Original Article

Muscle Injuries

Histological Study of Muscle Injuries to Observe the Effects of Environmental Pollutants on its Recovery and Regeneration

1. Muhammad Riaz Sheikh 2. Azhar Masud Bhatti

1. Assoc. Prof. of Anatomy, Postgraduate Medical Institute, Lahore

2. Ex-Senior Demonstrator of Forensic Medicine, KEMU, Lahore, DHS (EPI), Punjab & EDO (Health), Gujranwala

ABSTRACT

Objective: Humans are exposed to environmental pollution, food contamination and Cigarette smoking. Environmental pollution in addition to its effects on different systems of body, it also effect on recovery and regeneration of muscular injuries. In this study under different environments the recovery period and regeneration of muscular injuries will be studied on the basis of muscle histology.

Study Design: Experimental study.

place and Duration of Study: This study was conducted in Animal room of Anatomy Department, Postgraduate Medical Institute, Lahore from May 2011 to December 2011.

Materials and Methods: Study was carried out on total "100" animals. Control group comprises 28 animals exposed to (i) "Blunt trauma ", (ii) Incisional injury., (iii) thermal injury and (iv) chemical injury. Whereas other nine groups of animals following initiation of injury were exposed to heavy metal pollutants and non-heavy metal pollutants by orally, parentally or inhalation. Delayed wound healing was observed, because major factors limiting the ability of skeletal muscles to regenerate after trauma or diseases were a viable population of satellites cells, re-innervation and re-vascularization.

Results: The experimental group animals became more lethargic, inactive, death rate was more. Death occurred earlier in group " 6" & 8 as compared to rest of groups ($P < 0.01$). Injured muscle initially showed increase in circumference and then followed by resuming its normal size in two weeks. time interval.

Conclusion: In control group wound healing occurred in normal time whereas those exposed to metallic and non-metallic environmental pollutants showed weight reduction and delayed wound heating.

Key Words: Muscle, Injury, environment, pollutants, recovery, regeneration

INTRODUCTION

The word muscle is derived from Latin word. Mus. which means a mouse. The tail of mouse representing the tendon of the muscle skeletal muscles take part in, locomotion, speech, mastication etc. The Muscles have property of contractibility[1].

The muscles tissue is composed of excitable and contractile elongated cells called muscle cells. They are long cylindrical structure range 10---100Hm in diameter and form millimeters to many centimeters in length. The cell membrane is sarcolemma on its outer surface. In each fiber nuclei are 35 per mm of length. Nuclei are ovoid and situated peripherally those to sascolemma cytoplasm is occupied by parallel filamentous elements 1-3 um in diameter called Myofibrils. Section of skeletal muscle fibers show cross striations composed of light and dark bands. Myofibrils is further composed of actin and Myosin filaments. There is special smooth endoplasmic reticulum exist in the form of network of cisternal or membranous tubules which course between and around the myofibrils. These are longitudinal and transversally arranged tubules muscles are mesodermal in origin[2]. Skeletal muscles are voluntary muscles and under control of somatic nervous system. These muscles are situated and present at axial skeleton and appendicular skeleton. The skeletal muscles are red and white depending upon quantity of myoglobin. Red muscles are highly vascular as compared to white muscles[3].

A skeletal muscle capillary supply is very rich. Several capillaries have contact with each other muscle fiber. Each muscle fiber receives at least one motor nerve ending form the somatic nerves. One nerve fiber may innervate a single muscle fiber or many muscle fibers. A motor nerve fiber and muscle fiber together is motor unit (Richard S-snell-2005). A very peculiar property of skeletal muscle fibers is their power to regenerate following various kinds of degeneration causing injuries[4].

Regeneration: It is a process whereby lost specialized tissue is replaced by proliferation of surrounding undamaged specialized cells. There is no residual trace of previous injury. The causes of tissue loss or destruction are:

1. Traumatic excision, whether accidental or surgical
   a. Physical agents like trauma, extremes of temperature (Burns & deep cold)
   b. Chemical agents poisons Arsenic, cyanide mercuric Salt etc.

2. Physical, Chemical and microbial agents
   a. Physical agents
   b. Chemical agents
   c. Microbial agents i.e virus, Bacteria and parasites

3. Ischemid leads to infrarction.

Repair is the replacement of lost tissue by granulation tissue which matures to form scar tissue. Wound healing involved e.g movements of cells, division of

cells, rearrangement of tissues and biochemical changes. In wound healing following steps are important (i) wound contraction by which wound undergo reduction in size up to 80 %. So that only one-quarter to one-third of destroyed tissue has to be replaced. Contracting mechanism resides in granulation tissue at margins of wound i.e myofibroblast contract which form granulation tissue[5].

Wound healing occur by two phenomena's

(a) Healing by first intention means wounds with opposed edges. It is healing of clean, uninfected, surgical incision approximated by surgical sutures so called primary union or healing by first intension.

(b) Healing by second intention: When there is more extensive loss of cells and tissue occur in infarction, inflammatory ulceration and surface wounds. There is large tissue defect that must be filled. The original architecture cannot be restored. Abundant granulation tissue grows in from the margins to complete the repair. This healing called secondary union or healing by secondary intention. Extensive wound associated with ischemia. Factor influence wound healing are[6].

(i) Nutrition e.g low protein intake
(ii) Metabolic status e.g diabetes mellitus
(iii) Circulatory system i.e poor circulation
(iv) Harmon such as Glucocorticoids
(v) Infection
(vi) Movement

Environmental pollutants include Metallic and non metallic. Metallic environment pollutants are lead, mercury, Arsenic and Cadmium lead paints and lead pipes to deliver water to homes, packed juices are the great source of lead pollution. Average daily intake of lead is 0.2mg. Lead effect on neuromuscular junction and causes lead palsy i.e muscle weakness or palsy can occur. Mercury has a number of important industrial uses and poisoning from occupational exposure and environmental pollution by mercury vapors, which is 0,024 per days found in air. Lethal dose in blood is 4-5 ug/ml[7].

Arsenic is found in soil, water and air as a common environmental toxicant. It is also in high concentration in water. It is also found in fruit, vegetables (due to pesticide spray) and fish. Arsenic effects on skin causes necrosis, sloughing and hyper keratoris. Thus causes atrophy and degeneration. Cadmium is one of the major environmental pollutants. It is cumulative poison found in plastic and house hold utensils, shell fish, animal liver, kidneys, in cigarettes etc. Cadmium do constriction of large arterioles in skeletal muscles[8].

Peoples are exposed to non metallic environmental pollutants like air pollutants, solvents vapors and pesticides enter the body through inhalation. Five pollutants account for 98% of air pollution these are (i) Carbon Monoxide (52 %) (ii)Sulfur oxides (18 %) (iii) Hydrocarbons 121 (iv) particulate Matter (10 %) v. Nitrogen oxides (6 %). Solvent vapors such as gasoline, light fluids, Aerosol, Sprays floor and tile cleaners etc. Pesticides includes insecticides, rodenticides, fungicides etc. These compound are manufactured for the sole purpose of destroying some form of life[9].

MATERIALS AND METHODS

This research was carried out on "100" Sprague dawly strain albino rats housed in cages and fed on chicken diet No 4 and water ad libilum. All rats were divided into nine (9) groups. Control groups was comprised of " 28" animals and was divided into 4-sahgroups subject to different injuries it include:-

Group-I Blunt injury
Group-II incisional injury
Group-III Thermal injury
Group-IV Chemical injury like application of acids

Other eight groups of animals were administered two different doses of a specific pollutant for "one week" ; two weeks, three weeks and four weeks and so were further subdivided into 4-sub groups according to nature of injury applied to gastrocnemius muscle of lower limb under anaesthesia as that control group. Each group was sacrificed after " one, two, three and four weeks. Research was done according to following experimental design:

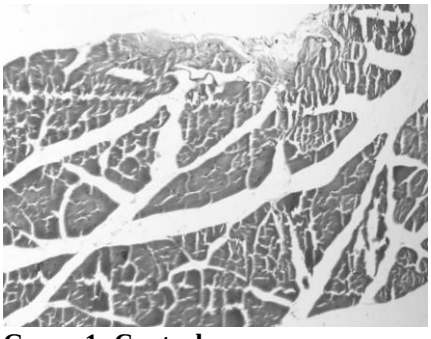
Sr.No.	Group	Dose	Sub groups as that of control
1	Control group		i. Blunt trauma ii Incisional injury iii Thermal injury iv. Chemical injury
2.	Lead group	i. 2.5 mg/kg body wt. orally ii. 4mg body wt. orally	i. ii iii, iv. As control sp
3.	Cadmium Chloride group	i. 0.1 mg/kg body weight orally ii. 1 mg/kg body weight	i, ii, iii, iv
4.	Arsenic group	i. 1.5 mg/kg body weight ii. 30 mg/kg body weight	i, ii, iii, iv
5.	Mercury group	1 mg /kg weight orally 3 mg/kg body weight orally	i, ii, iii, iv
6.	Carbon monoxide	i. 1000 PPM (Parts per million)/ 30 minute /day ii. 3000 PPM/30 minutes/day inhalation	i, ii, iii, iv
7.	Gasoline group	i. 10 mg /kg ii.100 mg/kg	i, ii, iii, iv
8.	Sulfur oxide group	i. 1 PPM/10 minutes/ day ii. 3 PPM/10 minutes/ day	i, ii, iii, iv
9.	Organophosphorus (pyrethramine group)	i. 25 mg/kg body weight orally ii.50 mg/kg body weight	i, ii, iii, iv

Injured muscle tissue was obtained " First week (day 1-7 days), seconds third and fourth week interval post injury than this tissue
was studied for following:
(1) cytology (2) Connective tissue
(3) Ratio of necrotic tissue and original surveying muscle fibers (4) Nucleoli
stain used was Mallory (PTAH) schofield's, silver impregnation method, haemotoxyline and eosin stain, van gerson's stain.

RESULTS

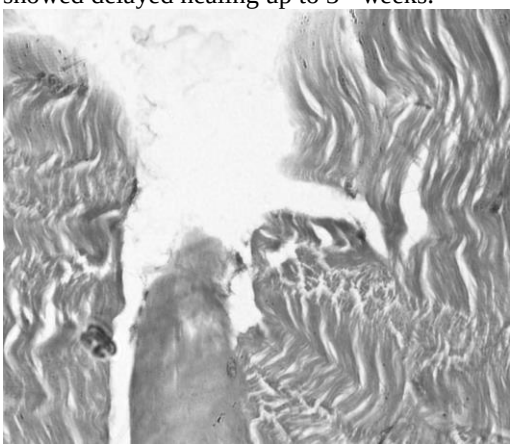
Macroscopic: The experimental group animals became more lethargic inactive, death rate was more, death occurred earlier in group 6 & 8 as compared to rest of groups. There was gradual decrease in mean animals weight ($P < 0.01$) injured muscle showed initially increase in girth to (12-14mm) to and then following by resuming its normal size (8 mm) in two weeks time interval.

Microscopic: In control group 4-types of injuries induced caused haemorrhage inflammation, non-necrotic degeneration and later regeneration were observed. In short time following blunt trauma, the damaged segments showed gross tearing and degeneration. A large number of mononuclear cells was seen in intercellular connective tissue and within damaged muscle Cells. By 24-48 hour there was an increase in the number of sarcolemmae nuclei .Some of which were likely of satellite cell origin. By day 3 regenerating muscle cells displayed central nuclei. By day 6th further progression of regeneration was seen. On days 14, 21 and 30 after trauma, the muscle appeared to have healed and no abnormalities could be found at the site of injury. In incisional injury healing occurred by contraction by which wound undergo reduction in size up to 80%. Contracting mechanism resides in granulation tissue at margins of would that is, myofibroblast. Which form granulation tissue. The only one quarter to one third of destroyed tissue was replaced by granulation tissue. Chemical and thermal injuries caused inflammatory ulceration and surface wounds with large tissue defect. Original architecture could not be restored even after 3rd weeks. Abundant granulation tissue growth was seen. Extensive wound ischemic was noted.

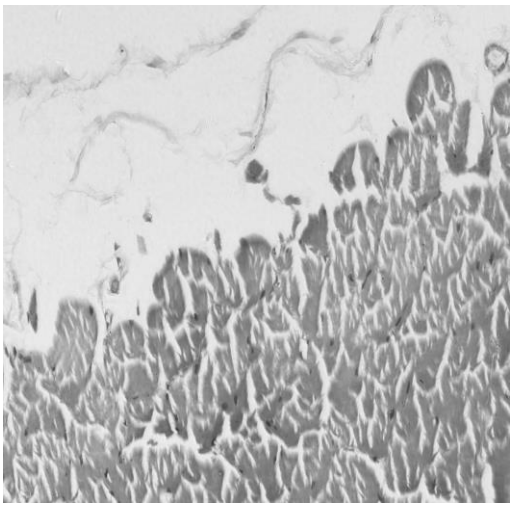


Group 1: Control

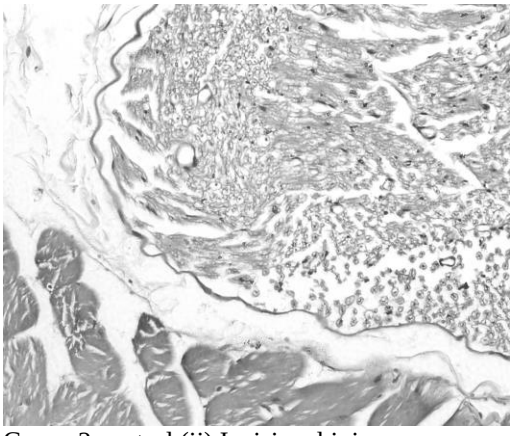
In groups 2 to 9 exposed to environmental pollutant incisional injury in all groups showed initial contraction to maximum reduction in size of wound, but one quarter of wound showed delayed appearance of granulation tissue up to 3rd week and ultimately healed in 4th week. Blunt trauma haemorrhage followed by non nectrotic regeneration phenomena was noted and showed delayed healing up to 3rd weeks.



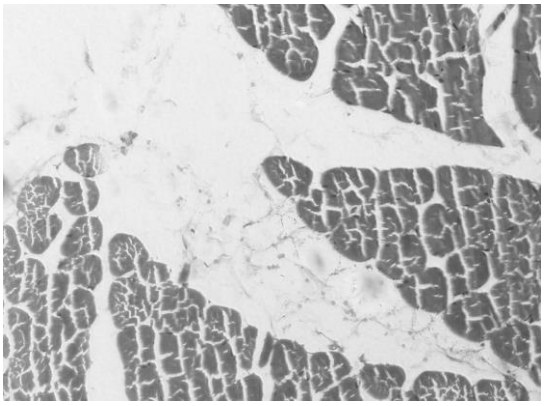
Group 1 Control (i.) Blunt trauma.



Group 1 control (i) Healing after blunt trauma.

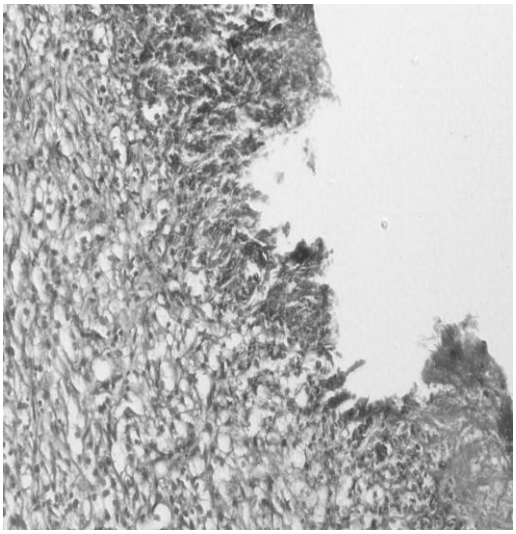


Group 2 control (ii) Incisional injury.

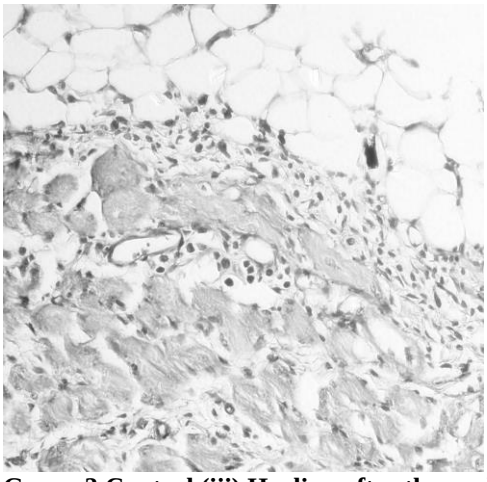


Group 2 Control (ii) Healing after incisional injury.

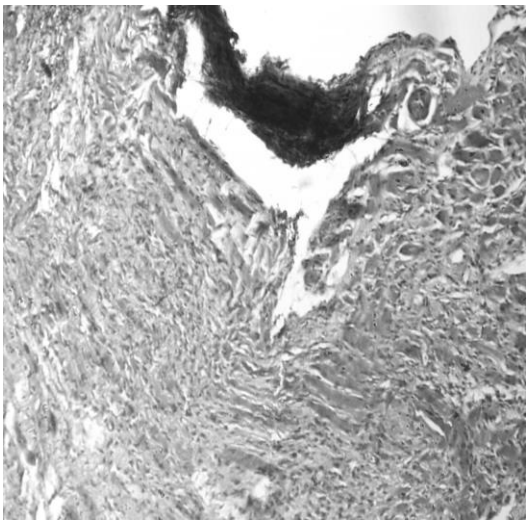
In chemical and thermal injuries there was extensive tissue loss, showed very much delayed healing phenomena, due to inhibition to muscle cells proliferation microangicpathy and nerve atrophy as caused by pollutants. Wound healing occurred with abundant evenly developed collagen tissue up to 4th week time.



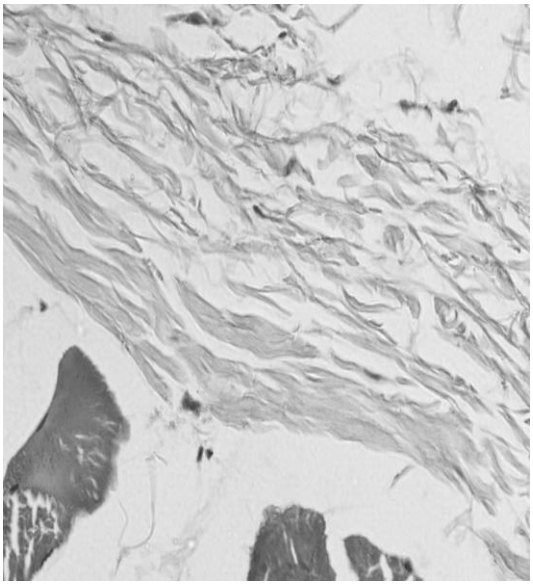
Group 3 Control (iii) Thermal injury.



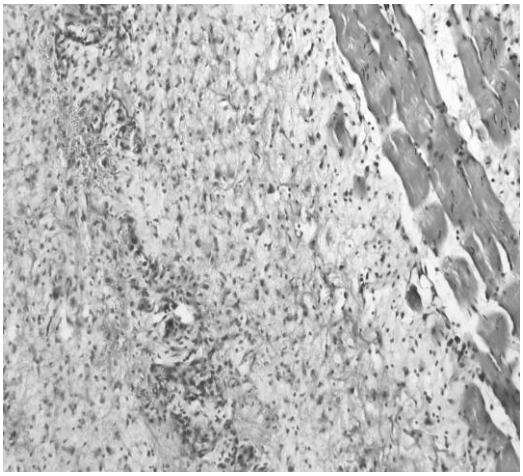
Group 3 Control (iii) Healing after thermal injury.



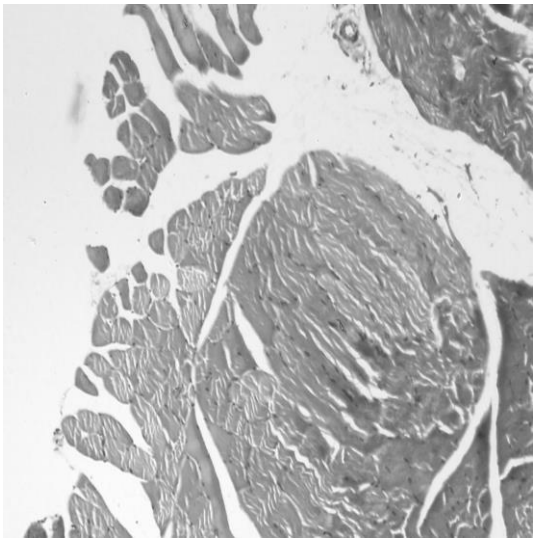
Group 4 Control (iv) Chemical injury.



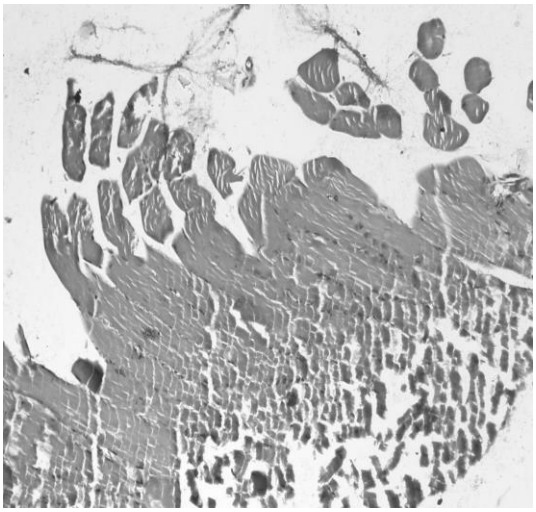
Group 4 Control (iv) Healing after chemical injury.



Group 5 Mercury group. Mercury exposed wound healing.



Group 8 sulphur oxide group. Wound healing after sulphur oxide exposure.



Group 9. Organophosphorus group. Wound healing after organophosphorus exposure.

DISCUSSION

This study highlighted the effect of environmental pollution on regeneration of muscle injury. In control group 4-types of injuries were induced and their normal healing process is almost completed in " 2 " weeks. Weight of animals remained constant during the period. In groups "2 to 9" exposed to various pollutants resulted in marked and gradual reduction in mean weight of animals ($P < 0.01$) and frequent death especially group 6 to 9. Each group after administration of pollutants incisional and Blunt wound healed in two weeks time, whereas other wounds which were chemically and thermal induced were wider wounds and showed damaged muscle cells and microangiopathy as indicated by black margins of wound. The wound healing delayed up to 4th week the group " 2" & "3" wounds undergo regeneration in mid of 3rd week whereas group " 4" to "9" resulted in much delayed healing up to end of 4th week.

It is correlated with GOODMAN and GILMAN'S[10] pharmacological statements of arsenic causes skin necrosis, sloughing and hyper karatosis. This causes atrophy and degeneration. Gutierrzz JMJ,[11] in his study chemical injury induced by snake venom caused disruption of the integrity of plasma membrane. Plasma membrane was interrupted in many portions. The good regeneration response may be explained by observation that chemical injury does not affect blood vessels nerves or basal lamina. Regeneration was completed at the end of 4th week. Bornemann et al proved that vimentin is a useful marker for regenerated muscle fibers. Zacharias LS.[12] Snyder R studied muscle regeneration is better in younger than older mice after imposed injury due to more satellite cells observed in younger muscle. Bichoff suggested that satellite cells are myogenic stem cells that can be induced te enter the cell cycle by an extract of crushed muscle. Wattig B,[13] proved the acceleration of muscle regeneration by nucleotide administration. Fisher BD, et al[14] studied that following acute blunt trauma, hemorrhage inflammation, non-necrotic regeneration was observed. Regeneration progressed at 6th day and completed upto 2nd week. Fisher concluded by its study of blunt injury the regeneration progressed at " 6th " day and completed up to 2nd week. Volodine[15] in it's study the causes of incomplete restoration of extensive skeletal muscle injury are accumulation of collagen, progressive secondary neurogenic atrophy, microangiopathy disorder of tissue nutrition resulted in chronicity of pathological process in the muscle. Hurme Timo et al[16] proved that proliferation of satellite cells was extensive in regenerating zone. Bodine –Fowler, Sue,[17] suggested direct injury caused by crushing pucturing, cutting, ischemia the major limiting factor is viable population of satellite cells, reinnervation and revascularization. Caldwell et al,[18] result of this study suggested that presence of empty basement membrane tubes is not essential for orientation of regenerating myoblast in skeletal muscle.

CONCLUSION

The environmental metallic pollutant are of greatest concern are lead, mercury, Arsenic, and cadmium. In the past lead paint was available for use in home and lead pipes to deliver water to homes. As a result, people were exposed to lead on daily basis. Mercury is a contaminant of our environment. Arsenic is found naturally in high concentration in drinking water. Cadmium has been classified as known human carcinogen. Non metallic environmental pollutants like carbon dioxide sulfur Oxide gases also affect human health. Wound healing is delayed in those exposed to these pollutants.

REFERENCES

1. Guyton AC. Medical physiology. Philadelphia: 2004.p.821-2.
2. Junqueira, Carneiro Basic Histology. 11th ed. 2005.p.182-196.
3. Copenhave WM, Delly GE, Wood RL. Bailey's text book of histology. Tokyo; 2005.
4. Richard S. Snell Clinical Neuroanatomy. 6th ed. 2006.p.475-481.
5. Walter JB. Walter and Israel General Pathology. 7th ed. 1996.p.165-178.
6. Gregory C. Sephal, Stephen C, Wood Ward Rubin's Pathology. 2004.p.84-116.
7. Abidi TS, Riaz M. Cadmium Induced Nephrotoxicity. Pak J Med Res 1994;33;2:108.
8. Zhang C, Thind GS, Joshua IG, Fleming JT. Cadmium-Induced Arteriolar Constriction in skeletal Muscle Microcirculation. AJH 1993;6:325-329.
9. Zhang C, Thind GS, Joshua IG, Fleming JT. Cadmium-Induced Arteriolar Constriction in skeletal Muscle Microcirculation. AJH 1993;6: 325-329.
10. Goodman, Gilman, editors. The Pharmacological Basis of Therapeutics. Heavy metals and Heavy metal Antagonists. 11th ed. 2006.p.1753-1775.
11. Gutirrez JM, Nunez J, Diaz C, et al. (Instituto Clodomoro Picado, Faculated de Microbiologia, Brazil.) Skeletal muscle degeneration and regeneration after injection of bothropstoxin II. Exp Mol Pathol. 1991;55(3):217-229.
12. Zacharias JM, Anderson JE. Muscle Regenration after imposed injury is Better in Younger than older MDX dystrophic mice. J Neurol Sci 1991;104(2):190-196.
13. Wattig BG, Schlow M, Madauss F, Warzok HR, Cervos-Navarro J. Acceleration of muscle Regenration. Zentralba Pathol 1991;137(5): 409-413.
14. Fisher. B, Baracos TK, Shnitka SW, Mendryk, REID DC. Ultrastructural events following acute muscle trauma. Med Sci Sports Exercise 1990; 22(2):185-193.
15. Volodina AV, Gurko NS, Yu V, Kuprenskii, Poznyakov OM. Analysis of the causes of incomplete structural and functional restoration of skeletal muscle after severe traumatic injury to the limb. Patol Fiziol Eksp Ter 1991;0(6):50-53.
16. Hurme, Timo, Kalimo H. (Dep. Pathol.,Univ. Turku Kiinamyllynakatu 10, SF 20520 Turkum Finland). Activation of myogenic precursor cells after muscle injury. Med Sci Sports Exercise 1992; 24(2):197-205.
17. Bodine-Flower, Sue. (Dep. Orthopaedic Surg., UCSD Sch Med., VA Med, Center, 3350 LA Jolla Village Drive, San Diego, CA 92161, USA) Skeletal muscle regeneration after injury: An overview. J Voice 1994;8(1):53-62.
18. Caldwell CDJ, Mattey, Weller RO. Role of the basement membrane in the regeneration of skeletal muscle. Neuropathol Appl Neurobiol 1990; 16(3): 225-238.

Address for Corresponding Author:
Dr. Muhammad Riaz Sheikh
Assoc. Prof. of Anatomy,
Post graduate medical institute
6- Bird wood road. Lahore
Cell No.: 0333-4239975
0331-4235082
Postal address: 171-G-Block Gulshan-e-Ravi Lahore.