Journal of Medical Laboratory Science, 2021; 31 (4): 20-33
http://jomls.org; info@jomls.org **Ibrahim Mohammed**
http://doi.org/10.5281/zenodo.5802199

Effect of Exosomes on Histological Changes of Spinal Cord Injury on Wistar Rats

Ibrahim Mohammed
Department of Histopathology, School of Medical Laboratory Sciences, Usmanu Danfodiyo University Sokoto, Nigeria

ABSTRACT

Introduction: Spinal cord injury is a neuro-damage occurring from a complete or incomplete, traumatic or non-traumatic that results to degeneration, structural, biochemical, and physiological changes of tissue. Subventricular Zone (SVZ) is a neurogenic region where stem cells produced the immature neuroblast that can be transported to the olfactory bulb and differentiate into interneurons and oligodendrocytes. Extracellular vesicles (EVs) are tiny lipid membrane bilayer vesicles secreted by different type of cells; they have an important physiological role to play in cell-cell communication, the application of EVs was very productive in neuro-protection, increasing successful brain remodeling, functional recovery. **Objective:** The aims of this research is to determine the effect of EVs on histological changes occurring as a result of spinal cord injury (SCI) on rats. **Methods:** The rats were allocated in to three groups: Sham group: Laminectomy without SCI (8 rats); SCI without treatment group: With SCI but no any treatment (8 rats); SCI + EVS group: SCI treated with dose of extracellular vesicles derived from SVZ (8 rats). Spinal cord injury was formed through weight compression method. Spinal cord was harvested on the epicenter of the injury, it was processed and stained by H &E and Cresyl violet staining techniques. **Results:** An injury was formed on the spinal cord, through observation of black dot on the injured area. H & E and cresyl violet staining shows much recovery of neural cells in EVs group compared to SCI group. **Conclusion:** It was concluded that EVs can pass freely through CSF into the injured area and make recovery

Keywords: sensory, autonomic, inflammation, ischemia, histology

Correspondence: ibrahmoh@yahoo.com +234-8036283229; ORCID: 0000-0002-4410-6507

Received: July/31, 2021; **Accepted**: November/21, 2021; **Published**: December/26, 2021.

Citation: Ibrahim Mohammed. Effect of Exosomes on Histological Changes of Spinal Cord Injury on Wister Rats. **J Med Lab Sci, 2021; 31 (4): 20-33**

INTRODUCTION

Spinal cord injury (SCI) is an incomplete or complete spinal cord motor, sensory, sphincter and autonomic dysfunctions a result of extreme attack on spinal cord, which is highly destructive disease in orthopedics and formed a significant physiological psychological damage to individuals (1). In some countries, SCI prevalence ratio is 20 to 40 per million populations (2). There are many causes of SCI that include traffic accidents (45.4%), falls (16%) and sport injuries (16.3%) (3). It was documented that final neurological damage of SCI is due to two ways that are primary injury and secondary injury. SCI is a very serious trauma that happens as a result of traumatic or non-traumatic damages depending on the origin of the injury and result to motor and sensory damages (4). SCI can bring different serious problems in some cases can even leads death due to its pathophysiology as well as lack of appropriate care and treatment (5, 6).

Primary injury occur as a result of the direct compression and contusion of the spinal cord via fracture and displaced bone fragments and disc material due to fracture-dislocation or burst fracture of the spine (7). The axons and nerves are disrupted and damaged neural cell membranes are ruptured. Blood vessels will be injured then micro hemorrhage will occur in the center of grey matter that spread out axially and radially and form spinal cord swelling and secondary ischemia (8). Ischemia, change ion balance and production of toxins from the disrupted neural membranes stimulate the secondary injury that increases the SCI (8). Primary injury occurred immediately after short period of time of the injury, damaged neurons, hemorrhage of endothelial tissues and necrosis appeared mostly in the gray matter of the spinal cord. These changes formed different complications such as quadriplegia and paraplegia (9). Secondary injury is the complex damage that occurred at a cellular level because of series mechanisms of pathophysiology stages involved which include excitotoxicity, oxidative stress, electrolytes imbalance, ischemia, inflammation, and massive cell death due to immune response to injury (7). Secondary injury starts with depolarization and voltage-depended opening of sodium, potassium and calcium ion channels. Overload of calcium ion result to mitochondrial malfunction and activation of phospholipase A2 and cytoplasmic nitric oxide synthase which leads to microvascular disruption and ischemia (10). Damages and blood capillaries produce toxic chemicals such as glutamate that affect nearby cells in a very disrupted process known as excitotoxicity (8). In many cases secondary injury is known as a result of microvascular damages, demyelination, electrolyte imbalances, edema, excitotoxicity, free radical production, neuroinflammation and cellular death(10). These processes results to different clinical complication such as cardiovascular diseases, respiratory and urinary tract dysfunctions and can results up to a maximum of 16.7% of morbidity and mortality(10).

Subventricular Zone (SVZ) is the largest neurogenic area in which stem cells produced the immature neuroblast that can be carried to the olfactory bulb and differentiate into interneurons (11), and many oligodendrocytes (12). In the human central nervous system (CNS), neural stem cell occurred in the SVZ of the lateral ventricles and dentate gyrus (DG) of the hippocampus. In vitro, neural/precausor cells (NPCs) differentiates and produce neurospheres, that are self-renewing and multipotent, also can be differentiated into neurons, astrocytes and oligodendrocytes (13). Different studies documented that the cells from SVZ can

safely and efficiently remove as a biopsy expand the resulting precursor cells in culture for the aim of autologous transplantation into the damaged CNS of same individual donor (14). It was revealed that there is possibility through the transplantation of NPCs on to SCI will restore neurological function through direct cell replacement or remyelination of affected axons (15). It will also result to other tissue healing mechanisms such as tissue regeneration, promotion of neuroprotection, as well as immunomodulation (16).

MATERIAL AND METHODS

Harvesting Extracellular vesicle from Subventricular Zone

Neural stem cells were isolated from SVZ as previously described by Aligholi et. al., (17). Adult rats were deeply anesthetized with ketamine (80 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.), and SVZ were dissected under sterile condition. The specimen was dissociated mechanically by cutting it into smaller pieces by surgical knife and enzymatically by adding 500 µL of 0.02% Trypsin/EDTA for 10 min at 37ºC. Trypsin was then inhibited by adding 500 µL of trypsin inhibitor. The dissociated cells were plated in Dulbecco's modified Eagle's medium/F12 containing 1% N2 supplement, 3% B27 supplement, 2 µg/ml heparin, 1% penicillin/streptomycin, 1% glutamax, 10 µg/ml basic fibroblast growth factor and 20 µg/ml epidermal growth factor were incubated at 37^{O}C at 5% CO2. The medium was changed every 3 days. The cells were grown during the primary culture as free-floating clusters (neurospheres). The spheres were passaged by mechanical and enzymatic dissociation every 5 days and re-plated into fresh growth medium. The total number of viable cells was determined before each passage by trypan blue exclusion as described by Azari et. al., (18). After passage 3 (85 % confluence) conditions medium was harvested every 72hrs. Briefly four centrifugation steps were carried out, the cells debris were collected through 3000 g for 10min and 20,000 g for 30min. Ultracentrifugation was then carried out at 110,000 g for 120min, and then the resulted plates were washed with PBS and ultra-centrifuged again. Final pellets were re-suspended in PBS.

Animal housing and groups

This study was carried out on 40 males Wistar rats (12 weeks old) weighing 250 to 300 g (Pasteur, Iran). The animals were housed in cages with ad libitum accessed to water and standard food for three days under controlled temperature with the light and dark cycle (12h for each) as described by Azari et. al., (18). The animals were handled in accordance with the guidelines of Iranian animal ethics society, Tehran University of Medical Sciences rules (19). The rats were allocated to three groups: Sham group: Laminectomy without SCI (8 rats); SCI without treatment group: With SCI but no any treatment (8 rats); SCI + EVS group: SCI treated with dose of extracellular vesicles derived from SVZ (8 rats).

Spinal Cord Injury

Spinal cord injury was formed through weight compression method; the animals were anesthetized by injection of ketamine (80 mg/kg) and xylazine (10 mg/kg), intraperitoneally (IP), and the unconscious reflex test was carried out for the confirmation of narcosis by severe pinch. The surgical area was shaved; and then the rats were placed on the stereotactic frame. Laminectomy was performed at T10 vertebra by fine Rongeur tool; briefly, the Dura mater was maintained intact during the laminectomy, and spinal cord was then

compressed using 50 g weight for 5 min using a rectangular plate, which was longitudinally oriented over the spinal cord; the area of plate was about 11.0 mm2 (2.2×5.0 mm) with a concave shape to ensure equal distribution of the pressure on the spinal cord tissue. The rats' body temperature during the surgery were checked and controlled in the range of 36- 37℃, the 3-0 Black Silk was used for the suturing of the skin as described by Farahabadi et. al.(19). To prevent dehydration after the injury, 1ml ringer solution was injected IP to each rat. Upon awakening, the rats were neurologically evaluated and monitored for food and water uptake and urine output for 72h.

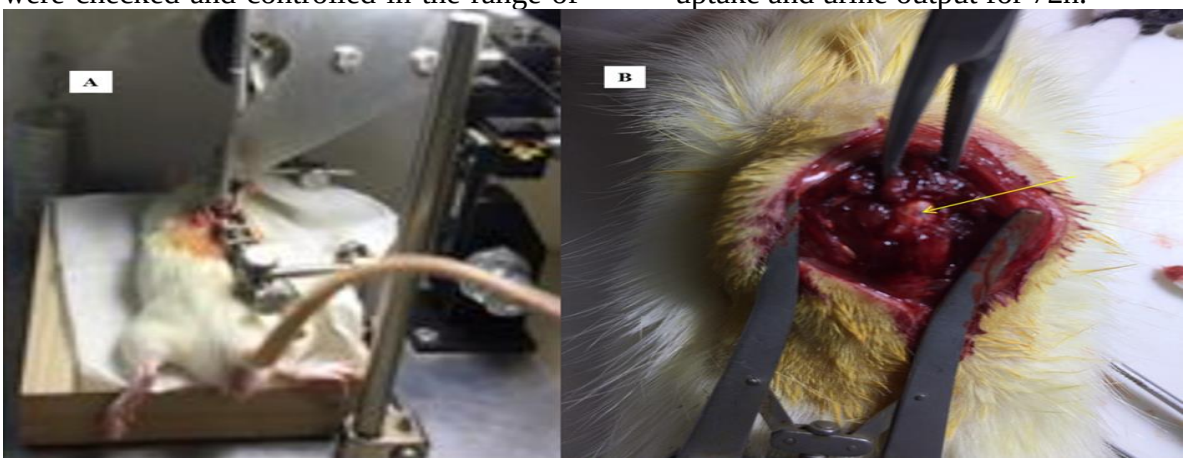

Figure 1A: shows making SCI by weight compression around incised T10 vertebra.
Figure 1B: Shows SCI on the spinal cord, yellow arrow showing black spot confirming SCI model.

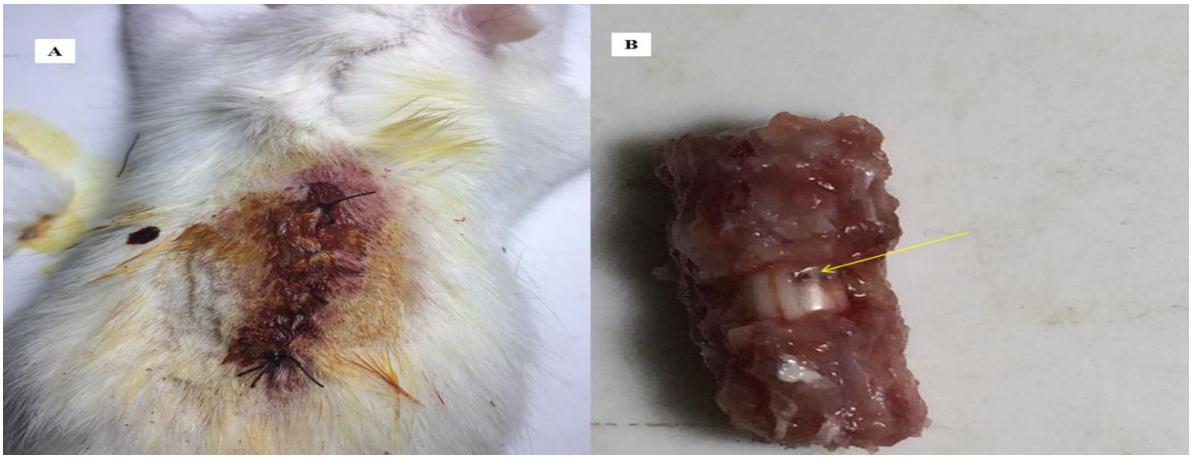

Figure 2A: Shows the sutured area after the surgery.
Figure 2B: Shows SCI tissue removed with vertebrate, yellow arrow showing a black spot of SCI model.

Injection of EVs

The animal was anesthetized by IP injection with ketamine (80 mg/kg) and xylazine (10 mg/kg). The rat was placed on at the end point of the board (20 x 30 cm), the back was fixed in a flexion position. Skin of the animal was shaved and incised longitudinally on L4-L5 vertebrae. 10 µl of (EVs /PBS) that

contained 10 µg of EVs per rat were aspirated in to 10 µl syringe (Hamilton) under sterile condition. The EVs were injected intrathecally into lumber cistern in a space between L4-L5 laminae slowly. A tail flicked known as a soft sign to assure that the needle entered correctly.

Histological Techniques

The rats were sacrifice through IP injection as described above four weeks after the injury and injection. Perfusion was carried out transcardially and fixed with saline and solution of paraformaldehyde 4 and glutaraldehyde 2.5% in PB (phosphate buffer) (0.1M pH 7.4) via the left ventricle. The spinal cord was removed corresponding to the epicenter of injury and post-fixed in the same fixative. After the spinal cord at the compression site was removed, the tissues were fixed in paraformaldehyde 4 and glutaraldehyde 2.5% in PB (phosphate buffer) (0.1M pH 7.4) and processed by paraffin wax technique; (Merk, Germany). Sections were cut with the Rotary microtome at 4µ and stained with Haematoxylin and Eosin (H & E) and Cresyl blue techniques.

RESULTS

Effects of EVs on Histological Changes on SCI Rats

EVs shows effect on histological changes on SCI rats stained with H & E and cresyl violet, shows differences between laminectomy, SCI and EVs groups. In laminectomy group no any destruction of neurons; white matter and gray matter are intact. SCI group after 3 days shows destruction in both grey matter and white matter with hemorrhage, vacuolization with many dark neurons and recruitment of polymorphonuclear cells compared with laminectomy group, appearance of much rbcs confirmed a good model of SCI. After intrathecal injection of EVs in EVs group, there was much improvement in EVs group after 4 weeks with no hemorrhage, the cavity reduced, with less dark neurons, gray matter and white matter recovered compared with SCI group (Fig 13). Figure 14 shows a cresyl violet staining technique, the results show intact gray matter and white matter in laminectomy group when compared with SCI group. The SCI group shows a unilateral SCI with much destructions of both gray matter and white matter, numerous vacuolization when compare with laminectomy group. After intrathecal injection of EVs in EVs group shows much recovery of gray matter and white matter with less vacuolization when compared with SCI group.

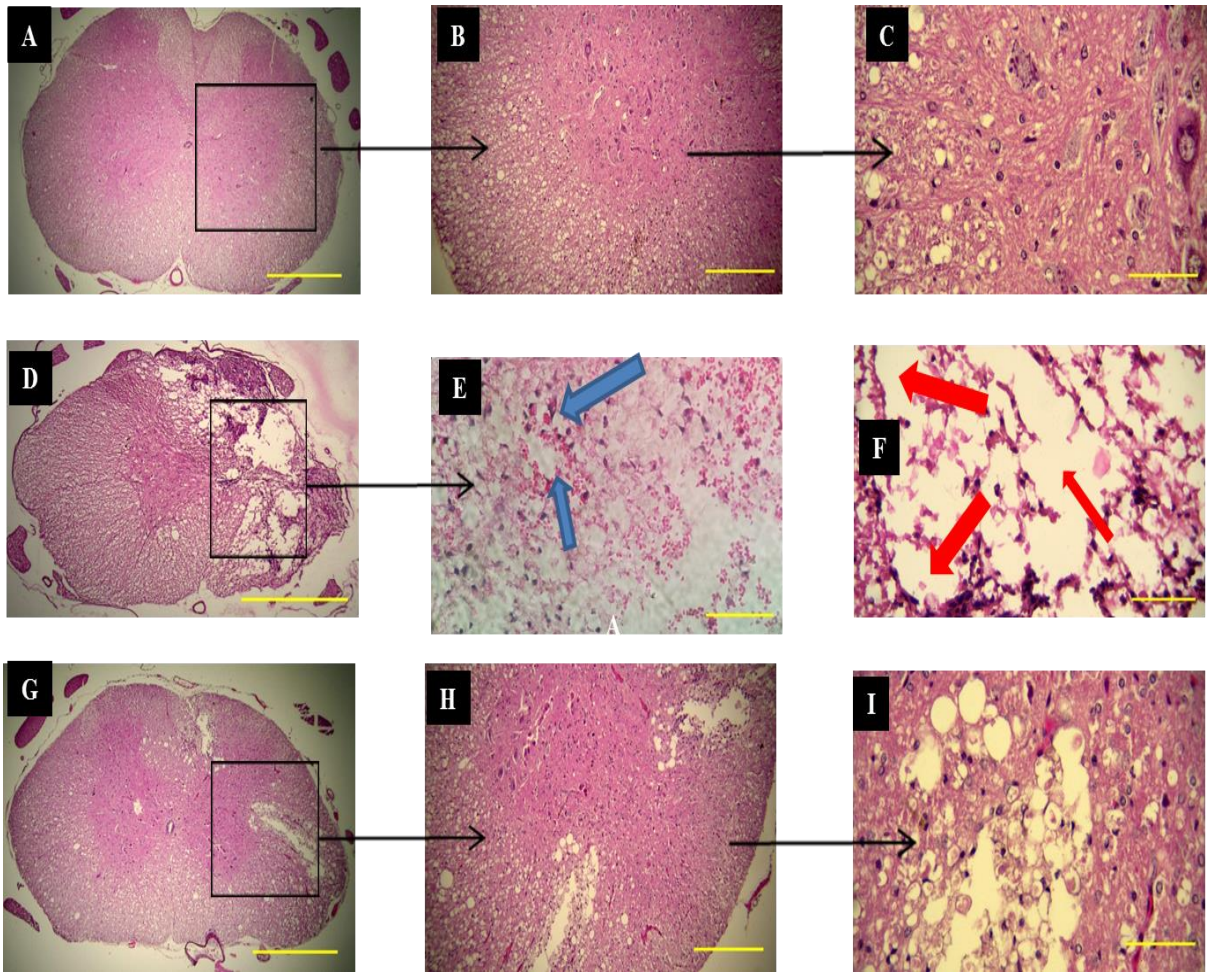

Figure 3: Transverse section of spinal cord at level of T10, H &E staining. **(A)** Laminectomy group shows intact spinal cord , scale bar = 1000 µm . **(B)** Laminectomy , scale bar = 400 µm . **(C)** Laminectomy , scale bar = 100 µm. **(D)** Massive destruction of grey and white matter unilateral SCI compared with laminectomy , scale bar = 1000 µm **(E)** SCI group after 3 days, green arrows showing hemorrhage confirming SCI model, scale bar = 100 µm . **(F)** SCI group after 4 weeks, red arrows showing vacuolization, scale bar = 100 µm x. **(G)** EVs reduced histological damage after SCI, scale bar = 100 0µm . **(H)** EVs group, scale bar = 400 µm . **(I)** EVs group showing cavity reduced with little hemorrhage, scale bar = 100 µm .

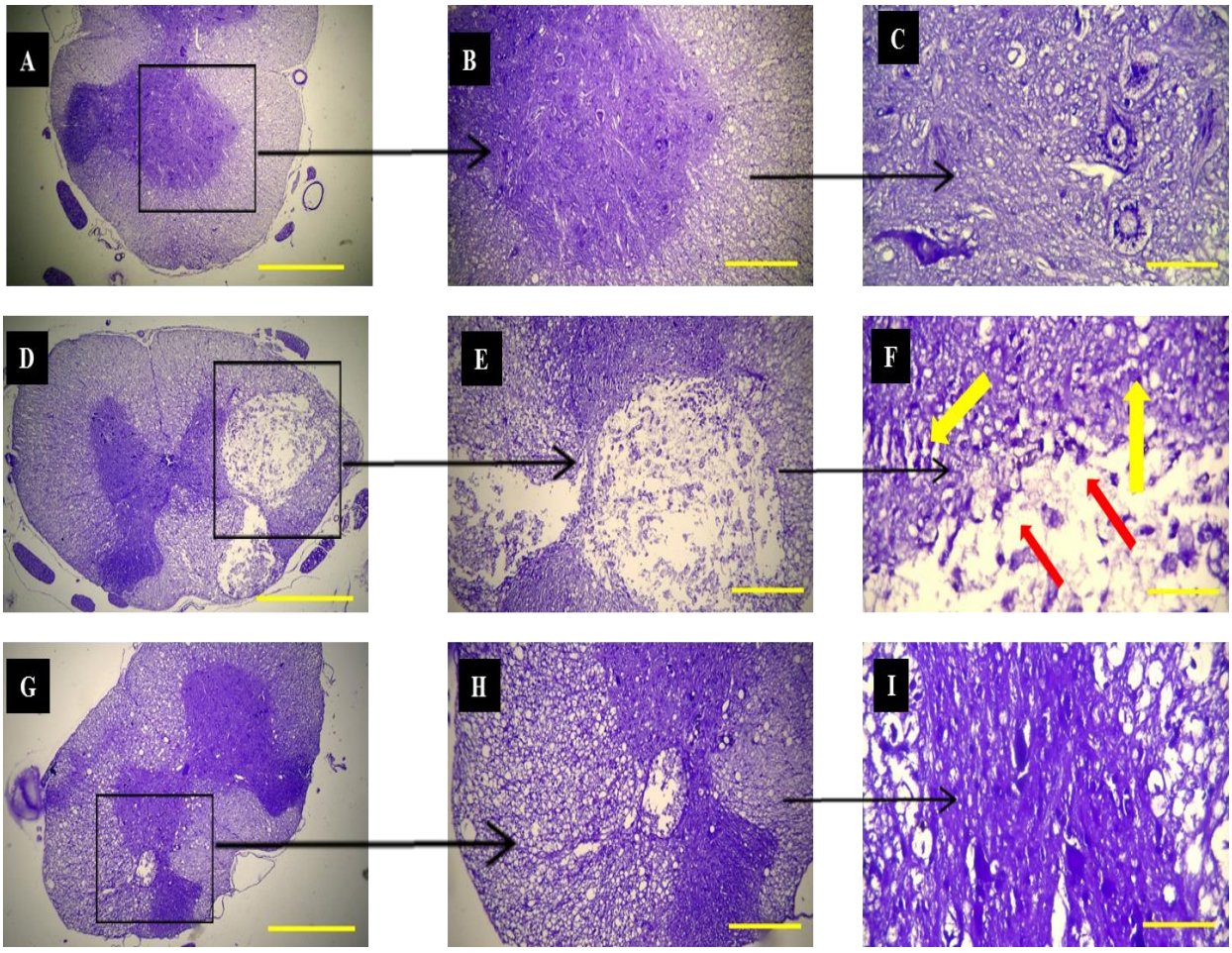

Figure 4: Transverse section of spinal cord at level of T10, stained with Cresyl violet. **(A)** Laminectomy group, scale bar = 1000 µm .**(B)** Laminectomy, scale bar = 400 µm. **(C)** Laminectomy, scale bar = 100 µm. **(D)** SCI group shows destruction of white & grey matter, scale bar = 1000 µm .**(E) SCI** group, scale bar = 400 µm . **(F)** SCI group, red arrows showing numerous cavities, yellow arrows showing dark neurons, scale bar = 100 µm. **(G)** EVs group with recovered grey and white matter scale bar = 1000 µm. **(H)** EVs group, scale bar = 400 µm. **(I)** EVs group showing less and smaller cavities with less dark neurons, scale bar = 100 µm.

DISCUSSIONS

Histological analysis (H & E and cresy violet) shows tissue damage on unilateral SCI with vacuolization, around the vacuolization with numerous hemorrhages, appearance of blood confirmed the formation of good model of SCI rats, activated astrocytes aggregated and makes gliosis, macrophages, polymorphonuclear cells, lymphocyte, and no normal neurons around the injury area compared to laminectomy slide. But after intrathecal injection of EVs in SCI rats, shows much recovery in histological analysis; good recovery of both grey and white matter of the spinal cord, it shows much reduction of hemorrhage, reduction of vacuole compared with SCI slide (fig. 10 and 11), as a result of these, this research came off with two hypotheses, there is possibility the treatment prevented the secondary injury, there is also regeneration of grey and white

matter after treatment. This is in conformity with results of study conducted by Sahar et al., 2019 (20) Mohammed et al., 2020 (21), this current study is also in a conformity with the studies conducted by Mohammadi et al., 2019; Mosavi et al., 2019; Lu et al., 2019 (22-24), but there is little variation with the current study in which they used Luxol fast blue and Nissl staining techniques.

EVs are cellular debris or unneeded toxic products that are excreted from cells, but based on their origin and mechanisms generation it was found out that EVs has a very important physiological roles to play in cell-cell communication (25). EVs can be found in every body fluid; also some other cells like immune and stem cells can be induced through different stimuli such as stressors and physiological mediators to release EVs(26). The mechanisms in which EVs is very important to cell include direct membrane fusion, caveolin-mediated uptake, clathrin-dependent endocytosis, macropinocytosis and phagocytosis mediated by specific receptors (27). EVs are known to be very important in establishing and maintaining cell and tissue polarity, it also act as a vehicles for morphogen and nucleic acid release and distribution at embryonic development as well as tissue regeneration (28). In this study, SCI was induced on Wister rats which resulted to damages on the spinal cord as shown in figure 3D and 4D but after injection of EVs from SVZ that was isolated from rats, it shows much improvement and recovery.

It was revealed that EVs involves in intercellular communication and function in the regulation of stem cell maintenance, tissue immunosurveillance and tissue repair (29). EVs has ability in transporting molecules as well as modulating biological functions in recipient cells (30). The superiority as well as multi functions of EVs like its small size have bring about the possibilities for their application in diagnosis, therapeutic and screening purposes for various diseases and trauma. This is what makes my mind to think using EVs as therapeutic agent in SCI on rat model, at the end a great success was achieved in recovery there by stopping the secondary damages following SCI on rat model.

Result from a recent study indicated that miR-133b has a great impact in neuronal apoptosis, neurite outgrowth and neuronal differentiation in the CNS (31). Another study shows that overexpression of miR-133b promotes motor function recovery following stroke in rats model (32). Exosomes, a good intercellular communicator, have been in application as biological agents for systemic or local transporter of miRNAs in the therapy of different diseases such as Parkinsons's disease and stroke (33). EVs are very tiny vesicles produced by cells that can likely contribute to cell-cell signaling by transferring bioactive lipids, proteins and RNA(34). The genesis of this vesicle was identified based on the specific pattern of expression of surface antigens(34). EVs released by NSCs show therapeutic effect on neurodegenerative diseases, inflammatory and ischemic(35). EVs likely to be good for regenerative therapy because they can evade the problems of direct stem cell transplantation such as tumorigenesis, low survival and de-differentiation (36). Application of stem cell for therapy may likely work predominantly through paracrine processes involving EVs(32), these are in conformity with the current study in which EVs was injected into the SCI rats, the results shows much recovery on the spinal cord.

SVZ derived EVs has many important characteristics the double lipid membrane content, protects the inner components from digestion and degradation(37). However, EVs can also pass freely in to cerebrospinal

fluid (CSF), which will help to reach the injured site of the spinal cord. All these characteristics are very important for motor recovery following SCI (38) In this research work, it was hypothesized that direct intrathecal injection of EVs can solve the problems associated with direct stem cell injection and suppress inflammasome complex formation as well enhanced motor recovery after SCI. Results of this study show that intrathecal injection of EVs to SCI animals downregulated formation of inflammation and result to motor recovery. This shows that injection of EVs in SCI patients can arrest the secondary damages. This is in conformity with several studies conducted that shows injection of EVs, neuronal cell, MSC or other agents can arrest the formation of inflammation and leads to secondary damages. Research conducted by Rong et. al., 2019 revealed that NSC-sEVs can successfully decrease neuroinflammation and neuronal apoptosis, thereby enhancing functional recovery following SCI in rats model (39). Yanhui et. al., shows that bone mesenchymal stem cell derived extracellular vesicle (BMSC-EV) can successfully hinder the migration of pericytes, and help to keep the integrity of blood spinal cord barrier following SCI. This also followed by axonal regeneration, reduction in neuronal cell death and motor function recovery; they also suggested that EVs might possibly serve as a good therapeutic agent for SCI (24).

In cell transplants for the purposed of treatment of CNS pathologies, there is need to reach the exact target area. While SCI trauma interferes with the blood spinal cord barrier, this is restored through endogenous repair. The systemic injection of cells like injection intravenously (40) , may likely have limited time window, which in animals like rats last up to a week for the gray matter (41). In the current study, the chronic stage treatment was intended, intrathecal injection of EVs into the CSF was chosen. It was shown that stem cell injections into the CSF is more effective than injection into the blood circulation (40), previous studies show that, most of clinical studies cells were injected through lumber puncture (41). In the current study, the subarachnoid space was accessed through lumber cistern between L4-L5 with aim of EVs to migrate to the injured area, and the results shows a great success of spinal cord recovery.

SCI brings about paralysis and dysfunction of some different parts of the body and may even result to death. Functional recovery is very difficult as a result of microenvironment changes, neuroinflammation and neuronal cell death (42). Because of this, there is need to create effective treatment and management of SCI. Exosomes has a very important parts of paracrine factors produced by MSCs (43). Results of this study demonstrated that, intrathecal injection of EVs improves neuronal regeneration, arrest secondary injury and prevent neuronal cell death, which is very important for motor function recovery after SCI. Several clinical researches have been conducted for many years on neuroprotective and neurodegenerative mechanisms, still SCI has poor prognosis generally (44). Sometimes even the neurons that can sustain the initial injury possibly lost as a result of traumatic event such as neuroinflammation, apoptosis and others. This secondary injury results to damage as well as loose of function (8). Activation of microglia is the early initiator of neuroinflammation and brings about spinal cord damage and disrupts the motor function (45). In this study it was demonstrated that SVZ derived EVs can down regulate neuroinflammation, microglia activation and reduce neuronal cells damage, thereby enhancing motor function recovery on SCI rat model.

Application of stem cell transplantation as a

way of therapy for CNS diseases was considered a good potential way of treatment, due to the ability of stem cells to differentiate into different cell types (46, 47). Several studies have revealed that NSCs safeguard surviving neurons and promote motor function recovery following SCI (48-50) as well as hypoxia-ischemia through decreasing inflammation processes (51-53). Many evidences from previous studies show a great success in different animal disease models, this can also be achieved in clinical application; many problems associated with CNS can be solved. In this study a great success was achieved in which transplantation of EVs reduced the formation of inflammation, apoptosis as well as motor function recovery on rat SCI models. The mechanisms behind this could be being an exosome a good intercellular communicator, local transporter of miRNAs in the therapy of different diseases such as neuronal diseases, can likely contribute to cell-cell signaling by transferring bioactive lipids, proteins and RNA into the injured area.

CONCLUSION

Spinal cord injury considered a very traumatic disease, and no specific treatment of SCI, from the results of this study, it was concluded that intrathecal inject of SVZ Derived EVs can regenerate the motor function and stop secondary damages of SCI. This study concluded that intrathecal injection of MSc directly into CSF is better route of injection than any other routes. This study also hypothesized that intrathecal injection of EVs prevent secondary injury, caused regeneration of grey and white matter after treatment, improved motor function recovery in SCI rats' model.

Acknowledgement: I would like to thanks and acknowledge the Department of Anatomical Sciences, Tehran University of medical sciences for giving me the courage and support for carrying out this research. I will never forget with Prof. Gholamreza Hassanzadeh for his maximum support and help for the success of this research. I would like to thank the International office of the Tehran University of Medical Sciences for their help, advices for the success of this research. I want to thank the Iranian Government for funding this research to success.

Funding: This study was funded by the **Iranian Government**

REFERENCES

1. Yune TY, Lee JY, Jung GY, Kim SJ, Jiang MH, Kim YC, et al. Minocycline alleviates death of oligodendrocytes by inhibiting pro-nerve growth factor production in microglia after spinal cord injury. Journal of Neuroscience. 2007;27(29):7751-61.

2. Price C, Makintubee S, Hemdon W, Istre GR. Epidemiology of traumatic spinal cord injury and acute hospitalization and rehabilitation charges for spinal cord injuries in Oklahoma, 1988–1990. American journal of epidemiology. 1994;139(1):37-47.

3. Chhabra H, Arora M. Demographic profile of traumatic spinal cord injuries admitted at Indian Spinal Injuries Centre with special emphasis on mode of injury: a retrospective study. Spinal Cord. 2012;50(10):745.

4. Mortezaee K, Khanlarkhani N, Beyer C, Zendedel A. Inflammasome: its role in traumatic brain and spinal cord injury. Journal of cellular physiology. 2018;233(7):5160-9.

5. Gholaminejhad M, Arabzadeh S, Akbari M, Mohamadi Y, Hassanzadeh G. Anti-oxidative and neuroprotective effects of flaxseed on experimental unilateral spinal cord injury in rat. Journal of Contemporary Medical Sciences. 2017;3(10):213-7.

6. Martinon F, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-β. Molecular cell. 2002;10(2):417-26.

7. Rowland JW, Hawryluk GW, Kwon B, Fehlings MG. Current status of acute spinal cord injury pathophysiology and emerging therapies: promise on the horizon. Neurosurgical focus. 2008;25(5):E2.

8. McDonald JW, Sadowsky C. Spinal-cord injury. The Lancet. 2002;359(9304):417-25.

9. Sekhon LH, Fehlings MG. Epidemiology, demographics, and pathophysiology of acute spinal cord injury. Spine. 2001;26(24S):S2-S12.

10. Hall ED, Springer JE. Neuroprotection and acute spinal cord injury: a reappraisal. NeuroRx. 2004;1(1):80-100.

11. Lois C, Alvarez-Buylla A. Long-distance neuronal migration in the adult mammalian brain. Science. 1994;264(5162):1145-9.

12. Menn B, Garcia-Verdugo JM, Yaschine C, Gonzalez-Perez O, Rowitch D, Alvarez-Buylla A. Origin of oligodendrocytes in the subventricular zone of the adult brain. Journal of Neuroscience. 2006;26(30):7907-18.

13. Anderson DJ. Stem cells and pattern formation in the nervous system: the possible versus the actual. Neuron. 2001;30(1):19-35.

14. Arias-Carrión O, Yuan T-F. Autologous neural stem cell transplantation: a new treatment option for Parkinson's disease? Medical hypotheses. 2009;73(5):757-9.

15. Rossi F, Cattaneo E. Opinion: neural stem cell therapy for neurological diseases: dreams and reality. Nature reviews Neuroscience. 2002;3(5):401.

16. Martino G, Pluchino S. The therapeutic potential of neural stem cells. Nature reviews Neuroscience. 2006;7(5):395.

17. Aligholi H, Hassanzadeh G, Azari H, Rezayat SM, Mehr SE, Akbari M, et al. A new and safe method for stereotactically harvesting neural stem/progenitor cells from the adult rat subventricular zone. Journal of neuroscience methods. 2014;225:81-9.

18. Azari H, Rahman M, Sharififar S, Reynolds BA. Isolation and expansion of the adult mouse neural stem cells using the neurosphere assay. JoVE (Journal of Visualized Experiments). 2010(45):e2393.

19. Farahabadi A, Akbari M, Pishva AA, Zendedel A, Arabkheradmand A, Beyer C, et al. Effect of Progesterone Therapy on TNF-α and iNOS Gene Expression in Spinal Cord Injury Model. Acta Medica Iranica. 2016;54(6):345-51.

20. Ijaz S, Mohammed I, Gholaminejhad M, Mokhtari T, Akbari M, Hassanzadeh G. Modulating Pro-inflammatory Cytokines, Tissue Damage Magnitude, and Motor Deficit in Spinal Cord Injury with

Subventricular Zone-Derived Extracellular Vesicles. Journal of Molecular Neuroscience. 2019:1-9.

21. Mohammed I, Ijaz S, Mokhtari T, Gholaminejhad M, Mahdavipour M, Jameie B, et al. Subventricular zone-derived extracellular vesicles promote functional recovery in rat model of spinal cord injury by inhibition of NLRP3 inflammasome complex formation. Metabolic brain disease. 2020;35(5).

22. Mohamadi Y, Moghahi SMHN, Mousavi M, Borhani-Haghighi M, Abolhassani F, Kashani IR, et al. Intrathecal transplantation of Wharton's jelly mesenchymal stem cells suppresses the NLRP1 inflammasome in the rat model of spinal cord injury. Journal of chemical neuroanatomy. 2019;97:1-8.

23. Mousavi M, Hedayatpour A, Mortezaee K, Mohamadi Y, Abolhassani F, Hassanzadeh G. Schwann cell transplantation exerts neuroprotective roles in rat model of spinal cord injury by combating inflammasome activation and improving motor recovery and remyelination. Metabolic brain disease. 2019:1-14.

24. Lu Y, Zhou Y, Zhang R, Wen L, Wu K, Li Y, et al. Bone Mesenchymal Stem Cell-Derived Extracellular Vesicles Promote Recovery Following Spinal Cord Injury via Improvement of the Integrity of the Blood-Spinal Cord Barrier. Frontiers in neuroscience. 2019;13.

25. Colombo M, Raposo G, Théry C. Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. Annual review of cell and developmental biology. 2014;30:255-89.

26. Farahabadi A, Akbari M, Pishva AA, Zendedel A, Arabkheradmand A, Beyer C, et al. Effect of progesterone therapy on TNF-α and iNOS gene expression in spinal cord injury model. Acta Medica Iranica. 2016:345-51.

27. Mulcahy LA, Pink RC, Carter DRF. Routes and mechanisms of extracellular vesicle uptake. Journal of extracellular vesicles. 2014;3(1):24641.

28. Clevers H, Loh KM, Nusse R. An integral program for tissue renewal and regeneration: Wnt signaling and stem cell control. science. 2014;346(6205):1248012.

29. Mousavi M, Hedayatpour A, Mortezaee K, Mohamadi Y, Abolhassani F, Hassanzadeh G. Schwann cell transplantation exerts neuroprotective roles in rat model of spinal cord injury by combating inflammasome activation and improving motor recovery and remyelination. Metabolic brain disease. 2019;34(4):1117-30.

30. Logozzi M, Mizzoni D, Bocca B, Di Raimo R, Petrucci F, Caimi S, et al. Human primary macrophages scavenge AuNPs and eliminate it through exosomes. A natural shuttling for nanomaterials. European Journal of Pharmaceutics and Biopharmaceutics. 2019;137:23-36.

31. Xia C, Cai Y, Lin Y, Guan R, Xiao G, Yang J. MiR-133b-5p regulates the expression of the heat shock protein 70 during rat neuronal cell apoptosis induced by the gp120 V3 loop peptide. Journal of medical virology. 2016;88(3):437-47.

32. Xin H, Katakowski M, Wang F, Qian J-Y, Liu XS, Ali MM, et al. MicroRNA-17–92 cluster in exosomes enhance

neuroplasticity and functional recovery after stroke in rats. Stroke. 2017;48(3):747-53.

33. Haney MJ, Klyachko NL, Zhao Y, Gupta R, Plotnikova EG, He Z, et al. Exosomes as drug delivery vehicles for Parkinson's disease therapy. Journal of Controlled Release. 2015;207:18-30.

34. Benito-Martin A, Di Giannatale A, Ceder S, Peinado H. The new deal: a potential role for secreted vesicles in innate immunity and tumor progression. Frontiers in immunology. 2015;6:66.

35. McCulloh CJ, Olson JK, Wang Y, Zhou Y, Tengberg NH, Deshpande S, et al. Treatment of experimental necrotizing enterocolitis with stem cell-derived exosomes. Journal of pediatric surgery. 2018;53(6):1215-20.

36. Baglio SR, Pegtel DM, Baldini N. Mesenchymal stem cell secreted vesicles provide novel opportunities in (stem) cell-free therapy. Frontiers in physiology. 2012;3:359.

37. Yang Y, Ye Y, Su X, He J, Bai W, He X. MSCs-derived exosomes and neuroinflammation, neurogenesis and therapy of traumatic brain injury. Frontiers in Cellular Neuroscience. 2017;11:55.

38. Liu W, Wang Y, Gong F, Rong Y, Luo Y, Tang P, et al. Exosomes derived from bone mesenchymal stem cells repair traumatic spinal cord injury by suppressing the activation of A1 neurotoxic reactive astrocytes. Journal of neurotrauma. 2019;36(3):469-84.

39. Rong Y, Liu W, Wang J, Fan J, Luo Y, Li L, et al. Neural stem cell-derived small extracellular vesicles attenuate apoptosis and neuroinflammation after traumatic spinal cord injury by activating autophagy. Cell death & disease. 2019;10(5):340.

40. Vaquero J, Zurita M, Oya S, Santos M. Cell therapy using bone marrow stromal cells in chronic paraplegic rats: systemic or local administration? Neuroscience letters. 2006;398(1-2):129-34.

41. Donnelly DJ, Popovich PG. Inflammation and its role in neuroprotection, axonal regeneration and functional recovery after spinal cord injury. Experimental neurology. 2008;209(2):378-88.

42. Kjell J, Olson L. Rat models of spinal cord injury: from pathology to potential therapies. Disease models & mechanisms. 2016;9(10):1125-37.

43. Ren K. Exosomes in perspective: a potential surrogate for stem cell therapy. Odontology. 2018:1-14.

44. Giger RJ, Hollis ER, Tuszynski MH. Guidance molecules in axon regeneration. Cold Spring Harbor perspectives in biology. 2010;2(7):a001867.

45. Hausmann O. Post-traumatic inflammation following spinal cord injury. Spinal cord. 2003;41(7):369.

46. Wyatt LA, Keirstead HS. Stem cell-based treatments for spinal cord injury. Progress in brain research. 201: Elsevier; 2012. p. 233-52.

47. Lescaudron L, Naveilhan P, Neveu I. The use of stem cells in regenerative medicine for Parkinson's and Huntington's diseases. Current medicinal chemistry. 2012;19(35):6018-35.

48. Stenudd M, Sabelström H, Frisén J. Role of endogenous neural stem cells in spinal cord injury and repair. JAMA neurology. 2015;72(2):235-7.

49. Zhu Y, Uezono N, Yasui T, Nakashima K. Neural stem cell therapy aiming at better functional recovery after spinal cord injury. Developmental Dynamics. 2018;247(1):75-84.

50. Chen N, Cen J-s, Wang J, Qin G, Long L, Wang L, et al. Targeted inhibition of leucine-rich repeat and immunoglobulin domain-containing protein 1 in transplanted neural stem cells promotes neuronal differentiation and functional recovery in rats subjected to spinal cord injury. Critical care medicine. 2016;44(3):e146-e57.

51. Braccioli L, Vervoort SJ, Adolfs Y, Heijnen CJ, Basak O, Pasterkamp RJ, et al. FOXP1 promotes embryonic neural stem cell differentiation by repressing Jagged1 expression. Stem cell reports. 2017;9(5):1530-45.

52. Zhang G, Chen L, Chen W, Li B, Yu Y, Lin F, et al. Neural stem cells alleviate inflammation via neutralization of IFN-γ negative effect in ischemic stroke model. Journal of biomedical nanotechnology. 2018;14(6):1178-88.

53. Koutsoudaki PN, Papastefanaki F, Stamatakis A, Kouroupi G, Xingi E, Stylianopoulou F, et al. Neural stem/progenitor cells differentiate into oligodendrocytes, reduce inflammation, and ameliorate learning deficits after transplantation in a mouse model of traumatic brain injury. Glia. 2016;64(5):763-79.