Original Article

Histological Study of the Effect of Bone Marrow-Derived Mesenchymal Stem Cells on Experimentally Induced Skeletal Muscle Injury in Adult Male Albino Rats

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ABSTRACT

Introduction: Muscle injuries are common and may be associated with impaired functional capacity, especially among athletes. The results of healing with conventional therapy are often inadequate, generating substantial interest in the potential for emerging technologies such as stem cells to enhance the process of soft tissue healing and to decrease time of recovery.

Aim of the Work: Evaluation the role of BM-MSCs in healing of experimentally induced skeletal muscle injury in adult male albino rat.

Materials and Methods: Forty-five adult male albino rats were used. They were divided into five groups. Group I: bone marrow was taken from both femur and tibia to prepare stem cells and the gastrocnemius muscle was obtained. Group II: gastrocnemius muscle was injured and specimens were taken at the same day within 6 hours. Group III: gastrocnemius muscle was injured and left without treatment. Group IV: gastrocnemius muscle was injured and immediately treated by intramuscular injection of stem cells. Group V: gastrocnemius muscle was injured and immediately treated by intramuscular injection of media used for suspension of bone marrow. Muscle specimens were excised from group III to V after 2 weeks and 4 weeks from injury and used for histological and electron microscopic study.

Results: The results revealed that stem cell treated group showed early and enhanced regeneration of skeletal muscle injury. This was evidenced by light and electron microscope and morphometric study. The stem cell treated group demonstrated early formation of many myotubes 2 weeks after injury and ultrastructural features of activated satellite cells in association with restoration of normal histological features of muscle fibers without fibrosis.

Conclusion: It could be concluded that a local injection of stem cells into the injured gastrocnemius muscle resulted in early activation, proliferation and differentiation of satellite cells and accelerated muscle healing.

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Key Words: Light & electron microscopes, muscle injury, stem cells.

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INTRODUCTION

Skeletal muscle is one of the most active tissues in the human body, consisting primarily of water (75%), protein (20%), and other chemicals. Sarcolemma surrounds a single muscle fibre and is made up of a combination of proteins that are physically related to the interior myofilament structure, particularly the actin protein[1].

Musculoskeletal injuries are the most common cause of severe chronic pain, physical disability and account for the majority of all sport related injuries[2].

Skeletal muscle goes through a well-defined series of events in response to damage, including inflammation, repair, and remodelling[3].

Satellite cells, which are resident muscle stem cells that proliferate, develop, and merge with existing myofibers or produce new myofibers, are responsible for repair[4].

Stem cell research holds significant promise for treating a wide range of degenerative and traumatic disorders for which no specific or effective treatment currently exists[5].

MATERIALS AND METHODS

Experimental animals

45 adult male albino rats were used in this study. Their sizes ranged from 200 to 250 g. All of the animals were housed in sufficiently clean and well-ventilated cages and fed a similar commercial laboratory diet and water, as per some researchers' recommendations[6]. The animals were left for acclimatization on the new environment.

Animal grouping

The rats were divided into the following five groups:

Group I (Control group): (10 rats) Both the femur and the tibia were used to extract bone marrow. and the gastrocnemius muscle was obtained to study the normal histological structure.

Group II: (5 rats) Gastrocnemius muscle injury was induced. The specimens were obtained at the same day within 6 hours.

Group III: (10 rats) Gastrocnemius muscle injury was induced as in group II and left untreated for spontaneous

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recovery. It was divided into 2 equal subgroups 5 rats each (sub group III A and sub group IIIB). They were sacrificed after 2 weeks and 4 weeks respectively[7].

Group IV: (10 rats) Gastrocnemius muscle injury was induced as in group II. The rats were treated immediately by a single dose of bone marrow mesenchymal stem cells suspended in its specific media injected locally into gastrocnemius muscle (1x106 MSCs cells)[7]. It was divided into 2 equal subgroups 5 rats each (sub group IVA and sub group IVB). They were sacrificed after 2 weeks and 4 weeks respectively according to (Andrade *et al*, 2015)[7].

Group V: (10 rats) Gastrocnemius muscle injury was induced as in group II. The rats were treated immediately by a single dose of media used for suspension of bone marrow mesenchymal stem cells injected locally into gastrocnemius muscle. It was divided into 2 equal subgroups 5 rats each (sub group VA and sub group VB). They were sacrificed after 2 weeks and 4 weeks respectively.

The animals were anaesthetized with sodium phenobarbital (50 mg/ kg) intraperitoneally after the proper period[8]. The muscles were dissected after they were sacrificed.

All specimens were collected and prepared for light and electron microscopic examination. After receiving approval from the Tanta Faculty of Medicine's Research Ethics Committee (REC), the sacrificed animals' remains were wrapped in a special packaging according to safety precautions and infection control measures and dispatched with the hospital biohazards.

Mesenchymal stem cell preparation for injection

Bone marrow harvesting[9]

Soft tissues were properly dissected from femurs, tibias, and humeri. A sterile scissor was used to cut the epiphyses at both ends of the bones. The bone marrow was flushed out from the diaphysis of the bones and cultured in freshly prepared media into tissue culture flask (RPMI, 10% FBS and 1% Antibiotic /Antimycotic mix) (RPMI from Lonza Com, Cat. No. BE12-702F, Swiss. FBS from Gibco, In *vitro* gen Com, Cat. No A11-151, Austria. Antibiotic/ Antimycotic mix from Lonza Com, Cat. No: 17–745 E, Switzerland). Culture flasks were kept at 37°C in 5% CO2 incubator. Non-adherent cells were removed after three days, and adherent cells were washed twice with phosphate buffered saline (PBS) before being cultivated.MSCs were distinguished from other BM cells by their tendency to adhere to tissue culture plastic flask[10]. Phase contrast microscope was used for examination and photographing.

Trypsinization, suspension, and counting of MSCs

When the confluence of the cells of passage 0 (p0) reached (70% - 80%) about (7-9) days after seeding, trypsinization and sub-culturing were carried out. The hemocytometer was used to determine the total cell count and the trypan blue 0.4% exclusion method was used to assess cells viability. The cells were injected at a dose of 1×10^6 MSCs /rat. All cell culture procedures were done in Tissue Culture Unit in Histology and Cell Biology Department, Faculty of Medicine Tanta University.

Induction of skeletal muscle (Gastrocnemius muscle) injury

The rats were sedated with ether inhalation before hair shaving and skin cleaning with betadine solution[11]. Gastrocnemius muscle was palpated and crushed between the blades of a hemostat at level 3 for 2 minutes, guided by tendon Achillis[12]. In group II, the specimens were examined at the same day within 6 hours. In group III, the injured muscles were left without treatment. In group IV, the injured muscles were injected immediately intramuscular by a single dose of bone marrow mesenchymal stem cell suspended in its media. In group V, the injured muscles were injected immediately intramuscular by a single dose of media used for suspension of bone marrow mesenchymal stem cells. The animals were restored to their homes after their recovery and given free chow and drink. At the end of 2nd and 4th week respectively, rats were sacrificed and the gastrocnemius muscles were excised and processed for light and electron microscopic examination.

Preparation of specimens for light microscopic study[13]

After extraction of the gastrocnemius muscle specimens from animals, they were immediately fixed in 10% formal saline for 24 hours, dehydrated in ascending grades of alcohol, then cleared in two changes of xylol. Impregnation was done in pure soft paraffin for 2 hours at 55ºC followed by embedding in hard paraffin to make paraffin blocks. Finally, sections of 5 microns thickness were cut by the microtome (Leica Biosystems, China) at the Histology Department, Faculty of Medicine, Tanta University, and were stained with haematoxylin & eosin (H&E).

Preparation of ultrathin sections for electron microscopic study[14]

Gastrocnemius muscle specimens were cut into small pieces and fixed in 4% phosphate buffered gluteraldehyde (0.1 M, pH 7.3), post-fixed with 1% phosphate-buffered osmium tetroxide, and then dehydrated in ascending grades of ethanol. After immersion in propylene oxide, the specimens were embedded in epoxy resin mixture. Ultrathin sections (80-90nm) were stained with uranyl acetate and lead citrate, to be examined by JEOL-JEM-100 transmission electron microscope (Tokyo, Japan) at the Electron Microscopic Unit, Faculty of Medicine, Tanta University, Egypt.

RESULTS

Light microscopic results

H&E staining

Group I (control group): H&E stained gastrocnemius muscle sections from group I were examined under the

microscope and revealed normal histological structure of the skeletal muscle.

In transverse section, Skeletal muscle was composed of bundles of skeletal muscle fibres separated by connective tissue (perimysium), which contained blood vessels. Connective tissue separated the individual muscle fibres (endomysium). Individual muscle fibres was polygonal in shape, with nuclei on the periphery (Figure 1A).

In longitudinal section, the skeletal muscle fibers appeared long, parallel, cylindrical and multinucleated with nuclei on the periphery directly under the sarcolemma, with their long axis parallel to the long axis of muscle fibers. The sarcoplasm appeared acidophilic with transverse striations (Figure 1B).

Group II (injury group): H&E stained transverse section obtained from animals of this subgroup showed normal skeletal muscle architecture was lost. Splitting, wide separation, fragmentation of muscle fibers and congested blood vessels were observed (Figure 2A). A longitudinal section showed transversely oriented nucleus of some fibers. Inflammatory cell infiltration and areas of hemorrhage were also observed (Figure 2B).

Group III (Gastrocnemius muscle was injured and left for spontaneous recovery): Subgroup IIIA (2 weeks after injury without treatment):

Examination of H&E stained transverse section obtained from animals of this subgroup showed variable sized widely separated muscle fibers and dilated blood vessels (Figure 3A). A longitudinal section showed loss of continuity of some muscle fibers with focal patches of pale acidophilic homogenous sarcoplasm. Nucleus internalization of some fibers was present (Figure 3B).

Subgroup IIIB (4weeks after injury without treatment): Examination of H&E stained transverse section obtained from animals of this subgroup showed variable sized muscle fibers with nuclear internalization. Some fibers show splitting of their myofibrils. Notice: the presence of fragmented muscle fibers (Figure 4A). A longitudinal section showed discontinuation of muscle fiber with nuclear internalization and inflammatory cells are observed between muscle fibers(Figure 4B)

Group IV (Gastrocnemius muscle was injured and treated with stem cells): Group IV depicted progressive improvement as many myotubes appeared with marked regression of the inflammatory reaction.

Subgroup IVA (2weeks after injury and treatment with stem cells): Examination of H&E stained transverse section obtained from animals of this subgroup showed many newly formed myofibers with acidophilic sarcoplasm and central nuclei, wide spaces between muscle fibers, focal areas of .pale acidophilic homogenous sarcoplasm. and inflammatory cell infiltration (Figure 5A). A longitudinal section showed row of central nuclei forming myotubes and discontinuation of muscle fiber (Figure 5B).

Subgroup IVB (4weeks after injury and treatment with stem cells): Examination of H&E stained transverse section obtained from animals of this subgroup showed marked improvement to be more or less similar to normal muscle fibers. In transverse section, polygonal muscle fibers with peripheral nuclei arranged in bundles separated by perimysium were seen. Blood vessels appeared in both endomysium and perimysium(Figure 6A). In longitudinal section, long, cylindrical, and parallel muscle fibres appeared., multinucleated, with acidophilic sarcoplasm and transverse striations. Muscle nuclei were peripheral and subsarcolemmal in position (Figure 6B).

Group V (Gastrocnemius muscle was injured and injected with media): This group revealed histological changes similar to that observed in group III.

Subgroup VA (2weeks after injury and injection with media): Examination of H&E stained transverse section obtained from animals of this subgroup showed variable sized widely separated muscle fibers, splitting of myofibrils and fragmented muscle fibers (Figure 7A). A longitudinal section showed loss of normal architecture of myofibrils, discontinuation of muscle fibers, splitting of myofibrils and pale acidophilic sarcoplasm (Figure 7B).

Subgroup VB (4weeks after injury and injection with media): Examination of H&E stained transverse section obtained from animals of this subgroup showed variable sized widely separated muscle fibers with nuclear internalization. Some fibers shows splitting of their myofibrils and others are fragmented (Figure 8A). A longitudinal section showed discontinuation of muscle fibers, nuclear internalization, splitting of myofibrils and Inflammatory cells in between muscle fibers (Figure 8B).

Electron microscopic results

Group I (Control group): Longitudinal ultrathin sections of gastrocnemius muscle revealed the normal ultrastructural features of skeletal muscle fibers. Myofibrils showed a regular pattern of bright (I) and dark (A) bands that extended parallel to the myofiber's long axis. Each A band was bisected by a paler H zone and a dark M line within it. A dark Z line bisected each I band. Regular sarcomeres appeared between successive Z lines (Figure 9A). small paired mitochondria were present around Z lines. Subsarcolemmal mitochondria were also present (Figure 9B). Satellite cells appeared separated from the muscle fiber by its own cell membrane with characteristic oval, heterochromatic nucleus surrounded by cytoplasm rich in ribosomes (Figure 9C).

Group II (specimens obtained from animals immediately after induction of muscle injury): Examination of ultrathin sections of gastrocnemius muscle of group II at the same day of injury revealed wide separation between myofibrils. Disruption of the myofibrils ranged from partial destruction to complete lysis with discontinuation of Z lines (Figure 10A). Irregularly detached sarcolemma with sub sarcolemmal lysis of myofibrils were observed (Figure 10 B).

Group III (Gastrocnemius muscle was injured and left without treatment):

Subgroup IIIA (2weeks after injury without treatment): Examination of ultra-thin sections obtained from animals of subgroup IIIA showed sever ultra-structural alterations in the form of loss of the normal architecture of skeletal muscle, focal lysis of myofibrils and mega mitochondria (Figure 11A). Focal loss of normal banding pattern of muscle fibrils , enlarged fused mitochondria and subsarcolemmal accumulation of mitochondria were observed. Satellite cell with increased condensation of peripheral chromatin and degenerated cytoplasm was also seen (Figure 11 B).

Subgroup IIIB (4weeks after injury without treatment): Examination of ultra-thin sections obtained from animals of subgroup IIIB showed partial destruction of myofibrils with loss of Z line, large mitochondria arranged longitudinally along Z line and satellite cell nucleus with its condensed peripheral chromatin (Figure 11 C).

Group IV (Gastrocnemius muscle was injured and treated with stem cells):

Subgroup IVA (2weeks after injury and treatment with stem cells): Examination of ultra-thin sections obtained from animals of subgroup IVA showed focal lysis of myofibrils, interrupted Z line, subsarcolemma accumulation of mitochondria, enlarged fused mitochondria and internalized myonucleus. Satellite cell with elongated cytoplasmic process was also observed (Figure 12 A).

Subgroup IVB (4weeks after injury and treatment with stem cells): Examination of ultra-thin sections obtained from animals of subgroup IVB showed fibers with regular banding pattern and regular sarcomeres were observed. Subsarcolemmal oval euchromatic myonucleus with thin condensed peripheral chromatin and prominent nucleolus was observed (Figure 12 B).

Group V (Gastrocnemius muscle was injured and injected with media): Examination of ultra-thin sections obtained from animals of revealed histological changes similar to that observed in group III

Subgroup VA (2weeks after injury and treatment with media): Examination of ultra-thin sections obtained from animals of subgroup VA showed disturbance of the normal architecture of skeletal muscle and partial destruction of myofibrils. The sarcoplasm contained subsarcolemmal accumulation of mitochondria and other mitochondria fused together forming mega mitochondria (Figure 13 A).

Subgroup VB (4weeks after injury and treatment with media): Examination of ultra-thin sections obtained from animals of subgroup VB revealed partial destruction of myofibrils with loss of Z line. The sarcoplasm showed subsarcolemmal accumulation of mitochondria and other mitochondria fused together forming mega mitochondria. Subsarcolemmal irregular myonucleus with indented nuclear membrane (Figure 13 B).

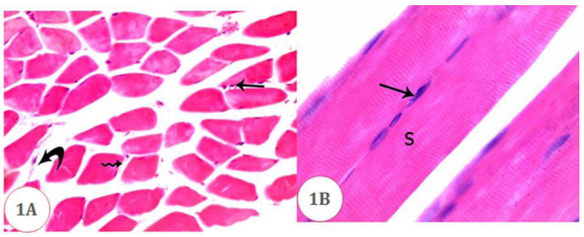

Fig. 1: A photomicrograph of the gastrocnemius muscle of group 1 (control group). (A) a transverse section showing bundles of polygonal muscle fibers with peripheral nuclei (arrow). They are separated by connective tissue perimysium (curved arrow). Endomysial connective tissue separates individual muscle fibers are also present (tortuous arrow). (H &E x400) . (B) A longitudinal section in the gastrocnemius muscle of groupI (control group) showing acidophilic sarcoplasm with transverse striations (S) and peripheral subsarcolemmal oval myonuclei (arrow). (H&E, x1000)

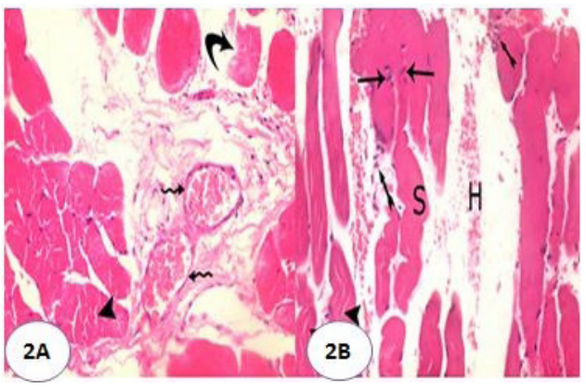

Fig. 2: A photomicrograph of the gastrocnemius muscle of group II (immediately after injury). (A) a transverse section showing fragmented muscle fibers (curved arrow), splitting of muscle fibers (arrow head). Notice: dilated congested blood vessels (tortuous arrows). (B) a longitudinal section) showing homogenous acidophilic sarcoplasm (S), splitting of muscle fibers (arrow head), transverse orientation of the nucleus (arrow). Notice: severe hemorrhage (H) and inflammatory cell infiltration (bifid arrows). (H&E X 400).

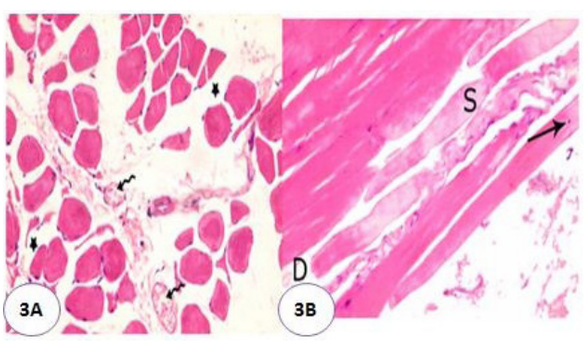

Fig. 3: A photomicrograph of the gastrocnemius muscle of subgroup IIIA (2 weeks after injury without treatment). (A) A transverse section showing widely separated muscle fibers (stars) and dilated congested blood vessels (tortuous arrows). (B) A longitudinal section showing irregularity and discontinuation of muscle fibers (D), areas of pale acidophilic sarcoplasm (S). Internalization of the nuclei of some fibers is also observed (arrow). (H&E X 400).

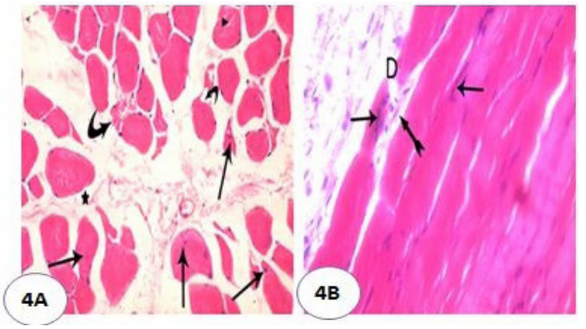

Fig. 4: A photomicrograph of the gastrocnemius muscle of subgroup IIIB (4weeks after injury without treatment). (A) A transverse section showing variable sized muscle fibers with nuclear internalization (arrows). Some fibers show splitting of their myofibrils (arrow heads). Notice: the presence of fragmented muscle fibers (curved arrow).B) (B) a longitudinal section showing showing discontinuation of muscle fiber (D) with nuclear internalization (arrows) and inflammatory cells are observed between muscle fibers (bifid arrow). (H&E X 400).

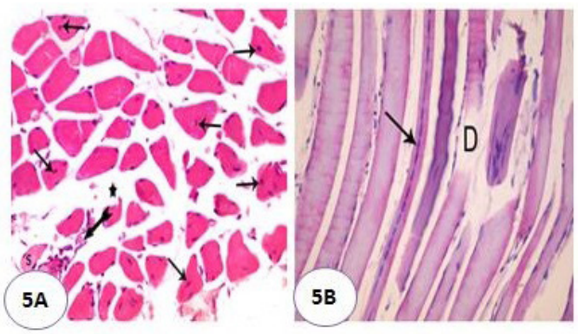

Fig. 5: A photomicrograph of the gastrocnemius muscle of subgroup IVA (2weeks after injury and treatment with stem cells). (A) A transverse section showing many newly formed myofibers with acidophilic sarcoplasm and central nuclei (arrows), wide spaces between muscle fibers (stars), focal areas of .pale acidophilic homogenous sarcoplasm (S). Notice: inflammatory cell infiltration is present (bifid arrow).(B) a longitudinal section in the gastrocnemius muscle showing row of central nuclei forming myotubes (arrow) and discontinuation of muscle fiber (D). (H&E X 400).

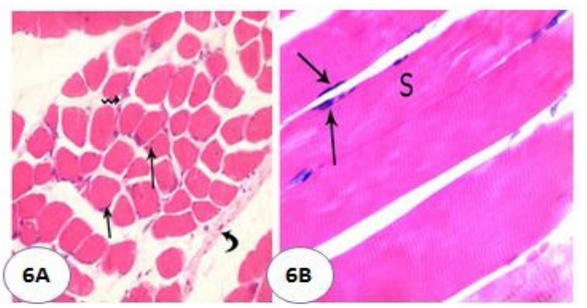

Fig. 6: A photomicrograph of the gastrocnemius muscle of subgroup IVB (4weeks after injury and treatment with stem cells). (A) A transverse section showing regenerated polygonal muscle fibers variable in size with peripheral nuclei (arrows). They are separated by connective tissue perimysium (curved arrow). Endomysial connective tissue separates individual muscle fibers (tortuous arrow) (H&E X 400). (B) a longitudinal section in the gastrocnemius muscle showing long parallel muscle fibers with acidophilic sarcoplasm, transverse striations (S) and peripheral myonuclei (arrows) to be more or less similar to control. (H&E, x1000)

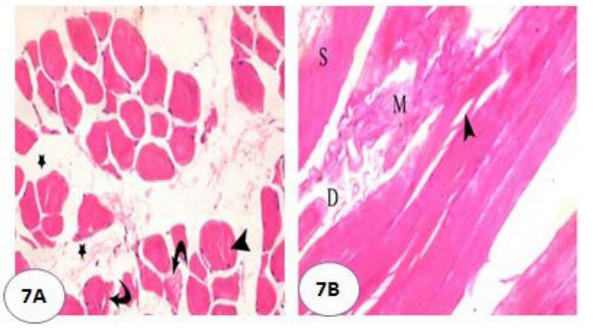

Fig. 7: A photomicrograph of the gastrocnemius muscle of subgroup VA (2weeks after injury and treatment with media). (A) A transverse section showing variable sized widely separated muscle fibers (stars), splitting of myofibrils (arrow head) and fragmented muscle fibers (curved arrows). (B) a longitudinal section in the gastrocnemius muscle showing, loss of normal architecture of myofibrils (M), discontinuation of muscle fibers (D), splitting of myofibrils (arrow head) and pale acidophilic sarcoplasm (S). (H&E X 400).

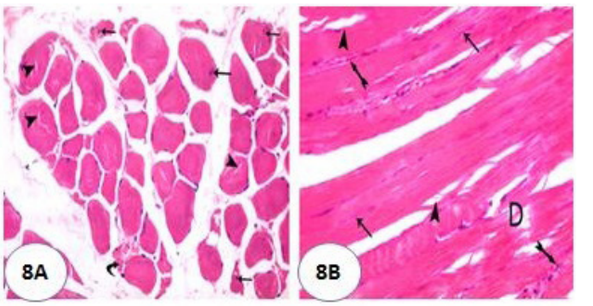

Fig. 8: A photomicrograph of the gastrocnemius muscle of subgroup VB (4weeks after injury and treatment with media) (A) a transverse section showing variable sized widely separated muscle fibers with nuclear internalization (arrows). Some fibers shows splitting of their myofibrils (arrow head) and others are fragmented (curved arrows). (B) A longitudinal section showing discontinuation of muscle fibers (D), nuclear internalization (arrows), splitting of myofibrils (arrow head). Inflammatory cells are observed in between muscle fibers (bifid arrows). (H&E X 400)

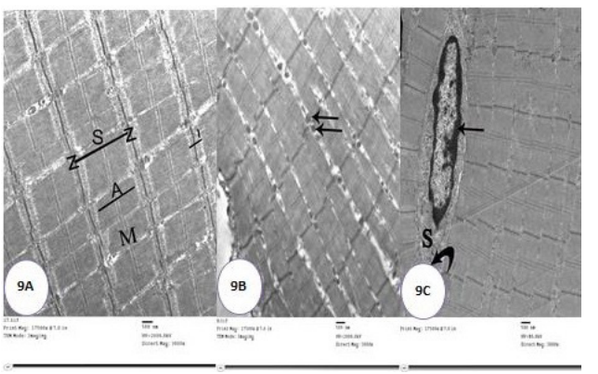

Fig. 9: An electron micrograph of a longitudinal ultrathin section of the gastrocnemius muscle of group 1 (control group) showing. (A) Normal banding pattern of the myofibrils with alternating light (I) and dark (A) bands. Z lines (Z) appear in the middle of I band and M line (M) appear in the middle of A bands. Sarcomeres (S) are observed in between two successive Z lines (B) paired mitochondria around Z lines (arrows). (C) Satellite cell (S) outside the sarcolemma. Its cytoplasm is rich in ribosomes (curved arrow) and contain oval nucleus with peripheral condensed chromatin (arrow). (Mic.mag. x3000)

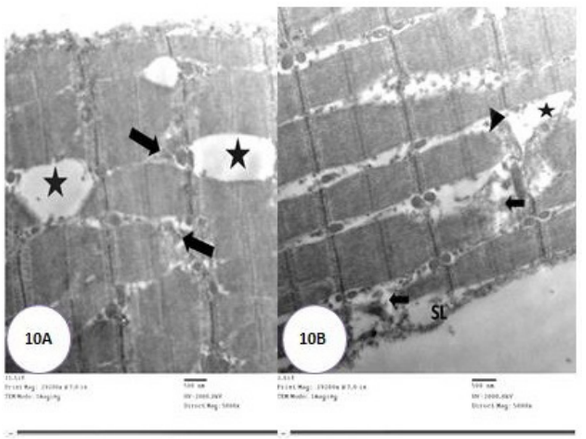

Fig. 10: An electron micrograph of longitudinal ultrathin section of gastrocnemius muscle of group II (immediately after injury) showing. (A) Wide separation between the myofibrils (stars) and partial destruction of myofibrils (thick arrow). (B) Detached sarcolemma and subsarcolemmal empty space (SL), wide spacing between the myofibrils (star) and areas of partial destruction of myofibrils (thick arrow) with discontinuation of Z line (arrow heads). (Mic.mag. x5000)

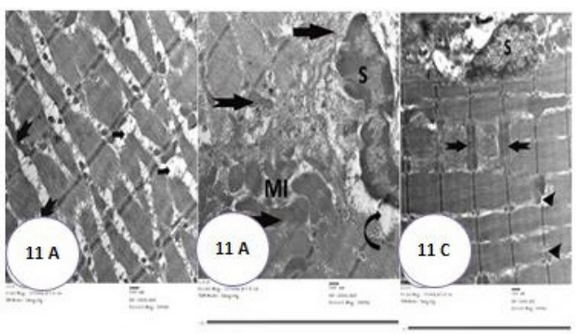

Fig. 11: An electron micrograph of a longitudinal ultrathin section of gastrocnemius muscle of subgroup IIIA (2weeks after injury without treatment) showing. (A) Focal lysis of myofibrils (thick arrow) and mega mitochondria (bifid arrow). (B) Focal loss of normal banding pattern of muscle fibrils (thick arrow), enlarged fused mitochondria (bifid arrows) and subsarcolemmal accumulation of mitochondria (MI). Notice: satellite cell (S) with increased condensation of peripheral chromatin and degenerated cytoplasm (curved arrow). An electron micrograph of a longitudinal ultrathin section of gastrocnemius muscle of subgroup IIIB (4weeks after Injury without treatment) showing (C) partial destruction of myofibrils with loss of Z line (arrow heads), large mitochondria arranged longitudinally along Z line (bifid arrows) and satellite cell nucleus with its condensed peripheral chromatin (S). (Mic.mag. x3000)

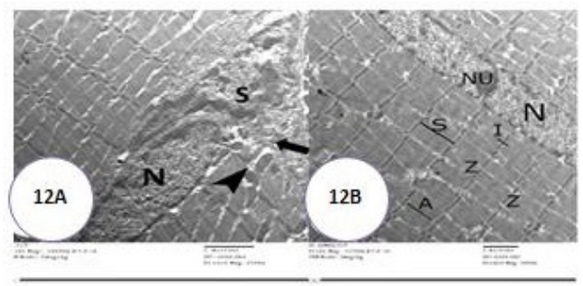

Fig. 12: An electron micrograph of a pancreas. (A) An electron micrograph of a longitudinal ultrathin section of gastrocnemius muscle in subgroup IVA (2weeks after injury and treatment with stem cells) showing focal areas of fragmentation of myofibrils (thick arrow), loss of z line (arrow head). Notice: satellite cell with long cytoplasmic process (S). (Mic. mag. X 2500). (B) An electron micrograph of a longitudinal ultrathin section of gastrocnemius muscle of subgroup IVB (4weeks after injury and treatment with stem cells) showing subsarcolemmal myonucleus (N), enlarged fused mitochondria (bifid arrow) and focal area of partial destruction of myofibrils (thick arrow) (Mic.mag. x3000). (C) subgroup IVB (4weeks after injury and treatment with stem cells) showing nearly normal muscle fibers with normal banding pattern with A bands, I bands and regular sarcomeres between Z lines. Notice: subsarcolemmal oval myonucleus (N) with prominent nucleolus (nu). (Mic.mag. x2000)

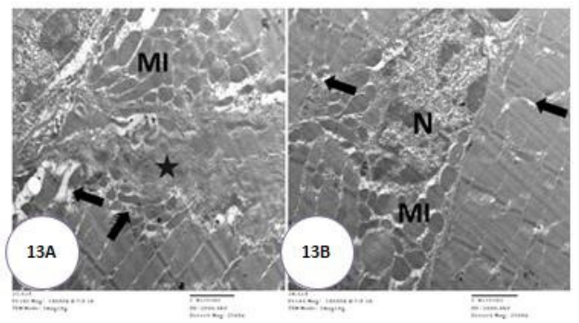

Fig. 13: An electron micrograph of a pancreas. (A) An electron micrograph of a longitudinal ultrathin section of gastrocnemius muscle of subgroup VA (2weeks after injury and treatment with media) showing partial destruction of myofibrils (thick arrows), loss of normal banding pattern (star) and subsarcolemmal accumulation of mitochondria (MI). (Mic.mag x3000) . (B) An electron micrograph of a longitudinal ultrathin section of gastrocnemius muscle in subgroup VB (4weeks of injury and treatment with media) showing subsarcolemmal accumulation of mitochondria (MI), subsarcolemmal irregular myonucleus with indented nuclear membrane (N) and partial destruction of myofibrils (thick arrows). (Mic. mag. X 2500)

DISCUSSION

The aim of this study was to evaluate the therapeutic effect of mesenchymal stem cells on skeletal muscle injury.

In this current study, light microscopic examination of gastrocnemius muscle sections obtained after induction of injury of group II revealed signs of muscle degeneration and necrosis. The most prominent features were hemorrhage, inflammatory cell infiltration in between muscle fibers. Some muscle fibers showed longitudinal splitting, discontinuation and wide spaces between them. In this work splitted myofibers were one of the most prominent findings.

This was in agreement with some authors[15,16] who stated that the initial degeneration and necrosis event of muscle injury is necrosis of the muscle fibres, which is predominantly owing to an uncontrolled calcium influx through sarcolemma lesions. This increase in cytoplasmic calcium activates proteases and hydrolases, causing further muscle injury. Soliman and Atteia[17] stated that muscle fibre splitting is an adaptive reaction that occurs when the fibre reaches a certain size when metabolite exchange

and oxygen supply are inefficient. In the current study, to detect the progress of healing process that may occur without management, group III specimens were examined after two weeks and four weeks. It showed persistence of the pathological changes observed in group II. In addition, more progressive findings in the form fragmented muscle fibers with pale acidophilic homogenous sarcoplasm. Features of regeneration began to appear as few muscle fibers with central nuclei. In this study the fragmented degenerated muscle fibers may be due to exposure to oxygen free radicals and activated neutrophils. Kalogeris and Baines[18] explained that during ischemia, muscle fibres are unable to maintain membrane integrity, resulting in the release of calcium, phospholipid A2, and production of polyunsaturated fatty acids and fatty acid radicals. When oxygenation is restored at that stage of ischemia, fatty acid radicals react with oxygen to produce lipid peroxidation, which enhances membrane permeability and drives leukocyte chemotaxis. When leukocytes are activated, oxygen-derived free radicals and proteolytic enzymes are released. This goes in line with Takhtfooladi and Shahzamani[19] who reported that neutrophils, inflammatory cytokines, nitric oxide, and reactive oxygen species are all likely involved in the skeletal muscle injury. In this study pale acidophilic homogenous sarcoplasm observed is in agreement with Carmo-Araújo and Dal-Pai-Silva[20] who stated that injured muscle cells exhibit necrosis features such as acidophilic cytoplasm and striation loss.

In this study muscle fibers appeared with central pale nuclei. This was previously observed by Saclier and Cuvellier[21] mentioned that regenerated myofibers refer to the myocytes with central nuclei. They also stated that the existence of central nuclei in striated skeletal muscle tissue subjected to injury suggests a stage of differentiation and healing. This was consistent with the observation of Gregory & Mars, (2004)[22] who suggested that it could be immature cells (myoblasts). They reported also that, Satellite cells are activated, and myoblasts are produced, which eventually unite with other myoblasts to form mature, multinucleated myofibers.

In this study, stem cells treated group at 2 weeks revealed some muscle fibers which appeared thin with central nuclei while others appeared with acidophilic sarcoplasm, peripheral nuclei and transverse striations to be more or less as the control. At 4weeks, marked improvement was observed in this period to be more or less similar to normal muscle fibers. In transverse section, polygonal muscle fibers with peripheral nuclei appeared, arranged in bundles separated by perimysium. Blood vessels appeared in both endomysium and perimysium and spaces inbetween muscle fibers also decreased. In longitudinal section, muscle fibers appeared long, cylindrical, parallel, multinucleated, with acidophilic sarcoplasm and transverse striations. Muscle nuclei were peripheral, pale, subsarcolemmal in position. This was in accordance with Andrade and Baldanza[23] who declared that when BM-MSCs were given to treat muscle injuries, the number of myotubes increased significantly when compared to the control group, showing a high level of regeneration activity. Shi and Garry[24] explained that BM-MSCs enhanced muscle fibre regeneration by either differentiating to satellite cells and then regenerating skeletal muscle fibres, or by fusing directly with muscle fibres, and that both methods coexist in the same tissue. Kholodenko and Konieva[25] added that the ability of BM-MSCs to create paracrine factors through release of a range of growth factors and cytokines, which could play a role in tissue regeneration, was crucial to their therapeutic efficacy.

This study's electron microscopic findings confirmed the light microscopic ones. In the present research, both group II (injury group) and group IIIA (non-treated) showed nearly the same ultrastructural changes involving alteration of sarcolemma, myofibrils, mitochondria, satellite cells and muscle nuclei. Non-treated group III showed signs of degeneration at 4weeks combined with focal regeneration while, group IV (stem cells treated) showed prominent regeneration starting from 2weeks and generalized regeneration at 4weeks. In this work irregular sarcolemma was present in group II (injured group) either being discontinuous or being corrugated. Kaminska, Curtis and Sewry,[26,27] explained that focal loss or breaks in the plasmalemma can be seen in conditions characterized by necrosis such as muscular dystrophies. Magaudda and Di Mauro[28] stated that two receptor complexes: dystrophin associated glycoproteins and integrins coupled with talin and vinculin, comprise the major system of cellular matrix interaction A study of human skeletal muscle through eccentric contraction revealed alterations in the dystrophin-glycoprotein complex and decreased expression of α-sarcoglycan shortly after eccentric contraction. Thus, The sarcolemma may become destabilised, resulting in changes in membrane permeability. The concentration of intracellular calcium may be influenced by the level of -sarcoglycan. As a result of [Ca2+] overloading in muscle fibres, [Ca2+]-dependent proteolytic pathways may be activated. In the present study, group II ultrathin sections showed disrupted myofibrils in the form of discontinuation of myofibrils or complete lysis. The same findings were observed by some authors[29] in damaged muscle after injury. They noticed disorganization of sarcomeres and variable sized vacuoles. Magaudda and Di Mauro[28] stated that increase in [Ca2+] level activates [Ca2+] dependent proteases such as calpain leads to disruption of myofibrils. Curtis and Sewry[27] added that myofibrillar structure is disorganized in degenerating and regenerating fibers. This finding was explained by Bainesp[30] who stated that partial degeneration and fragmentation of myofibrils or myofibrils disassembly occurred due to the action of proteinase which is activated by increased intracellular level of calcium ions after loss of integrity of the sarcolemma in necrotic muscle. He also declared that disassembly or fragmentation of myofilaments appeared due to digestion of Z lines, M lines, C proteins, tropomyosin and troponins T and I from the myofibrils. In addition, mitochondrial damage leadto insufficient energy generation, which is

required for cell homeostasis and cellular protein, may be another cause to myofibrillar disassembly. The observation of large fused mitochondria can be explained according to other authors[31,32] as a compensatory mechanism through which the cell protect itself from various kinds of injury and maximize oxidative capacity in response to any stress effects. This goes in line with Benard and Karbowski[33] who elucidated how mitochondrial fusion and fission work together to keep as many viable and healthy mitochondria as possible. When two mitochondria fuse, their genetic material can recombine, allowing damaged DNA to be exchanged. As a result, one of them can repair its DNA while the other accumulates more damaged DNA.

Some authors[34] added mitochondria with too much damaged DNA will be removed, but healthy mitochondria will be replenished by fission. In this study, extensive accumulation of mitochondria under the sarcolemma was also observed. This finding could be explained by Kavazis and Lejay[35,36] as a reaction to metabolic stress and a compensatory response by which the cell correct the respiratory chain deficiency and cytochrome C oxidase activity reduction. Interestingly, one of the prominent features in this study was the appearance of ultrastructural features of activated satellite cells in the form of long cytoplasmic processes and increase in their number.

This was in agreement with other authors[37,38] who described activated satellite cells as extensively elongated cells with the cytoplasm in peripheral regions, containing many mitochondria, a well-developed Golgi apparatus,and free ribosomes are all signs of high metabolic activity. Moreover, some authors[39-41] reported that the separating satellite cells had nuclei with condensed chromatin and high nucleus to cytoplasmic area ratio. They added that After activation, satellite cells proliferate and become enveloped by basal lamina prior to and maybe during division. Another mechanism by which stem cells exerted its effect in regeneration of muscle was by promoting proliferation, activation, differentiation of satellite cells and restoration of normal structure of muscle fibers. This is was in agreement with some authors[42] who stated that after therapy with mesenchymal stem cells, the functional abilities were fully recovered, and images revealed fully regenerated muscle tissue.

In current study, nuclear changes were observed particularly in group IIIB. The myonuclei appeared indented with corrugated nuclear membrane after 4 weeks of injury without treatment. This goes in line with Park and Hayashi[43] who attributed these changes to nuclear fragility and mechanical stress.

Abnormal position of myonuclei inbetween myofibrils in group IVA could be explained by some authors[26,44] as various cellular organelles were activated and involved in repair during regeneration, and muscle nuclei leave their peripheral position and migrate to the fiber's central region. In this study, the stem cell treated group (group IV A&B) showed moderate improvement after 2 weeks and the improvement was better after 4weeks as myofibrils appeared regularly arranged with normal banding pattern of alternating dark A bands bisected by M lines, light I bands bisected by Z lines and regular sarcomeres between successive Z lines. Subsarcolemmal oval muscle nucleus with its granular euchromatin, thin condensed peripheral chromatin and prominent nucleolus was observed. This improvement with stem cell was previously explained with light microscopic results[23,24,25].

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

دراسة هستولوجية لتأثير الخلايا الجذعية الميزنشيمية المشتقة من نخاع العظم على إصابة العضلات الهيكلية المستحدثه تجريبيا فى ذكور الجرذان البيضاء البالغة

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المقدمة: تعتبر إصابات العضلات الهيكلية من الإصابات الشائعة وقد تترافق مع ضعف القدرة الوظيفية ، وخاصة بين الرياضيين وغالبًا ما تكون نتائج الشفاء بالعلاج التقليدي غير كافية ، مما يولد إهتمامًا كبيرًا بإمكانية التقنيات الناشئة مثل الخلايا الجذعية لتعزيز عملية إلتئام الأنسجة الرخوة وتقليل وقت الإلتئام.

الهدف من الدراسة: تقييم دور الخلايا الجذعية الميزنشيمية المشتقة من نخاع العظام في التئام إصابة العضلات الهيكلية المستحدثة تجريبياً في ذكور الجرذان البيضاء البالغة.

المواد والطرق: تم إستخدام خمسة وأربعون من ذكور الجرذان البالغة و تم تقسيمهم إلى خمس مجموعات.

المجموعة الأولى للحصول على عضلة الساق الخلفية وجمع نخاع العظم لتحضير الخلايا الجذعية. المجموعة الثانية: إصابة عضلة الساق الخلفية. المجموعة الثالثة: إصابة عضلة الساق الخلفية وتركها دون علاج. المجموعة الرابعة: إصابة عضلة الساق الخلفية وعلاجها على الفور عن طريق الحقن العضلي للخلايا الجذعية. المجموعة الخامسة: إصابة عضلة الساق الخلفية وعلاجها على الفور عن طريق الحقن العضلي للوسيط المستخدم في تعليق الخلايا الجذعية وقد تم إستئصال عينات العضلات من المجموعة الثالثة الي الخامسة بعد إسبوعين و ٤ أسابيع من الإصابة .

النتائج: أظهرت نتائج هذه الدراسة أن العلاج بالخلايا الجذعية الميزنشيمية المشتقة من نخاع العظم قد أدى إلى تجديد مبكر ومعزز لإصابة العضلات الهيكلية. وقد تجلى ذلك من خلال الفحص المجهري الضوئي والإلكتروني والمورفومتري. وقد أظهرت المجموعة المعالجة بالخلايا الجذعية تكوينًا مبكرًا للعديد من الألياف مركزية النواة بعد إسبوعين من الإصابة والخصائص التركيبية للخلايا التابعه النشطة مع إستعادة السمات النسيجية الطبيعية للألياف العضلية بدون تليف.

الاستنتاج: يمكن الاستنتاج أن الحقن الموضعي للخلايا الجذعية في عضلة الساق الخلفية المصابة أدى إلى التنشيط المبكر والتكاثر والتمايز للخلايا التابعة مما أدى إلى تكوين عضلي مبكر ومعزز.