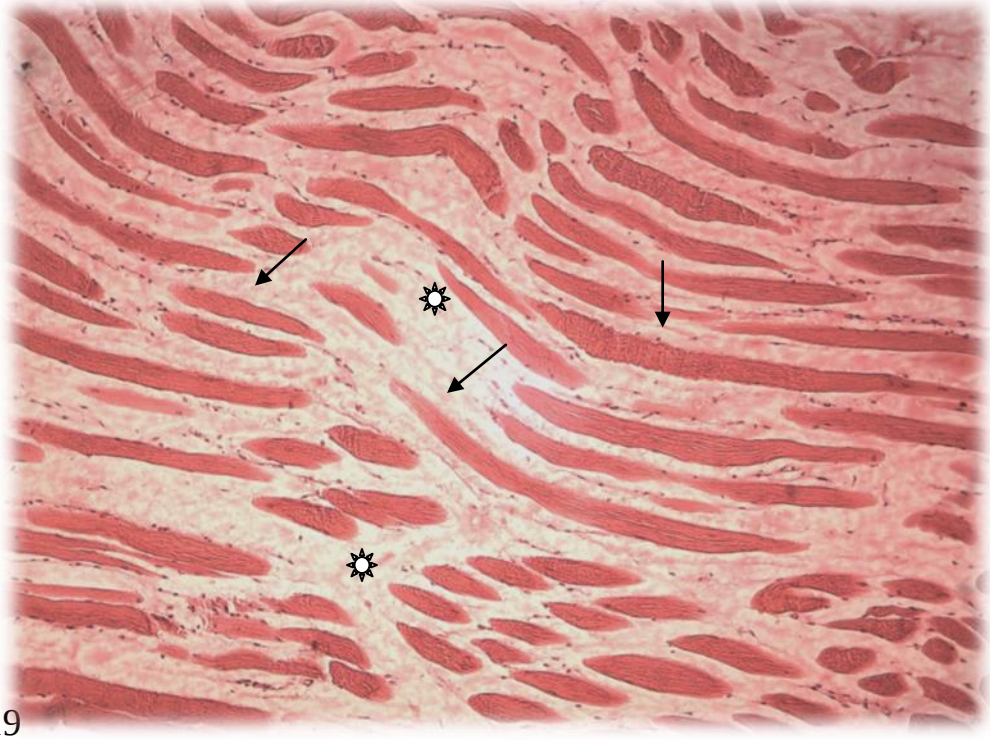
Pic. 17

High magnification on triceps muscle treated with (Bpvc) after (5d) of injection noticed increased with fibroblast (⟶),strands of collagenous fibers (⟹) also regenerated myotubes with peripheral nuclei clarified (▷) (H & E , 540x).



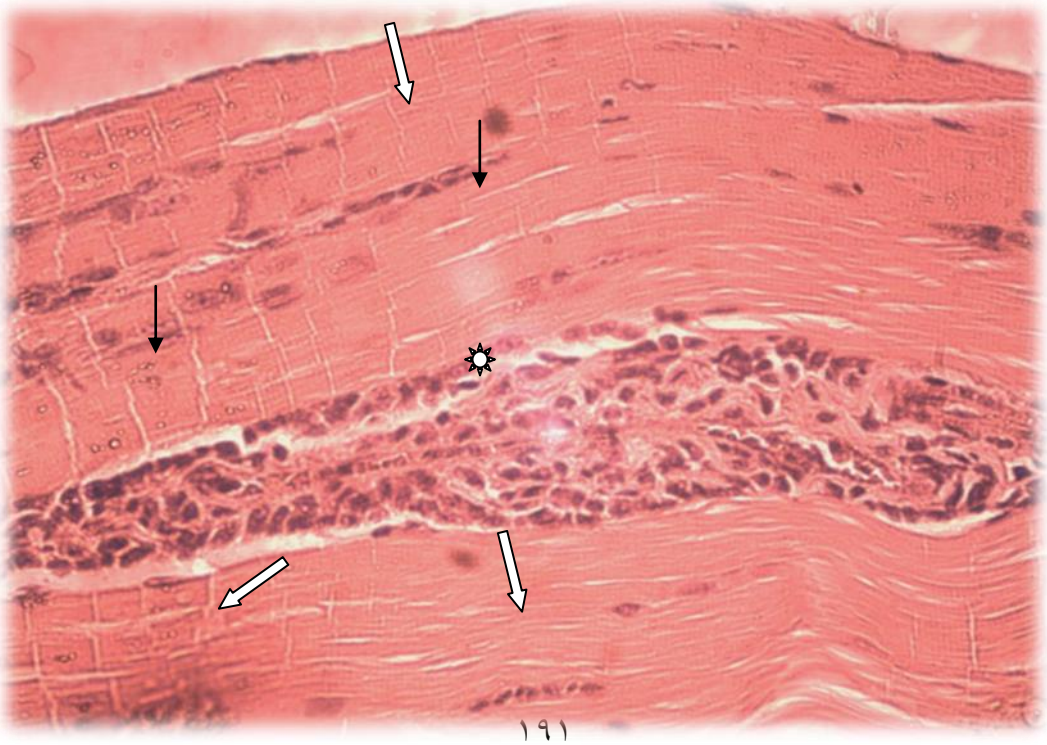
Pic. 18

Longitudinal section on triceps muscle treated with (Bpvc) at (5d) post injection showed regular nuclei as chain (⟶) dense nuclei of fibroblasts(▷) and irregular surface of muscle fibers appeared.(H & E ,540x).



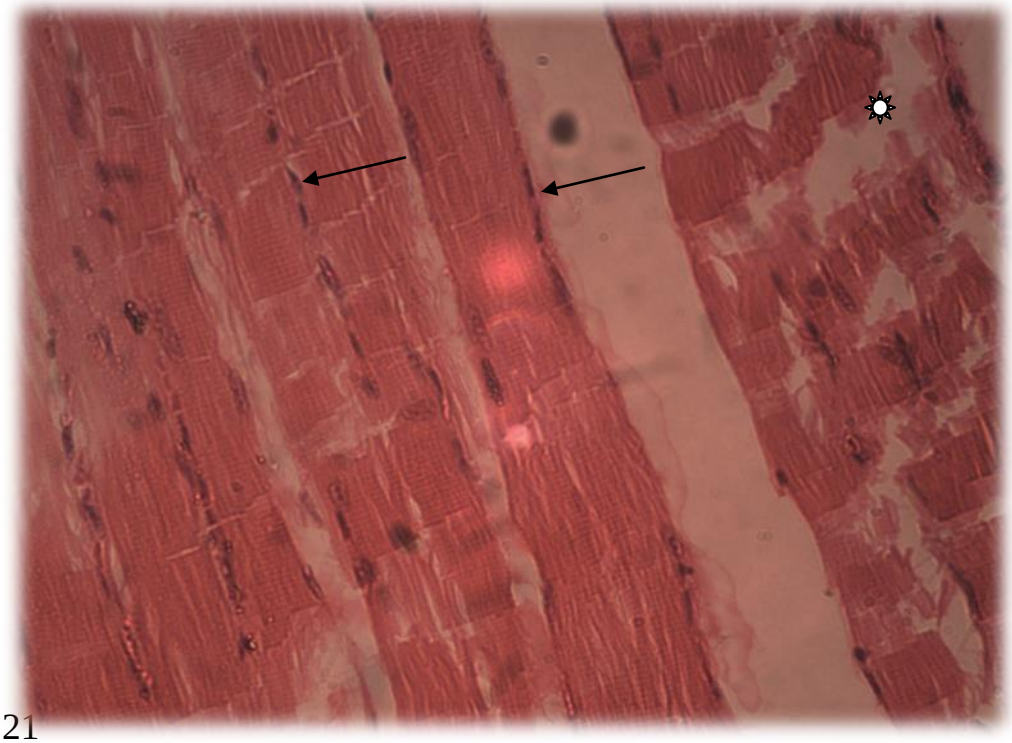
Pic. 19

Oblique section on triceps muscle injected with (Bpvc)at (5d) showed distinct units of regenerated myotubes (⟶) separated with connective tissue (✲) (H & E ,160x).



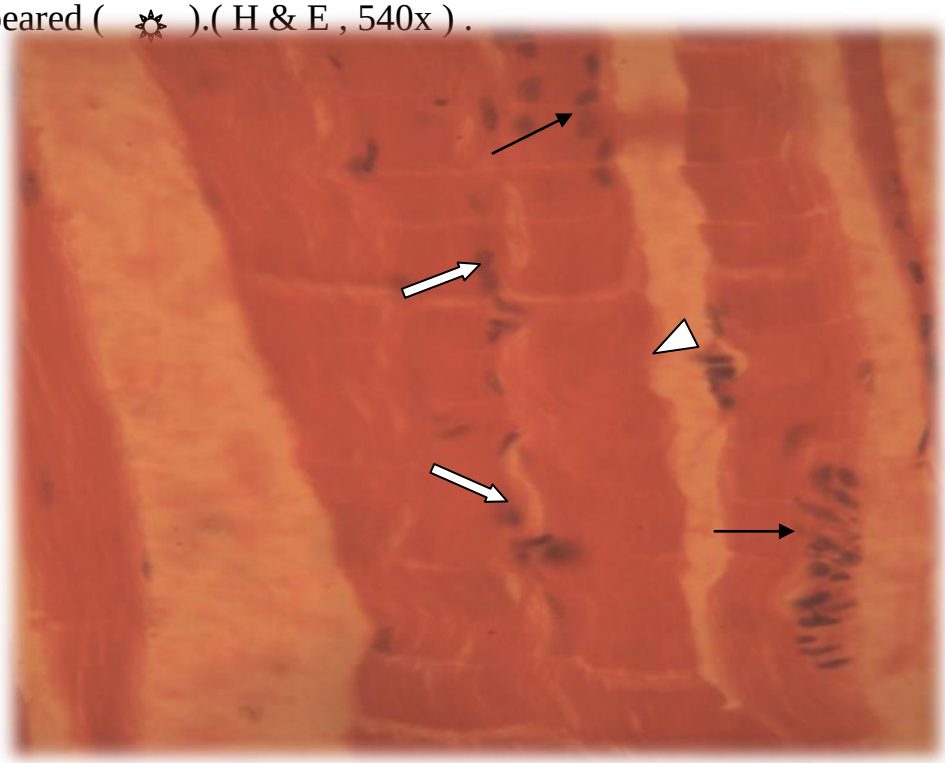
Pic. 20

Longitudinal section on gastronemius muscle after (10d) of (Bpvc) injection showed regenerated myotubes (⟶)with peripheral nuclei (⟹),also still edema , heavy inflammatory cell and myonecrosis (☼).(H & E ,540x) .



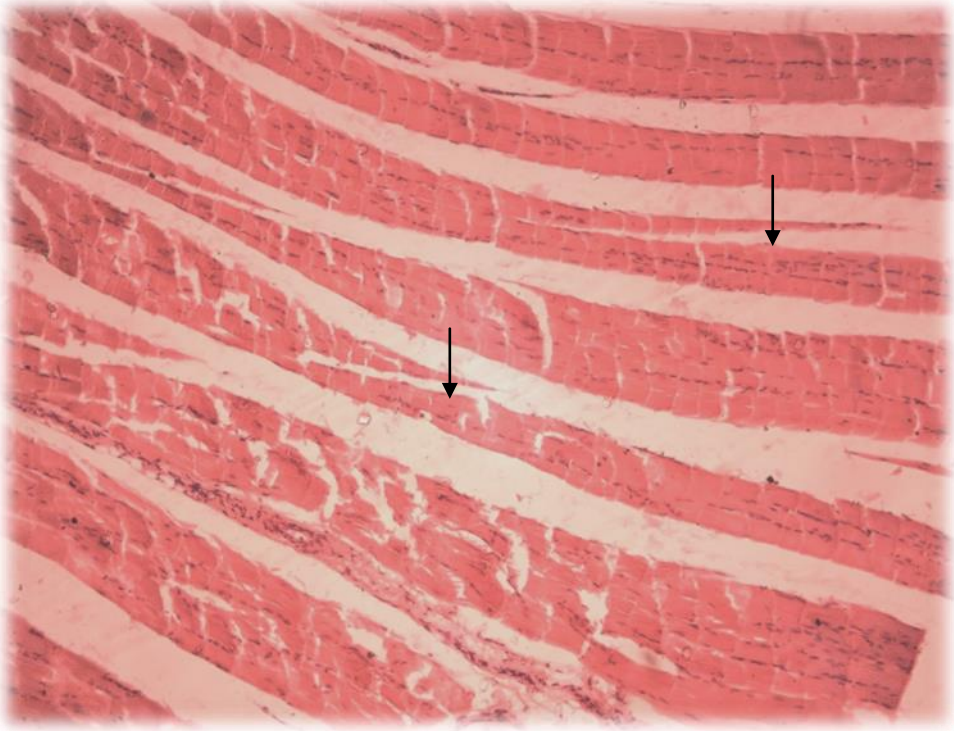
Pic. 21

Longitudinal section on Gastronemius treated with (Bpvc) at (10d) post injection showed regenerated myotubes (⟶), other damaged muscle fiber also appeared (☼).(H & E , 540x) .



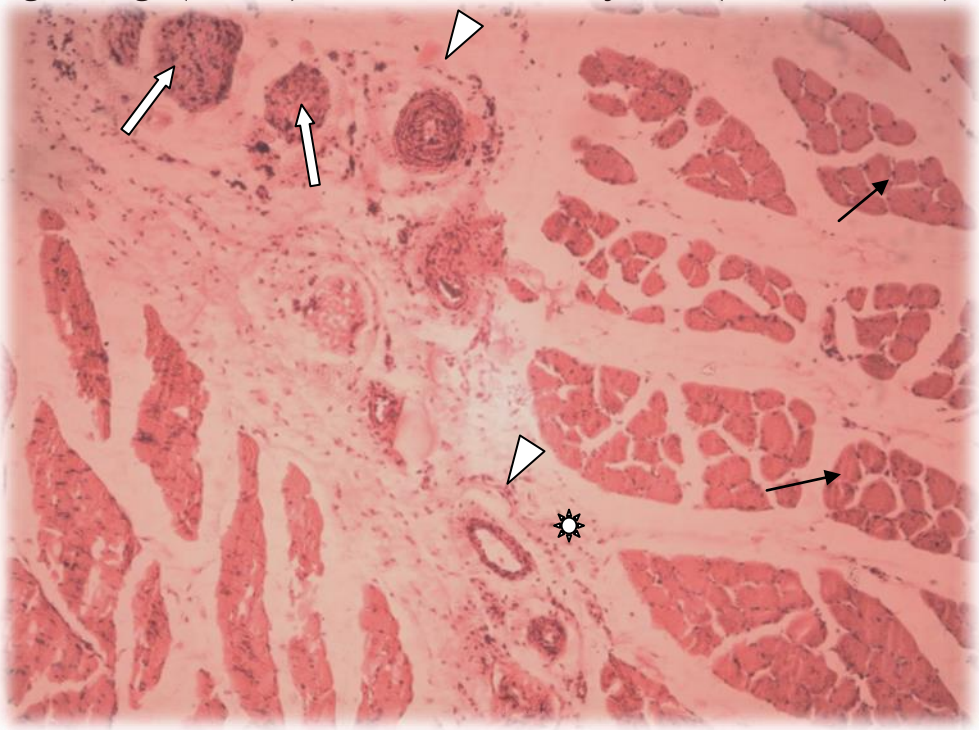
Pic. 22

Longitudinal section on Gastronemius muscle after (10d) of (Bpvc) injection showed regenerated fibers with multinuclei aggregated (⟶), Myofibroblasts (▷) with irregular external surface of muscle fibers.(H & E ,540x).



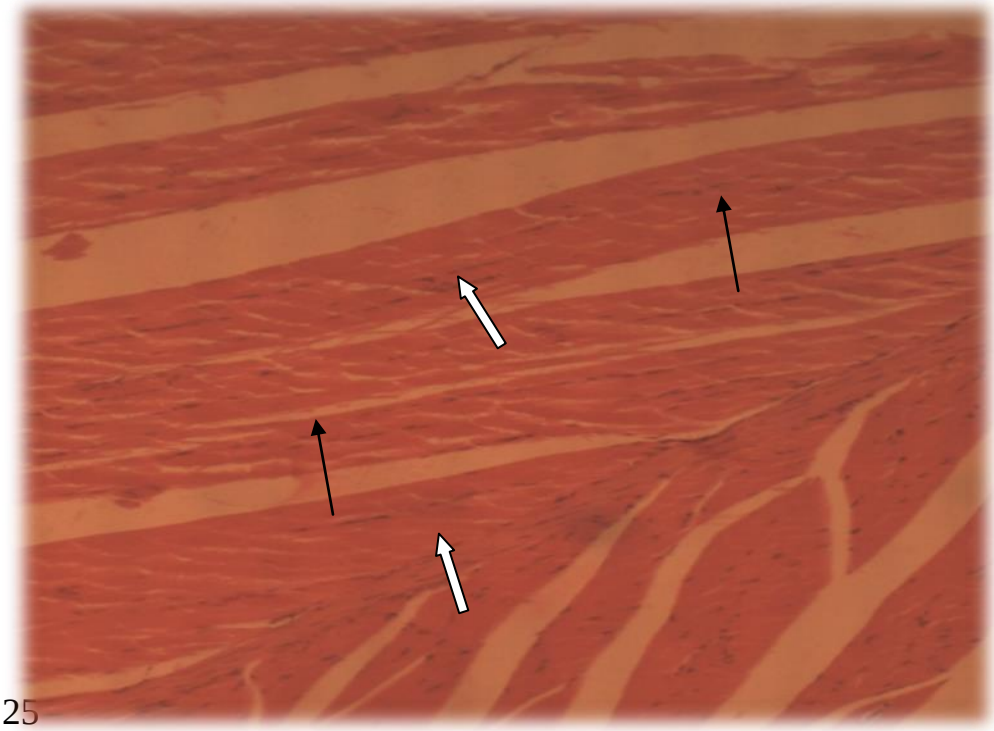
Pic. 23

Longitudinal section on triceps brachii muscle treated with (Bpvc) on (10d) post injection showed regenerated fibers with regenerated fibers ,bifurcate with tapering endings (⟶), other fibers still injuried .(H & E , 160x).



Pic. 24

Transverse section on treated triceps at (15d) post injection showed regenerated fibers with peripheral nuclei (⟶) , normal perimysium (☼) , normal nerve fibers (⟹) and rich vasculature (▷) .(H & E , 280x).



Pic. 25

Longitudinal section on Gastronemius muscle at (15d) postin jection showed bundle of parallel muscle fibers (⟶)with peripheral nuclei (⟹). (H & E , 280x).

4- Discussion

4-1 Biochemical analysis

Recent study indicated to significant increased at (2h , 2d , 5d) post injection with (Bpvc) in total serum protein concentration while there was no changes at (10d , 15d) post injection compared to control group , this result may be regarded to the toxicity caused by (Bpvc) which damaged the sarcolemma and myonecrosis which lead to fibers degeneration and increased the serum proteins, the study agreed with other studies illustrated that the injuries , damaged of muscle fibers caused sarcolemma disturbances , increased permeability and increased the serum proteins (Cherge & Rudnicki,2004).

The Bpvc effect lead to the activation of protease enzymes which known as Calpins that have ability to damaged myofibrills and degeneration of cytoskeleton proteins and released it to blood serum (Cabral *et al.*,2008).

The study also showed to an increased with significant at ($P \leq 0.01$) on (2h , 2d , 5d) post injection in (Ca) ion concentration compared to control group ,while there was no changes recorded at (10d , 15d) , this result may be clarified that (Bpvc) lead to sarcoplasmic reticulum degeneration and changes with ions permeability which in other hand result ions homeostasis , these result described by other researchers who showed that increased with (Ca) concentration related to Bpvc which penetrate the cellular membranes system because it is one of lipophilic agents that affect on structure of these membrane and caused disrupt intracellular Ca+2 homeostasis.(Zink *et al.*,2002 ; cherng *et al.*,2010).

Bpvc caused sarcosomes degeneration , an increased with oxidative enzymes , loss of permeability and increased with intracellular calcium , also there was increase in glutamate which mediated (Ca) metabolic pathway and all these factors lead to high level of (Ca) concentration (Cherng *et al.*,2010).

Our results showed increased with (Alp) at (2h ,2d ,5d) post injection while no changes with enzyme level at (10d , 15d) post injection compared to control group , this result related to the myotoxicity induced by Bpvc which lead to muscle fibers damaged , then increased serum enzyme concentration because this enzyme associated with cell membrane , this suggestion was discussed by (Safadi *et al.*,1991) who suggested that (Alp) determined by connective tissue components, endomysium cells and inflammatory cells which takeplace at severe muscle damage , also (Alp) activities associated with sarcolemma and endothelial cells lining capillaries.

4- 2 Histological study

Our findings identified that injection of normal saline into skeletal muscle caused dilation ,edema , with mild damaged but no signs of myonecrosis nor degeneration , this agreed with study of Zink *et al*.(2003) ; Cherng *et al*.(2010) who regarded the damaged fibers to mechanical effect of needle insertion and withdraw during the injection process and liquids size that push into muscles .

Recent work confirmed histological changes at (2h) post injection of (Bpvc) like damaged , hypercontracted fibers and dense infiltration of inflammatory cells , this related to myotoxicity induced by this local anaesthetic drug and its toxic effect .

This fact agreed with other studies established toxicity of (Bpvc) which induced muscle necrosis and degeneration (Plant *et al.*, 2005).

After (2d) , most muscle fibers showed necrosis , dense an inflammatory cells most of macrophages and (PMN) , with barely surface of muscle fibers , this related to acute inflammation which caused by (Bpvc) toxicity and the role of

macrophages and (PMN) to invade the damaged area , the result agreed with other studies suggested that injection of (Bpvc) in rat tibialis anterior muscles caused inflammatory response concluded invading macrophages to removed the debris of degenerated muscle fibers while other fibers appeared as chost with hyalinized cytoplasm (Rosenblatt & Wood ,1992; Nosaka,1996).

Circular and vacuolated fibers noticed at (2d) post injection of (Bpvc) , this may be aremanant of original fibers, previous study illustrated that vacuoles related to (Bpvc) toxicity and its effect on sarcoplasmic reticulum and the (Bpvc) caused intracellularly degeneration (Zink *et al.*, 2003) .

Histological exam on section from triceps and gastronemius at (5d) post injection showed variation from myonecrosis to hypercontracted fibers with central nuclei , regenerated myotubes and good vasculature this may be explained that at the same time when (Bpvc) caused myotoxicity there was also regeneration for some damaged fibers and the blood vessels play an important role to supply damaged tissue with oxygen and nutrients, the results also clarified previously by other studies indicated to that (Bpvc) induced apoptosis for myocytes and the skeletal muscles to preserve the tissue function , start complex regenerating mechanism (Cabral *et al*., 2008) .

Rosenblatt & woods (1992) illustrated that circular with central nuclei muscle fiber caused by incomplete formation of new muscle fibers , and the myonuclei migration from muscle fibre longitudinal axis to peripheral region still incomplete and partial .

Recent study referred that post (10d) of (Bpvc) injection inspite of active regeneration there was still bifurcated fibers with tapering endings and central nuclei this may be regarded that not all fibers developed to mature fibers , or this period was early stage of regeneration,this agreed with(carbal *et al.*,2008) who suggested that bifurcated fibers related to uncomplete fuse of regenerated fibers within the same basal lamina .

Also pictures of skeletal muscles injected with (Bpvc) on (15d) post injection showed the regenerated fibers more regular , parallel bundles with cross striations and located near vascular vessels this may be considered it was regenerated stage and the (Bpvc) less toxic , these results agreed with Manor & Sadeh (1989) ; Nosaka (1996) who indicated that the regenerated fibers more than the degenerated fibers and found near the supplied blood vessels .

Nishizawa et al. (2003) illustrated that the regenerated fibers more active , resistance against (Bpvc) which due to an intact blood vessels with absence of infiltration to form fibrous tissue .

In conclusion the recent study beneficial to understanding the myotoxicity induced by (Bpvc) and also to explained the most histopathological changes on different periods post injection , in addition to biochemical parameter which associated with these changes .

References

AstraZeneca,(2008).Product Monograph Sensorcaine, in, Vol. Mississauga, Ontario L4Y 1M4, AstraZeneca.

Belfieid,A. & Goldberg, D.M. (1971). Review assay for serum phenylphosphatase activity using 4-amino-antipyrine. Enzyme,12:561-573.

Cabral,A.; Machado,V.;Farinha,R.&Cabrita,A.(2008).Skeletal muscle regeneration : abrief review. Exp. Path.and health scie., 2(2):9-17.

Charge, SB. & Rudnicki, M.A. (2004). Cellular and molecular regulation of muscle regeneration .Physiol. Rev., 84(1):209-238.

Cherng, C.H. ;Wong, C.S. ; Wu,C.T. & Yeh,C.C. (2010). Intramuscular Bupivacaine Injection dose – dependently Increases glutamate release and muscle Injury in Rats. Acta. Anaesthesiol.Taiwan., 48(1):8-14.

Clark,W.L.; Balinski,E.L.; Marie,S.S. & Zak,B.(1975).Spectrometric study of adirect determination of serum calcium.Microchem.J.,20:22-32.

Couteaux,R.; Mira,J.C.& Albis,A.(1988).Regeneration of muscles after cardiotoxin injury I. cytological aspects.Biology of the cell., 62: 171- 182.

Hall-Craggs E.C. (1974). Rapid degeneration and regeneration of a whole skeletal muscle following treatment with bupivacaine (Marcain).Exp. Neurol., 43: 349-358.

Heavner, J.E. (2007). Local anesthetics. Curr. Opin. Anaesthesiol., 20:336-42.

Irwin, W. ; Fontaine, E. ; Agnolucci, L. ; Penzo, D. ; Betto, R. ; Bortolotto, S. & Reggiani, C. (2002). Bupivacaine myotoxicity is mediated by mitochondria. J. Biol. Chem. 277:12221-7.

Kindler, C. & Yost ,C.S. (2005).Two-pore domain potassium channels: new sites of local anaesthetic action and toxicity. Reg. Anesth. and Pain Med.,30(3):260-74.

Luna,L.G. (1968).Manual of histological staining methods of the Forces Institute of Pathology, 3rd ed. Mc Graw-hill, NY .

Manor,D. & Sadeh,M. (1989) . Muscle fibre necrosis induced by intramuscular injection of drugs.Br. J. Exp. Path.,70:457- 462.

Neymark, M.A. ; Kopacz,S.J. & Lee,C.P. (1980). Characterization of ATPase in sarcoplasmic reticulum from two strains of dystrophic mice.Muscle nerve., 3:316- 325 .

Nishizawa,T.; Tamaki,H.; Kasuga,N & Takekura,H. (2003) . Degeneration and regeneration of neuromuscular junction architecture in rat skeletal muscle fibers damaged by bupivacaine hydrochloride . J. Muscle Res. And Cell Moti.,24: 527 –537.

Nonaka, I.; Takagi, A.; Ishiura, S.; Nakase, H.& Sugita, H. (1983). Pathophysiology of muscle fiber necrosis induced by bupivacaine hydrochloride (marcaine). Acta. Neuropathol.,60(3-4): 167-174.

Nosaka , K. (1996) . Changes in serum enzyme activities after injection of bupivacaine into rat tibialis anterior. J. Appl. Physiol.,81:876-884 .

Plant, D.R.; Beitzel , F. & Lynch, G.S. (2005). Length - tension relationships are Altered in regenerating muscles of the rat after bupivacaine injection. J. Appl.Physiol.,98(6):1998-2003.

Punk ,K.; psinia ,L.;Welt, K.; Barth, W.; & Asmussen, G. (1999). Effects on skeletal muscle fibers of diabetes and Ginkgo biloba extracts treatment.Acta. Histochem., 101:53 - 69 .

Ragbetli , M.C. ; Yaluma, M.;Erdogan ,E.; Cengiz,N.;Kafi,I. and Ragbetli , C. (2009). Acute myotoxic effect of by infusion of prilocaine and lidocaine in Rats. J. of Ani. And veter . Adv. 8(11):2325- 2328.

Reddy, Y.S.; Reddy, N.B.; Mobley, B. A.; & Beesley, R.C.(1992). Myofibrillar and membrane – bound enzymes in skeletal muscle from myodystrophic mice.Int. J. Biochem., 24(4): 579 - 584.

Rosenblatt, J.D. & Woods, R.I. (1992) . Hypertrophy of rat extensor digitorum longus muscle injected with bupivacaine . A sequential histochemical, immuno- histochemical , histological and morphometric study. J. Anat., 181(1):11-27.

Safadi,A. ; livne, E. ; silbermann, M. & reznick,A.Z. (1991). Activity of Alkaline phosphatase in rat skeletal muscle localized along the sarcolemma and endothelial cell membranes. J.Histochem. Cytochem., 39(2):199-203.

Tietz, N. (1999) .Text book of clinical chemistry ,3rd ed. C. A. Burtis ,E. R. Ashwood, W.B. Saunders., 652- 657.

Zhang, C.;Phamonvaechavan,P.;Rajan,A.; Poon,D.Y.; Topcu-Yilmaz,P. &Guyton,D. L. (2010). Concentration-dependent bupivacaine myotoxicity in rabbit extraocular muscle. J. Am. Pedia. Ophthal. & Strabismus.,14(4): 323-327.

Zink,W. ; Graf,B.M. ; Sinner,B. ; Martin,E. ; Fink, R.H.A. & Kunst, G. (2002). Differential effects of bupivacaine on intracellular Ca++ regulation : potential mechanisms of its myotoxicity. Anesthesiol.,97(3)710 - 716.

Zink,W.; Seif,C.H.; Bohl, J.R.; Hacke,N.; Braun,P.M.;Sinner,B.; Martin,E.; Fink,R. & Graf,B. (2003).The acute myotoxic effects of bupivacaine and ropivacaine after continuous peripheral nerve blockades.Anesth.Analg., 97:1173-9.

الخلاصــة

هدفت الدراسة الحالية الى معرفة تأثير الحقن بالادوية المخدرة موضعياً كالبيفاكايين (Bpvc) في العضلات الهيكلية لحث السمية العضلية ، ولهذا فقد تم دراسة التلف العضلي ومن ثم اعادة التجديد فيما بعد للعضلات التالفة ومعرفة التغيرات النسجية المرضية للعضلتين الثلاثية الرؤوس والتوأمية في الفترات (٢ ساعة ، ٢ يوم ، ٥ يوم ، ١٠ يوم ، ١٥ يوم) بعد الحقن فضلا عن تقييم بعض المعايير الكيموحيوية المرتبطة مع السمية العضلية .

استعملت في هذة الدراسة الجرذان المختبرية نوع (Rattus norvegius) وبلغ العدد الكلي (٩٠) جرذا جميع الجرذان من الذكور وبأوزان تراوحت بين (٢٥٠ – ٣٠٠) غم وبعمر (١٢ – ١٤) اسبوع . قسمت الجرذان الى مجموعتين واعتبرت المجموعة الاولى كمجموعة سيطرة حقنت داخل العضلة بجرعة (0.6) مل من المحلول المتعادل في كل من العضلتين العضدية ثلاثية الرؤوس والعضلة التوأمية بينما المجموعة الثانية تم معاملة الجرذان فيها بالحقن داخل العضلة وبما يعادل (0.6)مل من (0.5%) من المخدر الموضعي (Bpvc) في كل عضلة لحث السمية العضلية .

خدرت الحيوانات ثم شرحت وازيلت العضلات من كل جرذ وخلال الفترات (٢ ساعة ، ٢ يوم ، ٥ يوم ، ١٠ يوم ، ١٥ يوم) بعد الحقن وأعدت هذة العضلات للفحص المجهري الضوئي ، بينما جمعت عينات الدم وفصل المصل وحفظ لأجراء بعض الاختبارات البايوكيميائية (محتوى البروتين الكلي ، وتركيز الكالسيوم ، انزيم الفوسفاتيز القاعدي) لكل من الجرذان المعاملة والسيطرة

تضمنت التغيرات النسجية المرضية بعد (٢ ساعة) من الحقن بالمخدر (Bpvc) التحلل والتنكس العضلي والتقلص الشديد وامتداد التلف الى الطبقات العميقة من العضلة بينما لوحظت الالياف العصبية والاوعية الدموية اعتيادية . بعد (٢يوم) من الحقن اوضح الفحص النسجي الياف عضلية تنكسية وارتشاح كثيف للخلايا الالتهابية معظمها خلايا ملتهمة وخلايا متعددة الاشكال النووية مع ظهور السطح الشعيري للالياف العضلية .

مقارنة مع الفترة (٥ يوم) بعد الحقن هناك تباين في التغيرات مثل فرط التقلص الشديد والالياف العضلية الفجوية ذات ليفات عضلية كثيفة وزيادة في الخلايا المولدة للالياف العضلية وظهور النبيبات العضلية كوحدات منفصلة .

أشارت الفترة (١٠ يوم) بعد الحقن الى وجود الياف عضلية متجددة مع وجود التنكس العضلي كما لازالت الالياف العضلية منقسمة وذات نهايات مستدقة ومقارنة مع الفترة اللاحقة (١٥ يوم) بعد الحقن معظم الالياف العضلية متجددة وتمايز الغلاف العضلي ومحتوياتة مع عدد قليل من الالياف التالفة .

مقارنةً مع مقاطع من العضلات الهيكلية لمجموعة السيطرة والتي حقنت بالمحلول المتعادل والتي أظهرت اتساع الفسح البينية ووذمة النسيج الرابط والحواجز ولم تكن هناك علامات عن التلف او التنكس العضلي

وبالاعتماد على التحليلات البايوكيميائية للاختلافات المعنوية بينت النتائج حدوث ارتفاع وبفارق معنوي عند ($P \leq 0.01$) في محتوى البروتين الكلي وتركيز الكالسيوم ومستوى انزيم الفوسفاتيز القاعدي عند الفترات (٢ ساعة ، ٢يوم ، ٥ يوم) ومقارنة مع مجموعة السيطرة . بينما لم يكن هناك اي زيادة معنوية في هذة المعايير عند الفترتين (١٠ يوم ، ١٥ يوم) بعد الحقن مقارنة مع مجموعة السيطرة وعند مستوى ($P \geq 0.01$) .